# FLOWER INITIATION AND DEVELOPMENT IN SELECTED CULTIVARS OF THE GENUS *PROTEA*

**BY**

**AUDREY INGA GERBER (nee TIMM)**

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**PROMOTER**

Prof. G. Jacobs - Department of Horticultural Science, University of Stellenbosch.

**CO-PROMOTER**

Prof. K. I. Theron - Department of Horticultural Science, University of Stellenbosch

## DECLARATION

I, the undersigned hereby declare that the work contained in this dissertation is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

## SUMMARY

Little is understood regarding flowering in the genus *Protea*. The information available on inflorescence initiation and development in the family *Proteaceae* was reviewed and discussed. A number of experiments were conducted to investigate inflorescence initiation and development, and their manipulation for commercial production, in selected *Protea* cultivars, in the Western Cape, South Africa (33°S, 19°E)

Protea species can be allocated into groups according to similar times of flower initiation and of harvest. The stages occurring during flower initiation, and their synchrony relative to shoot growth were investigated for three cultivars, viz. *Protea* cv. Carnival (*P. compacta* × *P. neriifolia*), *Protea* cv. Lady Di (*P. compacta* × *P. magnifica*) and *Protea* cv. Sylvia (*P. eximia* × *P. susannae*), when flower initiation occurred on the spring growth flush.

For all three cultivars the spring flush was preformed and enclosed in the apical bud before spring budbreak. During elongation of the spring flush the apical meristem produced floral primordia which differentiated into involucral bracts. After completion of the spring flush meristematic activity continued, to produce floral bracts with florets in their axils. The three cultivars showed differences and similarities in the time of budbreak, and the rates of shoot growth, appendage formation and flower development.

The presence of mature leaves on an over-wintering shoot is essential for inflorescence initiation on the spring growth flush of 'Carnival'. Inflorescence initiation in 'Carnival' started at spring budbreak, and production of involucral bracts occurred concurrently with spring flush elongation. Shoots were defoliated at different degrees of severity at intervals from pre- to post- spring budbreak. Total defoliation applied earlier than 6-7 weeks before spring budbreak prevented flowering. Defoliation closer to spring budbreak affected characteristics of the spring flush and the inflorescence subtended by the spring flush. Effects were most marked following total defoliation and diminished with less severe treatments imposed by partial defoliation. Total defoliation applied before spring budbreak resulted in slower inflorescence development and lead to later anthesis. Defoliation treatments applied after completion of spring flush elongation had no effect on either vegetative or reproductive spring growth.

The requirement for mature overwintering leaves to effect inflorescence initiation in 'Carnival' suggests that environmental factors, such as low temperature and daylength may play an inductive role. Shoots were in the induced state and committed to flowering 6-7 weeks before spring budbreak.

A change in source size and position subsequent to different severalties of defoliation in 'Carnival' lead to reduced dry mass accumulation and altered partitioning. Mature leaves on the overwintering shoot supported growth of the spring flush and the early stages of inflorescence development. When these leaves were removed by total defoliation dry mass accumulation in the spring flush was reduced. A hierarchy of

priorities between competing sinks was revealed by defoliation during growth of the spring flush and concomitant inflorescence development: formation of involucral bracts > leaf growth > stem elongation. Dry mass accumulation of the inflorescence subtended by the spring flush was supported by the spring flush leaves and was only indirectly affected by defoliation. Treatments which resulted in the production of a weaker spring flush lead to a reduction in dry mass accumulation of the inflorescence.

Different severalties of partial defoliation, whereby either upper or lower leaves were removed from a shoot, indicated that the position of leaves relative to the active sink is more important, with respect to source availability, than the number of leaves on the shoot.

Mature overwintering leaves are essential in 'Lady Di' for shoots to achieve the induced state for flowering, and are also crucial to the early stages of inflorescence initiation. Defoliation applied before formation of involucral bracts was complete prevented flowering. Defoliated shoots either remained vegetative or produced inflorescences which aborted. Reserve carbohydrates in the stem and leaves of overwintering shoots were low, and early growth and development of both the spring flush and inflorescence were, therefore, supported by current photosynthates from the overwintering leaves. Likewise, reserve carbohydrates available in the flowering shoot were insufficient to account for the dry mass increase during the major portion of growth of the spring flush and inflorescence. This rapid increase in dry mass occurred after elongation of the spring flush was complete and was supported by current photosynthates from the leaves of the spring flush. Defoliation treatments that

did not prevent inflorescence initiation, had no effect on inflorescence development, and flowering time of 'Lady Di' was not delayed by defoliation.

'Sylvia' has an open window for inflorescence initiation and can initiate flowers throughout the year. Despite the 'open window' inflorescences are initiated more readily on the spring flush, when it is subtended by one or more overwintering shoots. This may be the expression of a facultative response to inductive conditions for which 'Carnival' and 'Lady Di' have an obligate requirement.

The date of pruning affected flowering time of 'Sylvia' by influencing on which flush inflorescence initiation occurred, and the harvest could be manipulated to fall within the optimum marketing period for export to Europe. Flowers initiated on the spring flush reach anthesis in January and February; on the first summer flush predominantly in April and May; on the second summer flush in July and August; and on the autumn flush in November and December. Thus, shoots harvested within the optimum marketing period (September to February) initiated inflorescences on the autumn and spring flushes. Due to the readiness of shoots to initiate inflorescences on the spring flush many shoots harvested in January and February (following initiation in the previous spring) were short and were rendered unmarketable. For commercial production pruning in July is recommended. Long flowering stems will be harvested in October to November of the following year. Since the vegetative and reproductive cycles necessary to produce inflorescences on long stems span more than a year, a biennial cropping system is recommended.

# Bloeiwyse-inisiasie en –ontwikkeling, en die manipulasie daarvan, van geselekteerde cultivars van die genus *Protea*.


## Opsomming

Min word verstaan van blomvorming in die genus *Protea*. Die beskikbare inligting oor die bloeiwyse-inisiasie en –ontwikkeling in die familie *Proteaceae* is nagegaan en bespreek. 'n Aantal eksperimente is uitgevoer waarin geselekteerde *Protea* cultivars van die Wes-Kaap, Suid-Afrika (33°S, 19°O) se bloeiwyse-inisiasie en –ontwikkeling, asook die manipulasie daarvan vir kommersiële produksie ondersoek is.

Protea spesies kan in groepe ingedeel word op grond van blominisiasietye en oestye wat ooreenstem. Die verskillende stadiums van blominisiasie en hulle sinchronisering relatief tot stingelgroei is ondersoek vir drie kultivars, naamlik *Protea* cv. Carnival (*P. compacta* x *P. neriifolia*), *Protea* cv. Lady Di (*P. compacta* x *P. magnifica*) en *Protea* cv. Sylvia (*P. eximia* x *P. susannae*) tydens blominisiasie op die lentegroeistuwing.

By al drie die kultivars was die lentegroeistuwing reeds gevorm en omsluit in die apikale knop voor die lente-knopbreking. Gedurende die verlenging van die lentegroeistuwing het die apikale meristeem blomprimordia, wat in bloeiwyse-omwindselskutblare gedifferensieer het, geproduseer. Na voltooiing van die lentegroeistuwing, het meristematiese aktiwiteit voortgeduur en blomskutblare met blommetjies in hulle oksels is gevorm. Die drie kultivars het verskille en ooreenkomste vertoon tydens die periode van knopbreking, asook in die tempo van stingelgroei, aanhangselformasie en blomontwikkeling.

Die teenwoordigheid van volwasse blare op 'n oorwinteringstingel is noodsaaklik vir bloeiwyse-inisiasie op die lentegroeistuwing van 'Carnival'. Bloeiwyse-inisiasie in 'Carnival' het met lente-knopbreking begin en die produksie van bloeiwyse-omwindselblare het gelyktydig met lentegroeistuwing verlenging plaasgevind. Stingels is met tussenposes, van voor tot na die lente-knopbreking, en met verskillende grade van felheid, ontblaar. Algehele ontblaring vroeër as 6-7 weke voor die lente-knopbreking het blomvorming verhoed. Ontblaring nader aan die lente-knopbreking het 'n invloed gehad op die eienskappe van die lentegroeistuwing asook die bloeiwyse gedra deur die lentegroeistuwing. Die effek was die duidelikste sigbaar by algehele ontblaring en het verminder namate die behandeling minder fel geword het by gedeeltelike ontblaring. Algehele ontblaring wat voor die lente-knopbreking gedoen is, het gelei tot stadiger bloeiwyse-ontwikkeling en later antese. Ontblaringsbehandelings wat na die voltooiing van die lentegroeistuwing verlenging toegepas is, het geen effek op die vegetatiewe of die reproduktiewe lentegroei gehad nie.

Die nodigheid van volwasse oorwinteringsblare vir bloeiwyse-inisiasie in 'Carnival' dui daarop dat omgewingsfaktore soos lae temperature en daglengte 'n induktiewe rol kan speel. Stingels was in die geïnduseerde toestand en verbind tot blomvorming 6-7 weke voor die lente-knopbreking.

'n Verandering in oorspronggrootte en -posisie as gevolg van verskille in die felheid van ontblaring by 'Carnival', het gelei tot verminderde droë-massa-akkumulasie en

veranderde verdeling. Volwasse blare op die oorwinteringstingel het die groei van die lentegroeistuwing en die vroeë stadiums van bloeiwyse-ontwikkeling ondersteun. Toe hierdie blare verwyder is in 'n algehele ontblaring, het die droë-massa-akkumulasie in die lentegroeistuwing verminder. 'n Hiërargie van prioriteite tussen kompeterende sinke is blootgelê tydens ontblaring gedurende die lentegroeistuwing en saamlopende bloeiwyse-ontwikkeling: vorming van bloeiwyse-omwindselblare > blaargroei > stamverlenging. Droë-massa-akkumulasie van die bloeiwyse onderspan deur die lentegroeistuwing is ondersteun deur die blare van die lentegroeistuwing en is slegs op 'n indirekte wyse deur ontblaring geaffekteer. Behandelings wat tot die produksie van 'n swakker lentegroeistuwing gelei het, het tot 'n vermindering in die droë-massa-akkumulasie van die bloeiwyse gelei.

Verskille in die felheid van gedeeltelike ontblaring, waartydens óf die boonste óf die onderste blare van 'n stingel verwyder is, het aangetoon dat die posisie van die blare relatief tot die aktiewe sink belangriker is, met betrekking tot die beskikbaarheid van die oorsprong, as die aantal blare op die stingel.

By 'Lady Di' is volwasse oorwinteringsblare noodsaaklik vir stingels om die geïnduseerde stadium van blomvorming te bereik en hulle is ook van die uiterste belang in die vroeë stadiums van bloeiwyse-inisiasie. Waar ontblaring gedoen is voordat die vorming van bloeiwyse-omwindsel voltooi was, het blomvorming nie plaasgevind nie. Ontblaarde stingels het óf vegetatief gebly óf bloeiwyses geproduseer wat geaborteer het. Reserwe-koolhidrate in die stam en blare van die oorwinteringstingels was laag en die vroeë groei en ontwikkeling van beide die

lentegroeistuwing en die bloeiwyse is dus deur die bestaande fotosintate van die oorwinteringsblare onderhou. Net so was die reserwe-koolhidrate beskikbaar in die blomdraende stingels nie voldoende om die toename in droë massa gedurende die grootste deel van die groei van die lentegroeistuwing en die bloeiwyse te verklaar nie. Hierdie vinnige toename in droë massa het plaasgevind nadat die verlenging van die lentegroeistuwing voltooi was en is deur die bestaande fotosintate van die blare van die lentegroeistuwing onderhou. Ontblaringsbehandelings wat nie bloeiwyse-inisiasie verhoed het nie, het geen effek op bloeiwyse-ontwikkeling gehad nie en die blomtyd van 'Lady Di' is nie deur ontblaring vertraag nie.

'Sylvia' beskik oor 'n oop venster vir bloeiwyse-inisiasie en kan regdeur die jaar blomme inisieer. Ten spyte van die 'oop venster', word bloeiwyses tog meer geredelik in die lentegroeistuwing geïnisieer, wanneer dit deur een of meer van die oorwinteringstingels gedra word. Dit mag die uitdrukking wees van 'n fakultatiewe respons op induktiewe toestande wat vir 'Carnival' en 'Lady Di' 'n verpligte vereiste is.

'Sylvia' se blomtyd is deur die snoeidatum geaffekteer omdat die snoeidatum 'n invloed gehad het op die keuse van by watter groeistuwing bloeiwyse-inisiasie plaasgevind het. Die oestyd kon gemanipuleer word om binne die optimum bemarkingstydperk vir uitvoer na Europa te val. Blomme wat op die lentegroeistuwing geïnisieer is, bereik antese in Januarie en Februarie; dié wat op die eerste somergroeistuwing geïnisieer is, bereik antese hoofsaaklik in April en Mei; dié wat op die tweede somergroeistuwing geïnisieer is, bereik antese in Julie en Augustus en dié wat op die herfsgroeistuwing geïnisieer is, bereik antese in November en

Desember. Stingels wat in die optimum bemarkingsperiode (September tot Februarie) geoes is, het dus bloeiwyses op die herfs- en lente-groeistuwings geïnisieer. As gevolg van die gereedheid van stingels om bloeiwyses op die lentegroeistuwings te inisieer, was baie van die stingels wat in Januarie en Februarie geoes is, kort en kon nie bemark word nie. Vir kommersiële doeleindes word snoei in Julie aanbeveel. Lang blomdraende stingels sal in Oktober en November van die volgende jaar geoes word. Aangesien die vegetatiewe en reproduktiewe siklusse wat nodig is om bloeiwyses met lang stingels te produseer oor meer as 'n jaar strek, word 'n tweejaarlikse oesinsamelingstelsel aanbeveel.

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# CONTENTS

# 1. INTRODUCTION AND LITERATURE REVIEW -

## Inflorescence initiation and development in *Proteaceae*.

## Inflorescence initiation and development in *Proteaceae.*

Many genera of *Proteaceae* have potential as cut flower products. The main genera under production are: *Protea, Leucospermum, Leucadendron, Banksia, Serruria* and *Telopea.* The protea industry has developed rapidly in the last decade, both in South Africa and world-wide. Members of *Proteaceae* are produced for the floriculture industry in countries where indigenous species occur, e.g. South Africa, Zimbabwe and Australia, as well as many countries to which *Proteaceae* are exotic, e.g. New Zealand, Spain, Israel and U.S.A. (Hawaii and California). In South Africa the major development in the industry in recent years has been a decline in products harvested from natural stands, and this market share has been filled, and, indeed, extended, by produce grown in commercial plantations. To a large extent this trend was induced by an increasingly discerning consumer whose demands of a higher quality product necessitated a commercialisation of the industry.

In the floriculture industry the keywords to successful marketing are quality, uniformity, and a continuous supply. In addition to this, products exported from the Southern Hemisphere to the large flower markets in Europe attain higher prices and are more in demand during the European winter (September to February) when competition from locally grown flowers is low.

In order to meet the challenges of a developing industry, production of *Proteaceae* in South Africa, and other countries, needs to aspire to the sophistication of cultivation apparent in other floriculture crops.

Wild harvested produce tends to be of poor quality due to low, or non-existent, management standards to protect the plant against pest and disease damage. In addition, stems are often short and crooked. Cultivation in commercial plantations

following correct soil preparation allows optimum pest and disease control as well as application of management strategies, such as irrigation and fertilisation.

An improvement in the uniformity of products has been achieved by selections from seedling populations and dedicated breeding programmes to produce superior products. Visual characteristics and high production potential have been important selection criteria. More than 300 cultivars have been registered, with selections and hybrids originating from Australia, New Zealand, South Africa, Zimbabwe, and U.S.A. The establishment of efficient propagation techniques has enabled rapid multiplication and large scale production of clonal material.

Fulfilling the requirement for a continuous supply of superior products within the optimum marketing period has been more of a challenge to the industry. The natural variation in a seedling population tends to lead to extended production. Selection for specific criteria and clonal propagation of the selections removes this variability.

Fig. 1 displays the marketing figures for Protea species exported from South Africa during the 1997/98 season, largely grown in commercial plantations, but of seedling origin (Dücker, 1999). In all species production occurs over many months, much of which falls in the optimum marketing period between September and February.

The flowering times of *Protea* cultivars and selections, grouped according to "species type" visual characteristics (under which they are often marketed), illustrates how the advantage of extended production due to seedling variability is lost (Fig. 2-6).

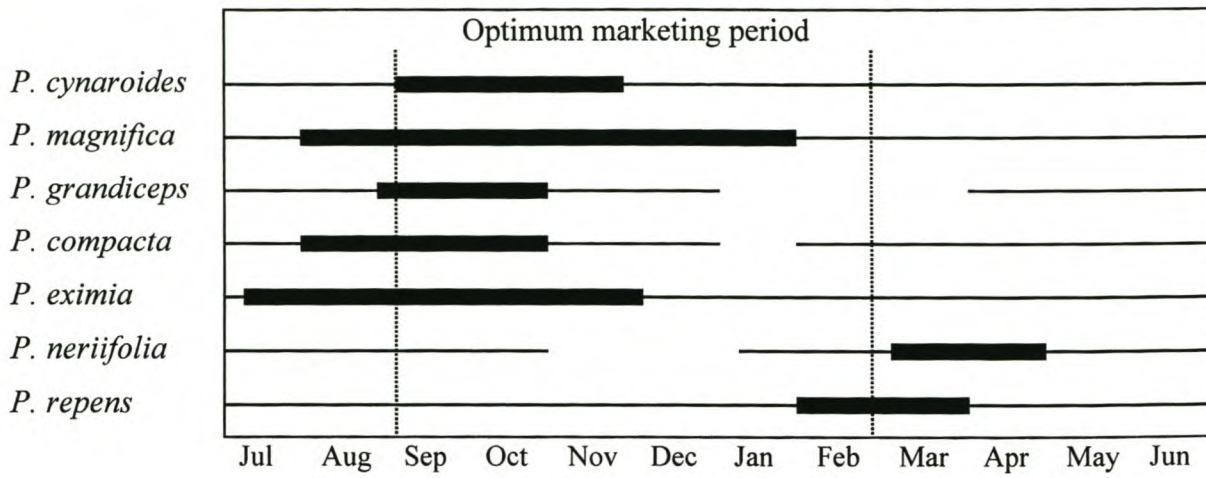



Fig. 1 . Time of production of *Protea* species in South Africa relative to the optimum marketing period (between vertical dotted lines). Horizontal lines indicate the harvesting period, with bold lines representing time of peak harvest (Dücker, 1999). (Figures for *P. repens* are for the Eastern Cape variant).

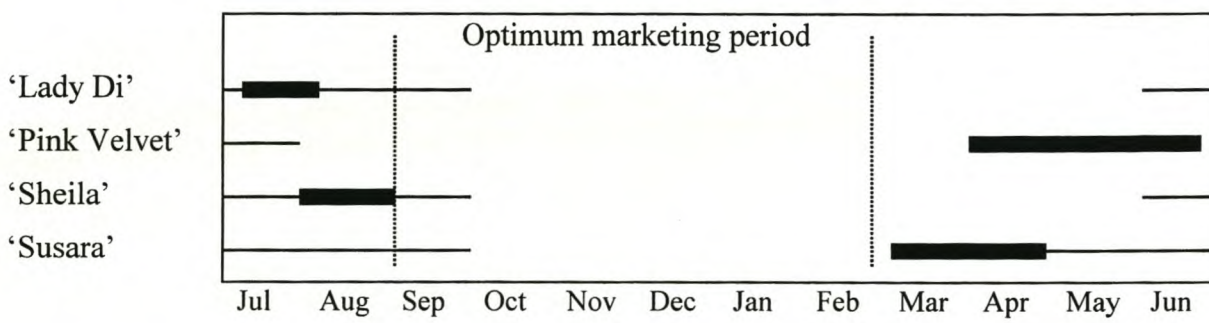



Fig. 2. Production time of "magnifica type" *Protea* cultivars in South Africa relative to the optimum marketing period (between vertical dotted lines). 'Lady Di' (*P. magnifica* × *P. compacta*); ' Pink Velvet' (*P. magnifica* × *P. compacta*); 'Sheila' (*P. magnifica* × *P. burchellii*); 'Susara' (*P. magnifica* × *P. susannae*). Horizontal lines indicate the harvesting period, with bold lines representing time of peak harvest (Dücker, 1999).

Of the main protea cultivars in production in South Africa few are harvested within the optimum marketing period. The harvest peak from *P. magnifica* seedlings falls almost entirely within the optimum marketing period (Fig. 1), while only the tail of production from "magnifica type" cultivars is harvested at this time (Fig. 2). From "compacta type" cultivars the early production is harvested toward the end of the optimum marketing period. No production occurs in the pre-Christmas period (Fig. 3). The same is true for "neriifolia type" cultivars (Fig. 4).

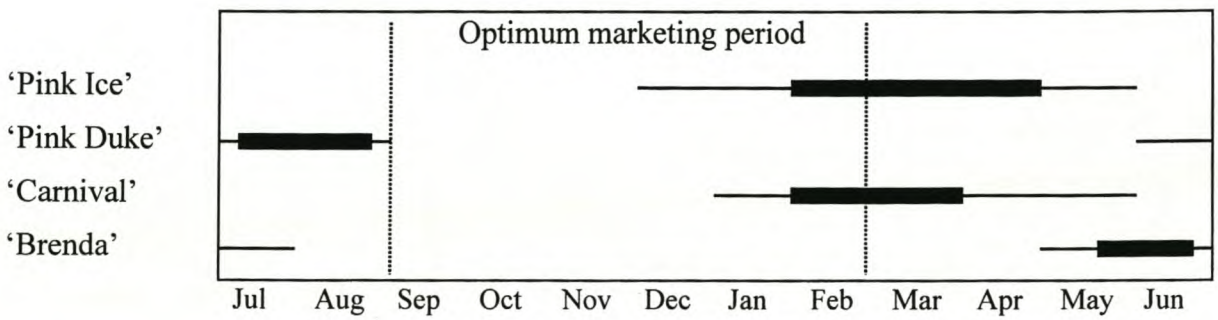



Fig. 3. Production time of "compacta type" *Protea* cultivars in South Africa relative to optimum marketing period (between vertical dotted lines). 'Pink Ice' (*P. compacta* × *P. susannae*); 'Pink Duke' (*P. compacta* hybrid); 'Carnival' (*P. compacta* × *P. neriifolia*); 'Brenda' (*P. compacta* × *P. burchellii*). Horizontal lines indicate the harvesting period, with bold lines representing time of peak harvest (Dücker, 1999).

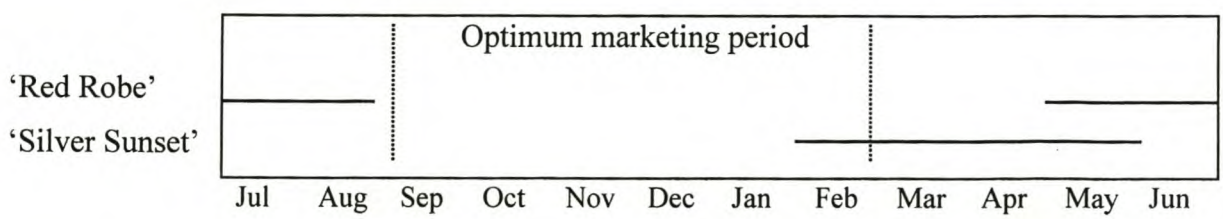



Fig. 4. Production time of "neriifolia type" *Protea* cultivars in South Africa relative to the optimum marketing period (between vertical dotted lines). Both cultivars are selections of *P. neriifolia*.. Horizontal lines indicate the harvesting period, production is too small to identify peaks.

Production in the later portion of the optimum marketing period is possible from "repens type" cultivars, but the year-round production achieved with seedling populations is unattainable (Fig. 5). Production of *P. eximia*, both of seedling origin and cultivars, is possible throughout the year (Fig. 1 and 6). This property has yet to be fully exploited to fill the optimum marketing period.

*P. cynaroides* holds a substantial market share of the industry in South Africa, but large scale production is still done from seedling populations. The few cultivars registered in South Africa are not readily available. There are no superior *P. grandiceps* selections available.

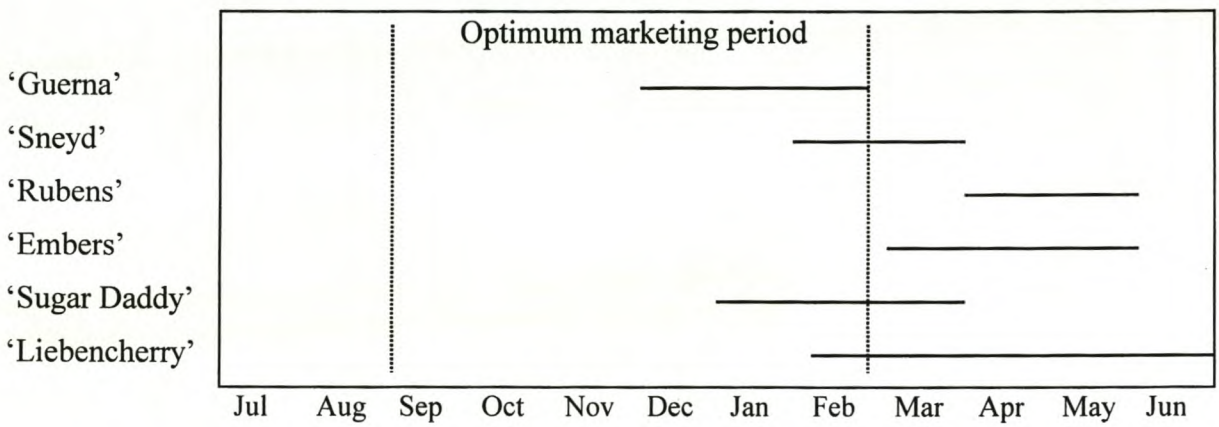



Fig. 5. Production time of "repens type" *Protea* cultivars in South Africa relative to the optimum marketing period (between vertical dotted lines). All cultivars are selections of *P. repens*, except 'Liebencherry' (*P. repens* × *P. longifolia*'). Horizontal lines indicate the harvesting period .

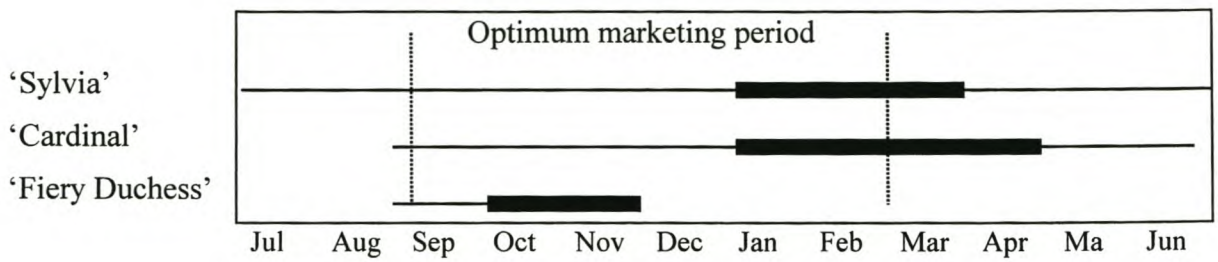



Fig. 6. Production time of "eximia type" *Protea* cultivars in South Africa relative to the optimum marketing period (between vertical dotted lines). 'Sylvia' (*P. eximia* × *P. susannae*); 'Cardinal'(*P. eximia* × *P. susannae*); 'Fiery Duchess' (*P. eximia*). Horizontal lines indicate the harvesting period, with bold lines representing time of peak harvest (Dücker, 1999).

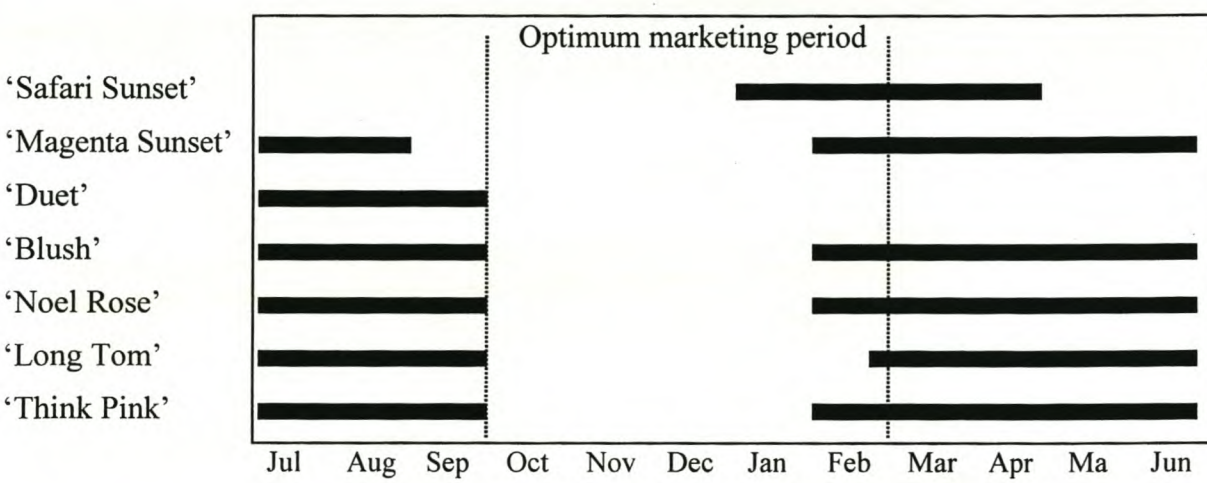



Fig. 7. Production time of *Leucadendron* cultivars available in South Africa relative to the optimum marketing period (between vertical dotted lines). Bold horizontal lines represent time of attractive appearance for marketing. All cultivars, except Duet (*L. stelligerum* hybrid) are *L. salignum* hybrids or selections.

*Leucadendrons* are harvested as fillers or foliage and the production time is not necessarily related to flowering time. Often market demand generates supply.

Cultivars, however, are harvested at a specific stage, generally associated with colour development. It is difficult to compare yield distribution between seedling and cultivar plantations purely from marketing figures, but few of the cultivars available are at their most attractive during the optimum marketing period (Fig. 7).

Flowering of *Leucospermum* species occurs naturally in the optimum marketing period and cultivars and selections have not lost this advantage, although little production occurs in January and February (Fig. 8). An understanding of the controlling factors of flower initiation has enabled extension, or delay of harvest to be achieved by disbudding (Jacobs et al., 1986), thus significantly improving the marketing period for *Leucospermum*.

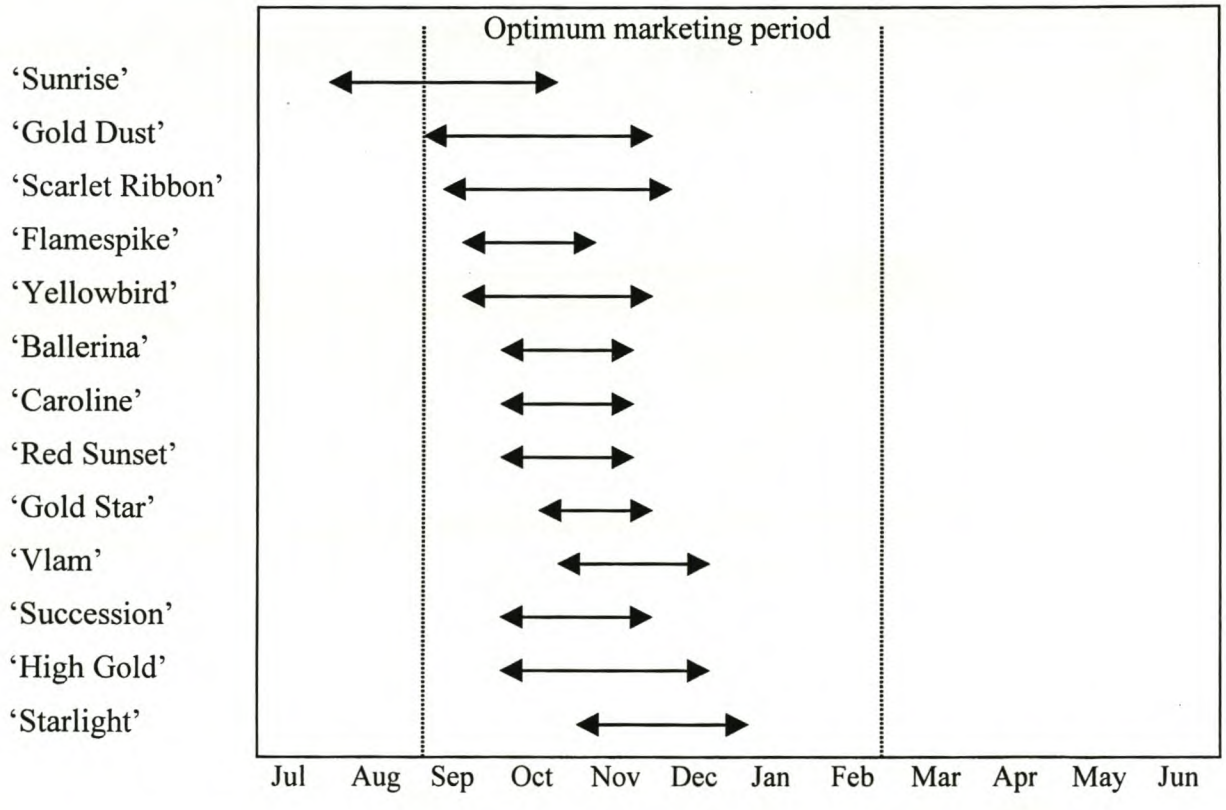



Fig. 8. Harvesting time of *Leucospermum* cultivars relative to optimum marketing period (vertical dotted lines). Arrows indicate the shift from normal flowering time to flowering time as a response to disbudding techniques (after Criley, 1998).

The majority of *Banksia* grown for cut flower production are of seedling origin. Although flower production occurs almost throughout the year, albeit in different species, many species individually have a limited period of flowering, and not much production occurs in the optimum marketing period (Fig. 9). Recent advances in breeding and selection have resulted in the introduction of cultivars to the industry (Sedgley, 1997). The seedling variation, apparent in lack of product uniformity and need for complicated management practices, is absent from these cultivars.

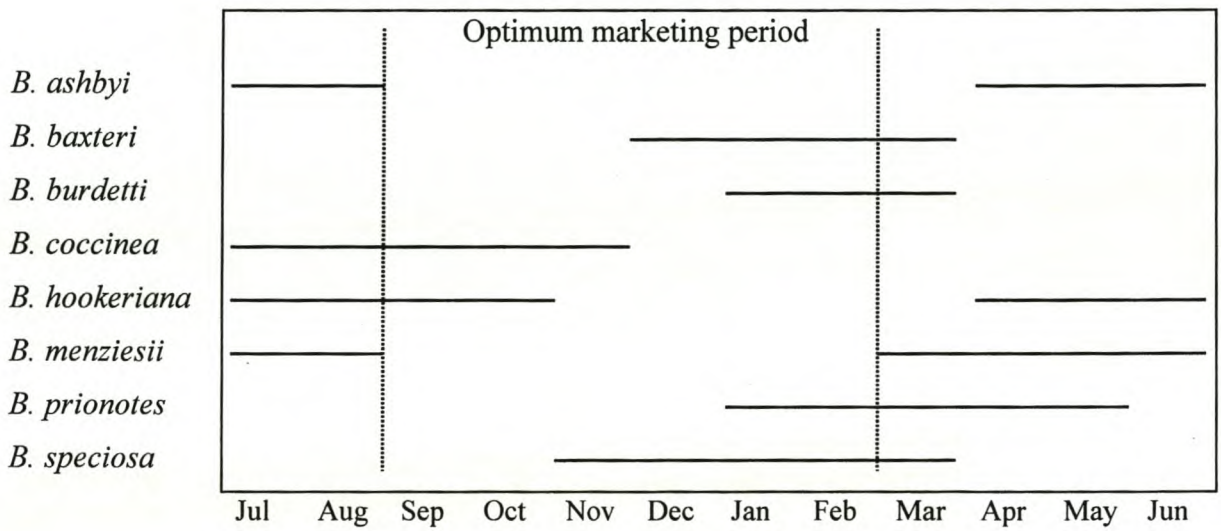



Fig. 9. Flowering times of the main species of *Banksia* cultivated in Australia for cut flower production relative to optimum marketing period (between the vertical dotted lines). Horizontal lines indicate flowering period (after Röhl et al., 1994).

Some progress has been made in manipulating flowering time of *Protea* by pruning (Gerber et al., 1995), but the most significant development in manipulating flowering time is in *Leucospermum*, and was afforded by a thorough understanding of inductive conditions for flowering and the patterns of flower initiation and development.

To summarise, although breeding and selection programmes have provided the industry with cultivars having superior characteristics the industry is still unable to produce a continuous supply of high quality products. An understanding of flower initiation and development in *Proteaceae* is essential before flowering time can be manipulated to fulfil market demands. A review of current knowledge on initiation and development in commercially important genera follows. Reviews on all aspects of *Leucospermum* (Criley, 1998) and *Banksia* (Sedgley, 1998) have recently been published and a review of *Protea* is in preparation ( Coetzee and Littlejohn, 1999).

*Flower initiation and development in Banksia*

In *Banksia* the floral structure is an inflorescence with many florets arranged in a spiral on a woody structure (Sedgley, 1998). The florets develop in pairs, each subtended by a floral bract, and the pair of floral bracts is subtended by a common bract. The bracts are inconspicuous and the colour of the inflorescence is due to the coloured perianths and styles. The species which have received most attention for commercial production as cut flowers bear flowers terminally, although many species produce axillary blooms.

Flower initiation in commercially important species occurs in late spring and early summer (October to December in the Southern Hemisphere). The start of flower initiation was identified by the production of involucral bracts, immediately preceded by a broadening of the apical meristem (Fuss and Sedgley, 1990). The link of flower initiation with a specific season indicates that temperature and daylength may be important environmental cues. Studies under conditions of controlled environment suggested that flower initiation in *B. coccinea* is influenced by daylength, and in *B. hookeriana* by temperature (Rieger and Sedgley, 1996). The positive flowering

response of *B. coccinea* to supplementary lighting may have been due to increased assimilate accumulation rather than the expression of a long day requirement. Both vegetative growth and flowering of *B. hookeriana* were inhibited by low temperatures. A short day-long day sequence was suggested to be necessary for flower induction in *B. ashbyi* (Wallerstein and Nissim, 1992).

Cessation of shoot growth is considered an essential pre-event to flower initiation (Wallerstein and Nissim, 1992). Vegetative growth in *Banksia* occurs from the terminal bud during each active growth season and continues until the tip is damaged or flower initiation takes place. Generally a single flush of growth occurs but two are occasionally produced (Fuss et al., 1992). Most vegetative growth occurs in spring and early summer. The likelihood of a shoot producing a flower is correlated with shoot age and size. Most flowers are initiated on 2-year-old shoots and only a few on 1- and 3-year-old shoots. A minimum shoot diameter, measured at the uppermost intercalation, has been identified as critical for flower initiation. In *B. coccinea* the critical diameter is 4.5 mm; in *B. menziesii*, 6 mm; in *B. hookeriana*, 8 mm; and in *B. baxteri*, 11 mm (Sedgley, 1998). This information is used in developing pruning management strategies for the different species (Sedgley and Fuss, 1992).

The rate of flower development varies between species resulting in different flowering times, despite similar times of flower initiation. Ten stages of flower development were identified from the transition of the vegetative to the reproductive state through to anthesis (Röhl et al., 1994). The difference in rate of flower development between *B. hookeriana* and *B. baxteri* was due to a delay in the macroscopic appearance of the flower bud of the former. Only the early stages of flower development occurred at different rates. In *B. menziesii* floret initiation began

in January and in April for *B. coccinea* (Fuss and Sedgley, 1990). The most rapid development was in *B. baxteri,* taking 3 months. Flowering of *B. hookeriana* occurred 5 months after initiation, and in *B. menziesii* flower development took 6-8 months, and 9-12 months in *B. coccinea* (Fuss et al., 1992). From these data the time from flower initiation to anthesis appears to be inversely related to the critical stem diameter. Abortion of individual florets, giving rise to abnormal blooms, was observed in *B. coccinea* and *B. menziesii,* and was thought to be due to low temperatures (Fuss and Sedgley, 1991).

*Flower initiation and development in Leucospermum*

In *Leucospermum* the flower is an inflorescence consisting of many individual florets arranged on an involucral receptacle (Criley, 1998). The involucral bracts remain small and, together with the coloured styles and perianths, provide the showy appearance of the inflorescence. The inflorescence develops from a distal axillary bud, but appears to be borne terminally.

Flower initiation occurs in late summer during declining daylength. Evidence for the short day requirement of *Leucospermum* for flower initiation came from experiments using artificial lighting (Malan and Jacobs, 1987; Malan and Jacobs, 1990). Long days were simulated by continuous incandescent lighting or night breaks, and prevented flowering. The short day response is quantitative and the critical daylength and the number of inductive days needed for flower initiation varies between species. In 'Red Sunset' the critical daylength is less than 12 hours (Malan and Jacobs, 1990). After 14 inductive short days the apical meristem was still conical and only flattened out after 28 days. The meristem was induced and committed to flowering after 42 days. The induced state is retained for 2-3 months then gradually

lost. The time of retention of the induced state varies from year to year and is lost sooner on short shoots (Jacobs, 1983) and under conditions of low light intensity (Napier and Jacobs, 1989). The short day requirement in *L. pattersonii* could be substituted for by low temperatures during long days (Wallerstein, 1989). Long days may be connected to the release of lateral buds from apical dominance, allowing flower initiation to occur.

Flower initiation only occurs after cessation of shoot growth and corresponding loss of apical dominance. The main period of vegetative growth occurs in summer, starting after flowering and ending with cessation of shoot growth in late summer. There was some indication that the ability of a shoot to flower was partly controlled by shoot diameter and leaf number (Jacobs, 1983). A decreased leaf starch content, as a result of shading, was associated with a reduced capacity to flower indicating the essential, yet non-determining role of carbohydrates in flowering.

Development of the inflorescence was divided into 4 stages (Napier et al., 1986): i) during the pre-floret stage peduncular bracts are formed and dry mass accumulation is low; ii) the growth rate remains slow during the floret initiation stage. Basal florets develop first; iii) the floret differentiation phase lasts about two months, during which a rapid accumulation in dry mass occurs; iv) organ development is completed in the floret enlargement phase, and the rapid increase in dry mass continues to anthesis.

The main factors affecting rate of flower development are temperature and shoot size. There is a linear relationship between mean daily temperature and the rate of inflorescence development (Criley et al., 1990), using a base temperature of 6°C (Jacobs and Honeyborne, 1979). Attempts to reduce temperatures and delay flowering by artificial shading were unsuccessful. Flower development of *L. cordifolium* cv.

Gold Dust was unaffected even under 80% shade. However, the decreased light intensity did reduce flower quality (Jacobs and Minaar, 1980). The inflorescence had fewer styles and a lower dry mass, and colour development was reduced.

The 6-10 buds immediately below the developing (primary) inflorescence also respond to inductive conditions but correlative inhibition prevents development beyond 5 mm in diameter (Malan and Jacobs, 1990). Development of the most apical secondary bud will continue if the primary inflorescence is removed during inductive conditions. The delay in continuation of development leads to a later flowering time of the secondary flower, and this technique, known as disbudding, is used in commercial practise. Cultivar differences exist in the responsiveness to disbudding and the length of the delay.

*Flower initiation and development in Protea*

The attractive appearance of *Protea* flowers is due to large, coloured involucral bracts which surround the many florets comprising the inflorescence. Floral bracts subtending the individual florets are small and inconspicuous. Florets and involucral bracts may be tipped with white or black hairs which enhance the general appearance. In the commercially important species flowers are borne terminally.

The factors affecting flower initiation in *Protea* are unclear. Unlike *Banksia* and *Leucospermum* where the time of flower initiation, and therefore the inductive factors, are similar for all species there is great variation in flowering in *Protea*. Many studies investigating flowering in *Protea* report macroscopic appearance of the flower bud as evidence of flower initiation. That flower initiation has indeed occurred is unequivocal, yet visual evidence of flowering provides no information as to the time or nature of inductive conditions. In *P. aristata* up to a year can elapse between

flower initiation and development, during which axillary shoot growth continues. This is also found, although to a lesser extent, in *P. repens* and *P. lanceolata*, and was suggested to be an adaptive mechanism, allowing extra time for shoot diameter growth to occur, both for increased mechanical strength and production of additional conducting tissue to support flower development (Le Maitre and Midgley, 1991).

Dupee and Goodwin (1990) stated that flower initiation in *P. neriifolia* cv. Salmon Pink occurs after spring flush growth in late October or early November, and in *P. cynaroides* there are two short periods during which flower initiation can occur, viz, May and December (Southern Hemisphere). In neither instance was a definition of flower initiation given. The presence of visible flower buds in late October on *P. neriifolia* in South Africa lead Heinsohn and Pammenter (1988) to conclude that the "flowering signal" was received and acted upon earlier in the growing season. Flower initiation in *P.* cv. Carnival (*P. neriifolia* × *P. compacta)* occurred mainly on the spring flush, but could also occur on the first summer flush, again intimating at initiation early in the season. At least two consecutive flushes were necessary for flower formation, although flowers were never initiated on the second summer or autumn flushes (Greenfield et al., 1994).

Some agreement was found in *Protea* with the interaction observed in *Banksia* between stem diameter and propensity to flower . The length and thickness of shoots of *P.* cv. Ivy greatly affected the ability of the stem to produce a flower (De Swardt, 1989). A greater percentage of shoots formed flowers in *P.* cv. Carnival when pruned for biennial bearing, which significantly increased stem length and thickness (Gerber et al., 1995).

The nutrient status of the soil affected flowering time of *P.* cv. Pink Ice (*P. compacta* × *P. susannae*) (Barth et al., 1996). On a soil with high fertility the harvest extended over an 8 month period, compared to 2-3 months on a poor soil. The study did not include investigations to determine whether the extended flowering was due to slower development or later initiation, either of which may have occurred due to competition with increased vegetative growth. In *Leucospermum* an increase in leaf starch levels occurred at the same time as inductive conditions and a link was tentatively suggested (Napier, 1985). The question of a relationship between carbohydrate levels and flowering was addressed in *P.* cv. Carnival (Greenfield et al., 1995). Reserve carbohydrates were low in two-year-old wood throughout the season and no connection was found between carbohydrate or nitrogen, or their ratio, and flowering.

*Flower initiation and development in Leucadendron*

*Leucadendron* stems are marketed when the large showy leaves produced on the distal portion of the stem are brightly coloured, generally red or yellow. Large leaves are produced upon cessation of shoot growth, and the appearance of colour may be, but is not always associated with flowering. Flowers, comprised of many individual florets, form terminally within these showy leaves but remain inconspicuous. Plants are dioecious and the appearance of the flowering stem can vary greatly between male and female plants of the same species (De Kock et al., 1994).

Flower initiation in *L. discolor* takes place in autumn, followed by flowering in spring. At the end of October (Northern Hemisphere) the meristem was identified as reproductive on a microscopic level, and covered by protective scales. By the end of December the flower was macroscopically visible. There is a period after flower

initiation during which reversion can occur and a compressed leafy shoot is produced instead of a flower. This reversion was a result of stress when the flowering stem was taken as a cutting to produce flowering pot plants (Ben-Jaacov et al., 1986). Once all the florets had been initiated flower development continued normally.

The time of cessation of shoot growth of *L.* cv. Safari Sunset was linked to the ability to initiate a flower (Wallerstein and Nissim, 1992). Shoot elongation stopped in late summer and, as was found for *L. discolor,* initiation took place in autumn. A similar pattern is likely in *L. rubrum*, where inflorescence differentiation took place from mid-winter (De Kock et al., 1994).

*Flower initiation and development in Serruria*

Both terminal and lateral flowers are formed, to produce a conflorescence, with 1-11 capitula attached to the shoot by a peduncle. Each inflorescence contains approximately 25 individual florets. Floral bracts are large and papery and provide the pink-cream colour of the inflorescence.

In *S. florida* vegetative growth occurs in spring and summer. Flowers are initiated in autumn, after cessation of shoot growth. Artificial long days produced by supplementary lighting prevented flowering, indicating that short days are inductive for flowering (Malan and Brits, 1990). Plants generally respond to inductive conditions in March (Southern Hemisphere), but, when the onset of inductive conditions was delayed by artificial lighting until early May, normal flower initiation still occurred. Anthesis was reached approximately 2 months later. If supplementary lighting was continued until late May an abnormal inflorescence formed with green, leaf-like bracts.

The terminal inflorescence reaches anthesis first, followed by the axillary inflorescences which reach anthesis 2-3 weeks later (Malan and Brits, 1990). Dry mass increase in the terminal inflorescence is initially slow, increasing after floret initiation is complete and continuing until anthesis.

*Flower initiation and development in Telopea*

The inflorescence of *Telopea* consists of 50-200 individual florets surrounded by involucral bracts. Both bracts and florets are coloured. Flowers are borne terminally, but secondary axillary inflorescences have been observed (Faragher, 1989).

Flower initiation occurs in midsummer. The first microscopic evidence of flower initiation was seen in mid-December (Southern Hemisphere) (Dupee and Goodwin, 1990). Floret initiation takes place from February to January. No specific inductive conditions have been identified for *Telopea.* Flower initiation does not occur until vegetative growth has ceased The improved flowering in commercial plantings compared with natural stands indicates that increased plant growth and higher light intensity are likely to promote flowering (Faragher, 1989). In natural stands *Telopea* is an understorey plant and grows in conditions of low light and high competition. Flower initiation in *Telopea* occurs during high light intensity and long days, but whether flowering is promoted by increased assimilate supply or photoperiod has yet to be determined.

Flower development occurs more rapidly on older shoots. Anthesis occurs from August to October. Development of the inflorescence can be interrupted and the meristem reverts to the vegetative state. Reversion can occur at different times during inflorescence development and mixtures of floral and vegetative growth are produced.

*Conclusion.*

Little is known regarding the factors controlling flower initiation and development in *Proteaceae*. An understanding of inductive factors for flower initiation in *Leucospermum* has lead to the development of disbudding techniques which provide the grower with a powerful tool with which to extend the natural harvest, or delay the harvest peak and avoid periods of oversupply. In *Serruria* the short day requirement for flower initiation has yet to be exploited in order to extend production. Flowers are marketed in the natural flowering period from May to October (Dücker, 1999). Flower development has been studied in *Banksia* and recent investigations into inductive signals are interesting, but need to be refined. The tremendous variation in flowering time of different *Protea* species suggests that no single mechanism will explain flowering in all species, as in *Leucospermum*.

**Literature Cited**

Barth, G.E., N.A. Maier, J.S. Cecil, W.L. Chyvl, and M.N. Bartetzko. 1996. Yield and seasonal growth flushing of *Protea* "Pink Ice" and *Leucadendron* "Silvan Red" in South Australia. Aust. J. Exp. Agric. 36:869-875.

Ben-Jaacov, J., R. Shillo and M. Avishai. 1986. Technology for rapid production of flowering pot-plants of *Leucadendron discolor*. Scientia Hort. 28:379-387.

Coetzee J.H. and G.M. Littlejohn. 1999. *Protea*: a floricultural crop from the Cape Floristic Kingdom. Hort. Rev. In preparation.

Criley, R.A. 1998. *Leucospermum:* Botany and Horticulture. Hort. Rev. 22:27-90.

Criley, R.A., P.E. Parvin and S. Leketwana. 1990. Flower development in *Leucospermum cordifolium* cv. Vlam. Acta Hort. 264:71-78.

De Kock, M., K.I. Theron, P. Swart, E.W. Weiler and D.U. Bellstedt. 1994. Cytokinins in the xylem sap of the dioecious fynbos shrub, *Leucadendron rubrum* Burm. f.: seasonal fluctuations and their possible interaction with morphological characteristics expressed in the two sexes. New Phytol. 127:749-759.

De Swardt, D. C. 1989. Aspekte van die vegetatiewe en reproduktiewe ontwikkeling by geselekteerde *Proteaceae* cultivars. M.Sc. Thesis. University of Stellenbosch.

Dücker., G. 1999. Evaluation of export figures and prices in the 1997/98 season in South Africa. Internal report. Department of Horticultural Science, University of Stellenbosch.

Dupee, S.A. and P.B Goodwin. 1990. Effect of temperature, daylength and growth regulators on flowering in *Protea*, *Telopea* and *Leucospermum*. Acta Hort. 264:79-86.

Faragher, J.D. 1989. *Telopea. In*: A.H. Halevy (ed.) Handbook of Flowering Vol VI pp 593-597 CRC Press: Boca Raton, Fla.

Fuss, A.M. and M. Sedgley. 1990. Floral initiation and development in relation to the time of flowering in *Banksia coccinea* R.Br. and *B. Menziesii* R.Br. (Proteaceae). Aust. J. Bot. 38:487-500.

Fuss, A.M. and M. Sedgley. 1991. Variability in cut flower production of *Banksia coccinea* R. Br. and *Banksia menziesii* R. Br. at six locations in southern Australia. Aust. J. Exp. Agric. 31:853-858.

Fuss, A.M., S.J. Pattison, D. Aspinall and M. Sedgley. 1992. Shoot growth in relation to cut flower production of *Banksia coccinea* and *Banksia menziesii* (Proteaceae). Scientia Hort. 49:323-334.

Gerber, A. I., K.I. Theron, and G. Jacobs. 1995. Pruning of *Protea* cv. Carnival to optimise economic biomass production. Acta Hort. 387:99-106.

Greenfield, E. J., K.I. Theron, and G. Jacobs. 1994. Effect of pruning on growth and flowering response of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 4(1):42-46.

Greenfield, E. J., K.I. Theron, and G. Jacobs. 1995. Seasonal changes in carbohydrate and nitrogen levels in the bark and wood of pruned and unpruned plants of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 5(1):25-28.

Heinsohn, R-D. and N.W. Pammenter. 1988. Seasonality of shoot growth and flowering in the fynbos shrub *Protea neriifolia* cultivated in a summer rainfall area. S. Afr. J. Bot. 54(5):440-444.

Jacobs, G. 1983. Flower initiation and development in *Leucospermum* cv. Red Sunset. J. Amer. Soc. Hort. Sci. 108(1):32-35.

Jacobs, G. and G.E. Honeyborne. 1979. The relationship between heat unit accumulation and the flowering date of *Leucospermum* cv. Golden Star. Agroplantae 11:83-85.

Jacobs, G. and H.R. Minnaar. 1980. Light Intensity and flower development of *Leucospermum cordifolium.* HortScience. 15(5):644-645.

Jacobs, G., D.N. Napier and D.G. Malan. 1986. Prospects of delaying flowering time of *Leucospermum*. Acta Hort. 185:61-65.

Le Maitre, D.C. and J.J. Midgley. 1991. Allometric relationships between leaf and inflorescence mass in the genus *Protea (Proteaceae)*: an analysis of the exceptions to the rule. Funct. Ecol. 5:476-484.

Malan, D.G. and G. Jacobs. 1987. The influence of photoperiod on flower induction of *Leucospermum* cv. Red Sunset. Protea News 6:12-13.

Malan, D.G. and G. Jacobs. 1990. Effect of photoperiod and shoot decapitation on flowering of *Leucospermum* 'Red Sunset'. J. Amer. Soc. Hort. Sci. 115(1):131-135.

Malan, D.G. and G.J. Brits. 1990. Flower structure and the influence of daylength on flower inititation of *Serruria florida* knight (Proteaceae). Acta Hort. 264:87-92.

Napier, D. R. 1985. Initiation, growth and development of *Leucospermum* cv. Red Sunset inflorescences. M. Sc. Thesis. University of Stellenbosch.

Napier, D.R. and G. Jacobs. 1989. Growth regulators and shading reduce flowering of *Leucospermum* cv. Red Sunset. HortScience. 24(6):966-968.

Napier, D.R., G. Jacobs, J. Van Staden,. and C. Forsyth. 1986. Cytokinins and flower development in *Leucospermum*. J. Amer. Soc. Hort. Sci. 111(5):776-780.

Rieger, M.A. and M. Sedgley. 1996. Effect of daylength and temperature on flowering of the cut flower species *Banksia coccinea* and *Banksia hookeriana*. Aust. J. Exp. Agric. 36:747-753.

Röhl, L.J., A.M. Fuss, J.A. Dhaliwal, M.G. Webb and B.B. Lamont. 1994. Investigation of flowering in *Banksia baxteri* R. Br. and *B. hookeriana* Meissner for improving pruning practices. Aust. J. Exp. Agric. 34:1209-1216.

Sedgley, M. 1997. New *Banksia* cultivars for cut flower production. Acta Hort. 453:77-79.

Sedgley, M. 1998. *Banksia*: New proteaceous cut flower crop. Hort. Rev. 22:1-25.

Sedgley, M. and A. Fuss. 1992. Correct pruning lifts banksia yields. Aust. Hort. 90(7):50-53.

Wallerstein, I. 1989. Sequential photoperiodic requirement for flower initiation and development of *Leucospermum patersonii* (Proteaceae). Israel J. Bot. 38:25-34.

Wallerstein, I. and A. Nissim. 1992. Control of growth and flowering in *Banksia ashbyi*, *Leucospermum patersonii* and *Leucadendron* "Safari Sunset". Acta Hort. 316:73-80.

# 2. PAPER I - Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars.

## Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars.

Control of inflorescence initiation in *Proteaceae* is poorly understood. Short days induce flowering in *Leucospermum* cv. Red Sunset (Malan and Jacobs, 1990) and *Serrurria florida* (Malan and Brits, 1990). *Protea* species differ not only in the time of year when anthesis occurs, but certain species, such as *P. eximia*, can flower at any time of the year. Most species, however, have a flowering time restricted to a particular time of year.

The inflorescence initiation and development of three protea cultivars, viz. 'Carnival' (*P. compacta* × *P. neriifolia*), 'Sylvia' (*P. eximia* × *P. susannae*), and 'Lady Di' (*P. magnifica* × *P. compacta*), was studied. Shoot growth in these cultivars occurs in flushes, and they have different flowering times. Inflorescence formation of 'Carnival' is essentially limited to the spring shoot growth flush when this flush is subtended by one or more previous flushes (Greenfield et al., 1994). It rarely flowers on the summer flush which follows the spring flush. Over the course of a year 'Carnival' plants pruned in late winter put on three or four shoot growth flushes before flowering occurs on the spring flush of the following year (Greenfield et al., 1994).

Inflorescence formation of 'Lady Di' is comparable to 'Carnival' in that it is restricted to the spring growth flush subtended by one or more previous flushes (personal observations). 'Lady Di' does not usually form more than two shoot growth flushes per year and flowers are harvested from May to June. Flowering of 'Sylvia' can occur all year round on shoots composed of two or more shoot growth flushes (personal observations). In this study we report on the characteristics of the spring flush that subtends an inflorescence; the time of inflorescence initiation; the progression of

inflorescence differentiation; and the processes involved in the formation of successive shoot growth flushes.

## Materials and Methods

**PLANT MATERIAL.** Plants of 'Carnival', 'Sylvia', and 'Lady Di' used in this study were grown in commercial plantations in the Stellenbosch district (lat.33°15';long.19°07'), South Africa. In 1995 'Carnival' and 'Lady Di' plants were 5 years old, and 'Sylvia' plants were 6 years old. The plants were spaced 1 m in the row, and 4 m between rows, clean cultivated and were not fertilised or irrigated. The Stellenbosch climate is Mediterranean, with cool, wet winters and hot, dry summers. Annual rainfall is 600 - 700mm.

**SUCCESSIVE SHOOT GROWTH FLUSHES IN 'CARNIVAL'.** To study the manner in which shoot growth flushes developed 'Carnival' plants were pruned on 20 August 1996. Pruning consisted of heading back all shoots to leave the basal 15-20 cm (bearer), as described previously (Gerber et al., 1995), on which new shoot growth originated from axillary buds. At the first sign of budbreak in spring similar sized bearers were tagged. When the first flush was complete individual shoots of similar size were tagged, to further reduce variation, and the production of subsequent summer and autumn growth flushes studied.

At regular intervals from bud sprouting in spring (18 September 1996), five buds or developing shoots were collected, shoot length measured, and appendages counted using the stereomicroscope. This was continued until all three shoot growth flushes were completed (7 August 1997). At the completion of each growth flush the number of appendages which constituted the flush became known. This number was subtracted

from the total number of appendages present in the system, to identify when appendages belonged to the following flush.

**APPENDAGE FORMATION DURING THE SPRING GROWTH FLUSH.** During winter, and before commencement of the spring growth flush in 1996, shoots were tagged. Shoots of 'Carnival' and 'Sylvia' were composed of three shoot growth flushes and 'Lady Di', two shoot growth flushes. 'Sylvia' shoots which flowered on the spring flush were selected. At regular intervals five samples of the apical bud or the developing spring growth flush were collected for dissection under the stereomicroscope. The number of appendages constituting the apical bud and the developing spring growth flush were counted. This continued until completion of elongation of the spring growth flush. After this it became impossible to count the appendages on the developing inflorescence, due to their small size. It was not possible to distinguish the transition of appendages destined to become leaves or involucral bracts morphologically. Since the average number of appendages constituting the spring growth flush was known after completion of the spring growth flush, this number could be subtracted from the number of appendages present in the system to yield the progression in the formation of involucral primordia. The length of the spring growth flush was also measured.

**INFLORESCENCE AND FLOWERING SHOOT CHARACTERISTICS.** Five flowering shoots of 'Carnival', composed of the following shoot growth flushes: spring 1995, summer 1995, autumn 1996 and spring 1996, which subtended an inflorescence, were cut from plants on 24 February 1997. Similarly five flowering shoots of 'Sylvia' and 'Lady Di' were collected on 20 January 1997 and 9 June 1997, respectively. 'Sylvia' shoots were composed of the inflorescence subtended by the spring shoot growth flush of 1996 and three earlier shoot growth flushes of the previous year. Shoots of 'Lady Di' were

composed of the spring shoot growth flush of 1996, subtending an inflorescence and the autumn 1996 plus the spring 1995 shoot growth flushes. The oldest flush of flowering shoots originated from an axillary bud on a bearer.

Shoots were cut at the point of inception and brought to our laboratories. The length of each flush unit of a shoot was measured separately and the number of budscales, transition leaves and true leaves were counted. The basal inflorescence diameter was measured and the involucral bracts and florets that constituted the inflorescence were counted.

**INFLORESCENCE DEVELOPMENT.** Ten shoots per cultivar were tagged in the field when the inflorescence bud was approximately 10 mm in basal diameter, whereafter the diameter was measured at two-weekly intervals.

During inflorescence development samples of apical buds of 'Carnival' were placed in FAA (formaldehyde: acetic acid: 50 % ethanol, in the ratio 1:1:18 by volume). These apical buds were used to follow the morphological changes taking place in the bud during inflorescence development by scanning electron microscopy (SEM). After removal from the FAA the buds were ethanol-dehydrated, critical-point-dried with $CO_2$ and sputter-coated with gold at 1kV for 5 min in an Edwards Auto 306 ioncoater. The buds were viewed on a Joel JSM 6100 SEM at an accelerating voltage of 5kV. Micrographs were taken with a Joel HR 80018 camera using Agfa 120mm, 100 ASA film.

## Results

**SUCCESSIVE SHOOT GROWTH FLUSHES IN 'CARNIVAL'.** Pruning 'Carnival' shoots released rudimentary buds in axillary positions on the bearer from correlative inhibitions, allowing meristematic activity in the buds to resume. The first visual signs of bud expansion were noticed 29 days after pruning, on 18 September 1996. Bud expansion

continued for a further 21 days before flush extension started in early October. During this 50 day period from pruning the meristem produced the total number of 28 appendages which constitute the first (spring) growth flush (Fig. 1). Only once all the appendages had been produced did the spring flush start elongating.

During elongation of the first spring flush the meristem continued to produce the elements necessary for the subsequent, summer flush (Fig. 1). Almost the full complement of appendages necessary for the summer flush was contained in the apical bud when expansion of the spring flush was complete in early December. A lag of about 2 weeks separated the end of spring flush growth and the start of enlargement of the terminal bud to produce the summer flush.

This developmental pattern was repeated for the autumn growth flush. While the summer flush was elongating the meristem formed the appendages which would comprise the autumn flush (Fig. 1). When elongation of the summer flush was complete the majority of the autumn flush appendages had been formed, and there was a lag of one week before growth of the autumn flush began. Autumn flush elongation was accompanied by the formation of appendages for the next spring flush. When elongation of the autumn flush was complete (at the end of April) the apical bud did not yet contain all the appendages for the second spring flush. Meristematic activity continued through winter to produce the full complement of appendages for the spring flush. Before spring budbreak, in early September, the entire spring flush was preformed and enclosed in the apical bud. The preformed leaf primordia, for all flushes, were minute in comparison with preformed leaves of the apple (Verheij, 1996). During bud sprouting the preformed leaves differentiated and developed while covered by scale-like bracts. Development continued and enlargement occurred during elongation of the internodes.

Neither appendage formation, nor flush elongation occurred at a constant rate (Fig. 1). Extension growth of the flushes of 'Carnival', especially the spring growth flush, were sigmoidal. Slow rates of growth were apparent at the beginning and end of elongation, with a phase of rapid growth in between.

**APPENDAGE FORMATION ON FLOWERING SPRING GROWTH FLUSHES.** At the time when terminal budbreak occurred in spring, terminal buds contained 67 ± 7.8, 78 ± 14.0 and 66 ± 3.8 appendages for 'Carnival', 'Sylvia' and 'Lady Di', respectively (data not presented). These numbers equalled or exceeded the average final number of appendages that constituted the spring growth flush for the three cultivars (Table 1). The appendages for the spring growth flush were thus preformed before elongation began. Subtracting this number from the number of appendages present in the system yielded the progression in the formation of involucral bract primordia. It is evident for all three cultivars that the involucral bract primordia are formed during extension growth of the spring shoot growth flush (Fig. 2). The number of appendages present at the time of completion of the spring flush was comparable to the number of involucral bracts present on the mature inflorescence (Table 2).

In relation to length of the spring flush the rate of appendage formation in 'Carnival' and 'Sylvia' was described by a quadratic function (Fig. 2). During the early stages of extension growth the rate of appendage formation was high, but decreased toward the end of elongation of the spring flush. The rate of appendage formation relative to spring flush elongation of 'Lady Di', however, was constant throughout extension growth indicating a linear relationship.

**INFLORESCENCE AND FLOWERING SHOOT CHARACTERISTICS.** The characteristics of flowering shoots of 'Carnival', 'Sylvia' and 'Lady Di' are presented in Tables 1 and 2.

The characteristics of the spring shoot growth flush, which subtended the inflorescence, differed from flushes lower down on the stem in the following ways. For all three cultivars the number of leaves and scales, and, therefore, the total number of appendages was greater (Table 1). The length of the spring flush of 'Carnival' and 'Lady Di', but not 'Sylvia', was longer than preceding flushes. In all three cultivars, starting with the most basal flush, the flushes preceding the spring flush of 1996 progressively contained more appendages. In the case of 'Carnival' and 'Lady Di' this was due to an increase in the number of scales, and for 'Sylvia' an increase in both scales and leaves.

'Lady Di' is a larger inflorescence, as indicated by the basal diameter of 60 mm compared with 40 and 44 mm for 'Carnival' and 'Sylvia', respectively, and this greater diameter is reflected by the larger number of involucral bracts (Table 2). The number of florets contained in 'Lady Di' inflorescences was similar to that of 'Carnival', and 'Sylvia' inflorescences contained fewer florets.

**INFLORESCENCE DEVELOPMENT.** The rate of inflorescence development, as approximated for by the increase in diameter, was similar for the three cultivars (Fig. 3). 'Sylvia' and 'Carnival', with similar sized inflorescences, completed development within the same time period. The larger size of 'Lady Di' inflorescences necessitated that development continued for longer and anthesis was delayed by ten weeks.

The stages of apical meristem development during floral initiation in 'Carnival' are shown in Fig. 4. In the vegetative state the apical meristem is dome shaped, becoming flat and broad for the production of involucral bracts. This is similar to meristem changes seen in *Leucospermum* during production of peduncular bracts, as is the return to the domed, or conical, state during the initiation of floral bracts (Malan et al., 1994). Floral

bracts and individual florets initiate and develop acropetally, as is seen in inflorescence development in *Banksia* (Fuss and Sedgley, 1990)

**Discussion**

Shoot growth in proteas occurs by elongation of successive growth flushes. With the exception of the flush originating from a rudimentary axillary bud, a flush develops from a preformed shoot in the terminal bud. The leaf primordia in the terminal bud are differentiated during elongation of the previous flush and the full complement is present at budbreak. Shoot growth is due to extension of preformed internodes and development of preformed leaf primordia. This is the same pattern of development that describes rhythmic growth in the oak (Crabbé, 1987)

Most of the appendages for the shoot growth flush developing directly from a rudimentary axillary bud are initiated only once correlative inhibition is removed by pruning. Leaf primordia differentiate after budbreak, but before elongation of the flush. A degree of plasticity in the formation of leaf primordia is, thus, apparent, where primordia for a specific flush can form during budbreak and during elongation of the previous flush, but the full complement is always present before shoot elongation occurs. Although sequential growth of flushes was not studied for 'Sylvia' and 'Lady Di', the preformed nature of the spring flush growth intimates that the same pattern applies.

The inflorescence bearing spring growth flush differed from other flushes in the number of appendages formed (Table 1). An increase in the number of budscales and transitional leaves with successive growth flushes and a large increase in the number of leaves on the flush subtending the inflorescence were common features for all three cultivars. However, the spring flush subtending the inflorescence was much longer than the other flushes for 'Carnival' and 'Lady Di', but not for 'Sylvia'.

It is unclear if the increase in appendage number is a prerequisite for flowering. The correlation between an decrease in plastochron and the transition from the vegetative to floral state has received much attention (Fulford, 1966; Verheij, 1996). The plastochron was not measured in these investigations and little can be concluded regarding the interrelationship between meristem activity and its fate, although it is apparent from SEM photographs that the phyllotaxy does change during the progression from production of leaf primordia through the different stages of inflorescence initiation.

With respect to inflorescence initiation on the spring growth flush it is clear that, for the three cultivars studied, the initial stages of inflorescence initiation and differentiation coincided with elongation of the spring growth flush. Inflorescence development had advanced to the stage where all the involucral bracts had initiated by the time elongation of the spring growth flush was complete, and initiation of floral bracts had begun. Floret initiation and differentiation occurred after completion of the spring growth flush. Three phases of inflorescence development can, therefore, be distinguished in 'Carnival', 'Sylvia' and 'Lady Di': a phase of involucral bract formation, which occurs during extension growth of the shoot flush which subtends the inflorescence; a phase of floret initiation, which occurs after completion of the subtending shoot growth flush; followed by an inflorescence enlargement phase.

The characteristics of 'Carnival' and 'Sylvia' inflorescences, when subtended by the spring flush, were similar. The rate of development was similar and they were harvested within the same time period. The start of spring budbreak of 'Lady Di', signalling commencement of inflorescence initiation, was delayed by approximately 8 weeks. This, together with the longer period needed for development, lead to a greatly delayed harvest time in May and June compared with 'Carnival' and 'Sylvia', which were harvested in

February and March, despite also being initiated on the spring flush. The reason for the delay of spring budbreak in 'Lady Di' is unclear, but may be due to a photoperiodic requirement which is only fulfilled by the long days occurring after the September equinox. Vegetative growth in *Protea* cv. Ivy was inhibited under short day conditions in winter and was stimulated by daylength continuation with the supply of artificial light (unpublished results).

In conclusion, three sinks are active during the development of a shoot growth flush: growth of preformed leaves, extension growth of preformed internodes, and formation of new appendages in the apical meristem. The appendages give rise either to new leaves for the next flush of shoot growth, or involucral bracts of the inflorescence. All three cultivars studied have similar strategies of inflorescence initiation and development when borne on the spring flush. Inductive factors which restrict inflorescence initiation to the spring flush in 'Carnival' and 'Lady Di' and allow flowering throughout the year in 'Sylvia' are the subject of future work.

## Literature Cited

Crabbé J. 1987. Aspects particuliers de la morphogenése caulininaires des végétaux ligneux et introduction à leur étude quantitative. Centre d'Etude de la Reproduction Végétale, Brussels.

Fulford, R.M. 1996. The morphogenesis of apple buds. III. The inception of flowers. Ann. Bot. 30:207-219.

Fuss, A.M. and M. Sedgley. 1990. Floral initiation and development in relation to the time of flowering in *Banksia coccinea* R.Br. and *B. menziesii* R.Br. (Proteaceae). Aust. J. Bot. 38:487-500.

Gerber, A. I., E.J. Greenfield, K. I. Theron, and G. Jacobs. 1995. Pruning of *Protea* cv. Carnival to optimise economic biomass production. Acta Hort. 387: 99-106.

Greenfield, E.J., K. I. Theron, and G. Jacobs. 1994. Effect of pruning on growth and flowering response of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 4(1): 42-46.

Malan, D.G. and G. Jacobs. 1990. Effect of photoperiod and shoot decapitation on flowering of *Leucospermum* 'Red Sunset'. J. Amer . Soc. Hort. Sci. 115(1):131-135.

Malan, D.G. and G.J. Brits. 1990. Flower structure and the influence of daylength on flower initiation of *Serruria florida* knight (Proteaceae). Acta Hort. 264:87-92.

Malan, D.G., J.G.M. Cutting, and G. Jacobs. 1994. Correlative inhibition of inflorescence development in *Leucospermum* 'Red Sunset'. J. S. Afr. Soc. Hort. Sci. 4(1):26-31.

Verheij, F.A. 1996. Morphological and physiological aspects of the early phases of flower bud formation in apple. Dissertation Agric. Univ. Wageningen 147pp.

Table 1. Characteristics of the flowering shoot at anthesis when the inflorescence is subtended by a spring flush (mean of ten samples ± 1 SE).

| *Protea* cv. Carnival | | | | |
|---|---|---|---|---|
| | Spring 1995 | Summer 1996 | Autumn 1996 | Spring 1996 |
| Length (mm) | 273 ± 25.8 | 239 ± 30.0 | 261 ± 33.1 | 395 ± 29.9 |
| Budscales | * | 10 ± 0.9 | 16 ± 1.9 | 19 ± 1.9 |
| Transitional leaves | 4 ± 1.3 | 6 ± 0.5 | 6 ± 1.0 | 10 ± 1.4 |
| Leaves | 25 ± 2.6 | 25 ± 2.3 | 26 ± 2.4 | 36 ± 3.2 |
| Total appendages | 29 ± 2.6 | 41 ± 2.9 | 49 ± 2.6 | 65 ± 4.9 |

| *Protea* cv. Sylvia | | | | |
|---|---|---|---|---|
| | Flush 1[z] | Flush 2[z] | Flush 3[z] | Spring 1996 |
| Length (mm) | 181 ± 50.5 | 187 ± 34.6 | 164 ± 30.7 | 164 ± 32.8 |
| Budscales | * | 11 ± 1.5 | 15 ± 2.4 | 28 ± 5.5 |
| Transitional leaves | 4 ± 1.2 | 3 ± 0.8 | 3 ± 0.8 | 6 ± 0.9 |
| Leaves | 12 ± 2.7 | 15 ± 2.0 | 17 ± 2.3 | 25 ± 5.3 |
| Total appendages | 16 ± 2.7 | 29 ± 2.9 | 35 ± 3.5 | 59 ± 8.0 |

[z]Flushing is less synchronous in 'Sylvia' and specific seasonal flushes are difficult to identify.

| *Protea* cv. Lady Di | | | |
|---|---|---|---|
| | Spring 1995 | Autumn 1996 | Spring 1996 |
| Length (mm) | 216 ± 27.8 | 162 ± 15.0 | 420 ± 82.5 |
| Budscales | * | 12 ± 1.0 | 20 ± 3.3 |
| Transitional leaves | 4 ± 0.9 | 5 ± 0.7 | 7 ± 2.3 |
| Leaves | 21 ± 2.1 | 22 ± 1.9 | 47 ± 8.0 |
| Total appendages | 26 ± 1.3 | 39 ± 2.3 | 74 ± 9.4 |

* budscales of the first flush become concealed in the junction between bearer and shoot

Table 2. Characteristics of the inflorescence at anthesis when subtended by a spring flush (mean of ten samples ± 1 SE).

| | |
|---|---|
| *Protea* cv. Carnival | |
| Basal diameter (mm) | 40.5 ± 5.54 |
| Bracts + involucral bracts | 99 ± 4.9 |
| Florets | 232 ± 10.4 |
| *Protea* cv. Sylvia | |
| Basal diameter (mm) | 43.8 ± 2.65 |
| Bracts + involucral bracts | 98 ± 1.1 |
| Florets | 205 ± 15.3 |
| *Protea* cv. Lady Di | |
| Basal diameter (mm) | 60.5 ± 3.30 |
| Bracts + involucral bracts | 127 ± 5.5 |
| Florets | 240 ± 18.4 |

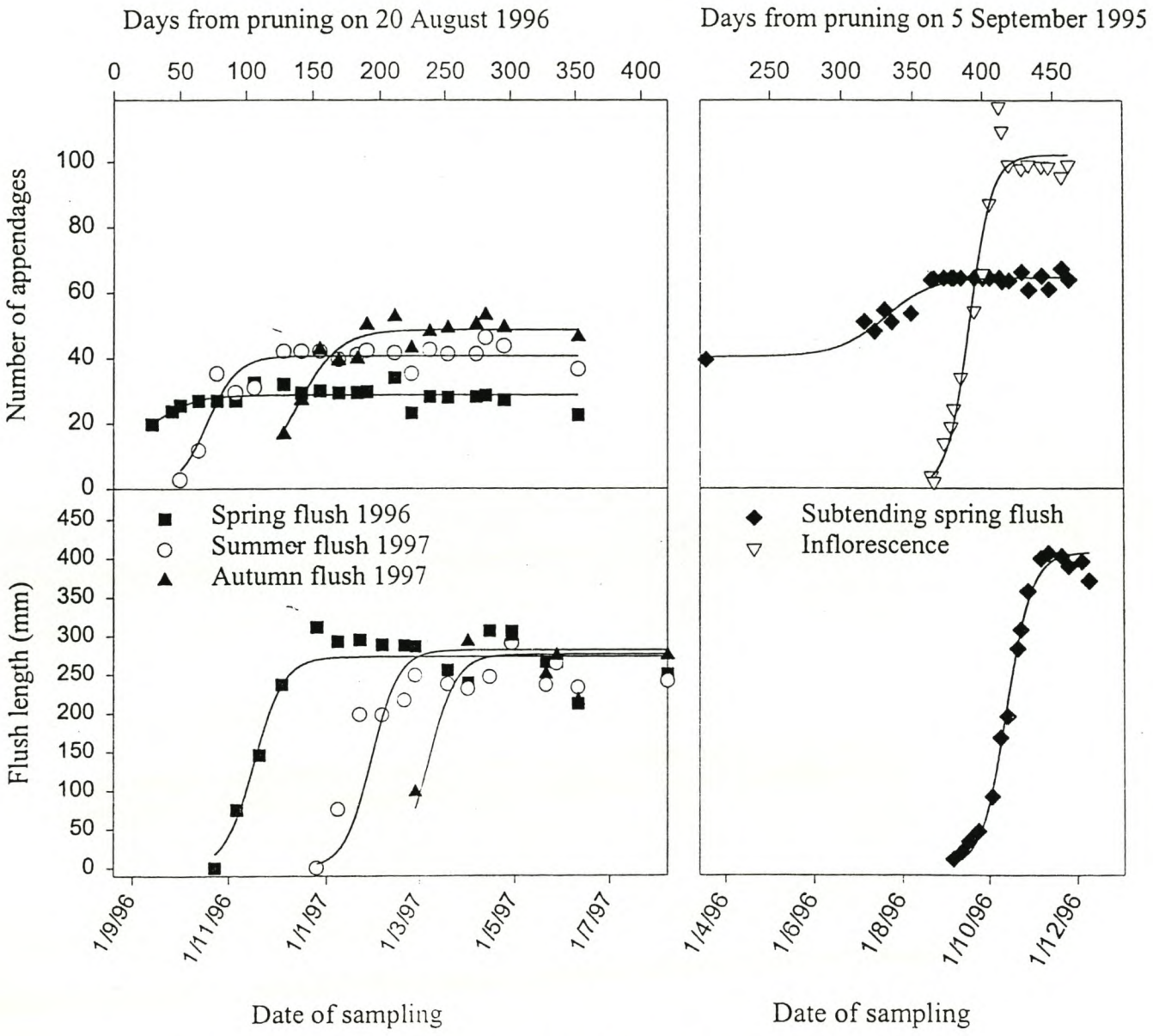



Fig. 1. Appendage formation and length of growth flushes of *Protea* cv. Carnival over time.
(The vegetative and reproductive cycles are presented in phenological, not chronological, order)

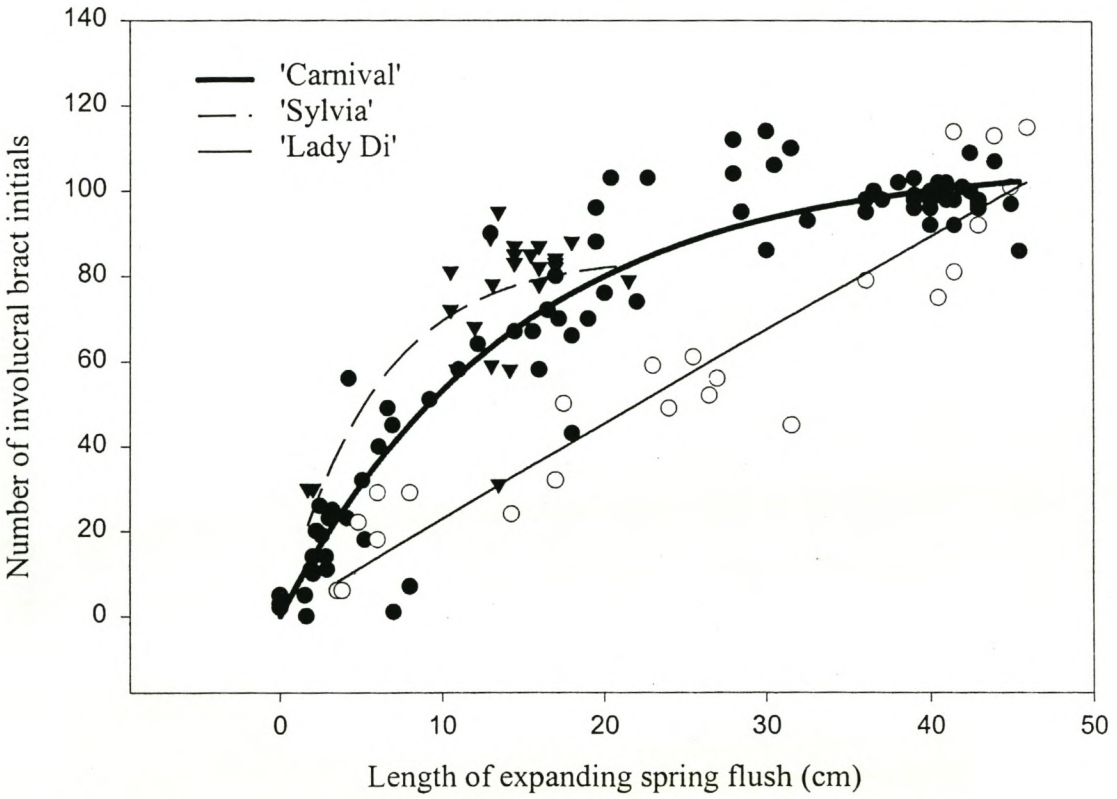



Figure 2. Appendage formation during elongation of the spring growth flush which subtends the inflorescence in three *Protea* cultivars, 'Carnival' (filled circles), 'Sylvia' (filled triangles), and 'Lady Di' (open circles)

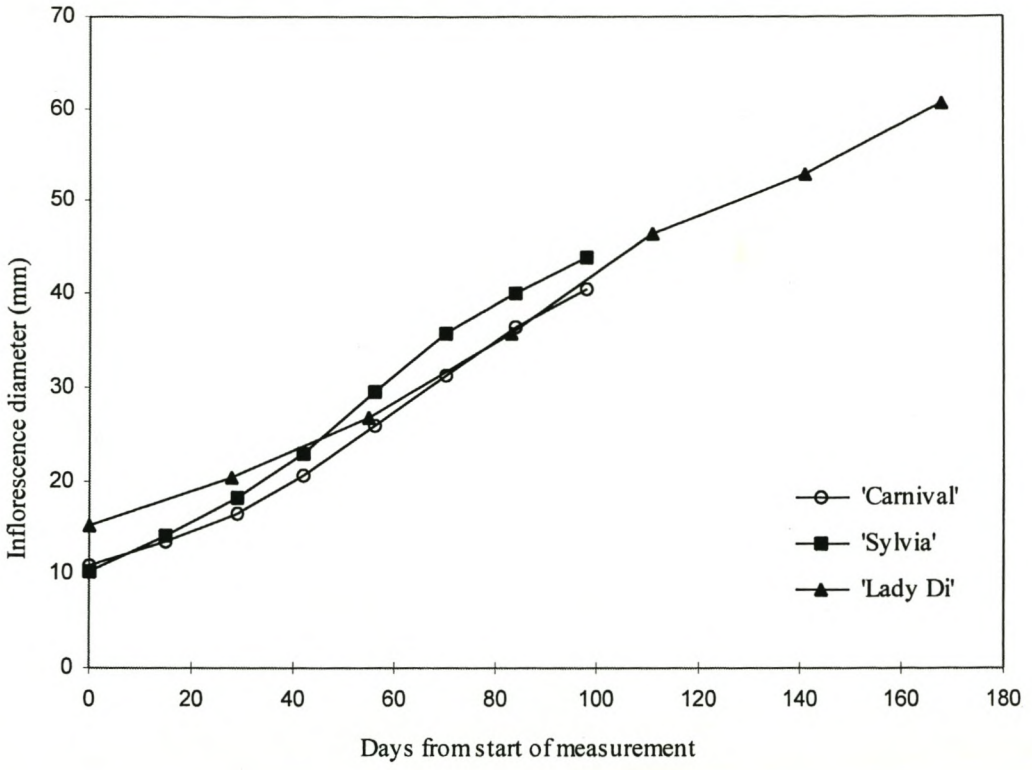
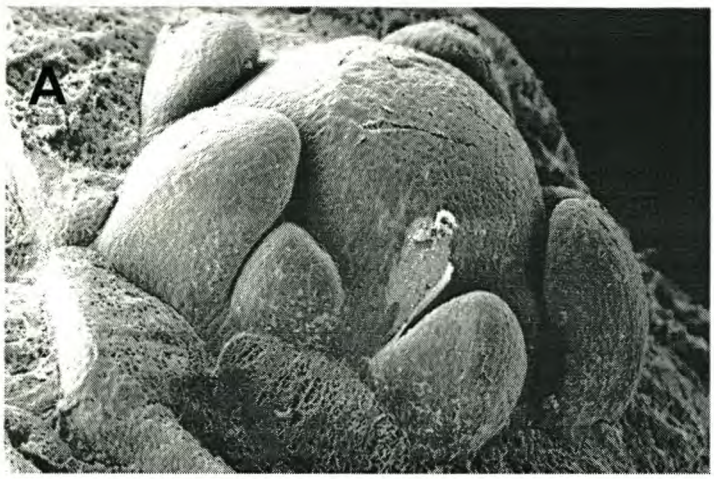
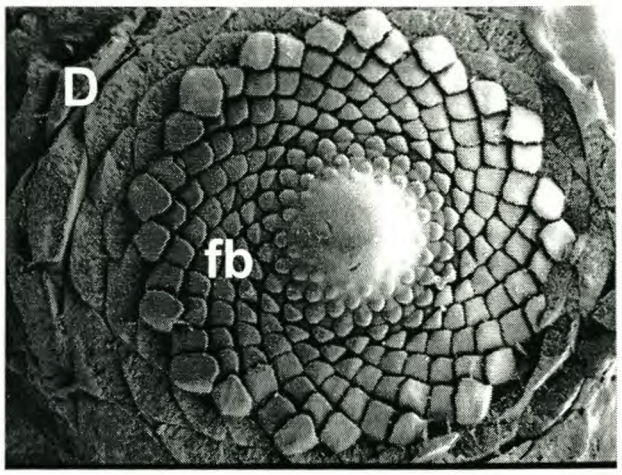
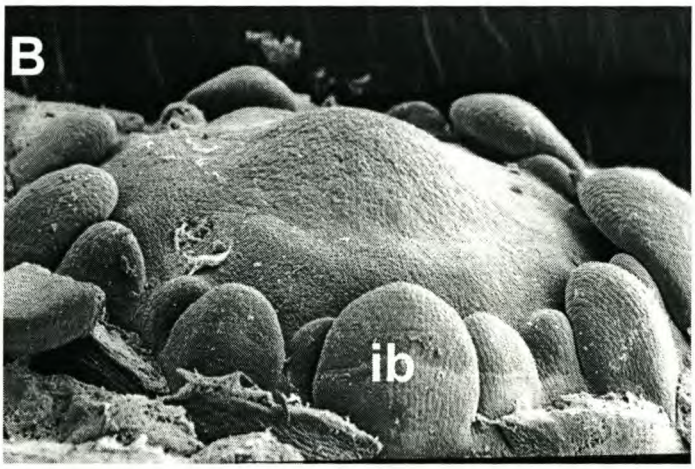
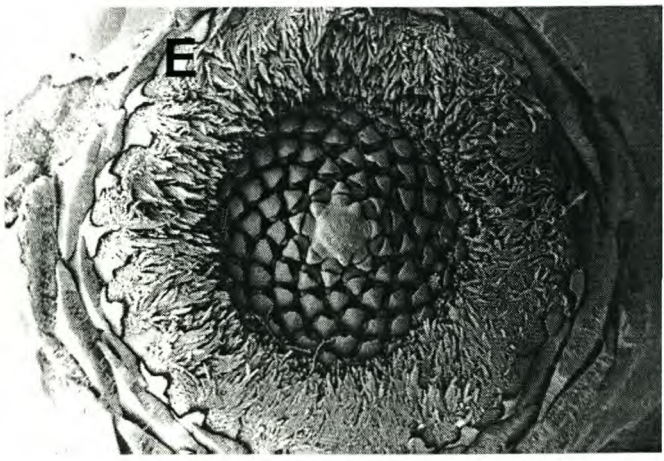


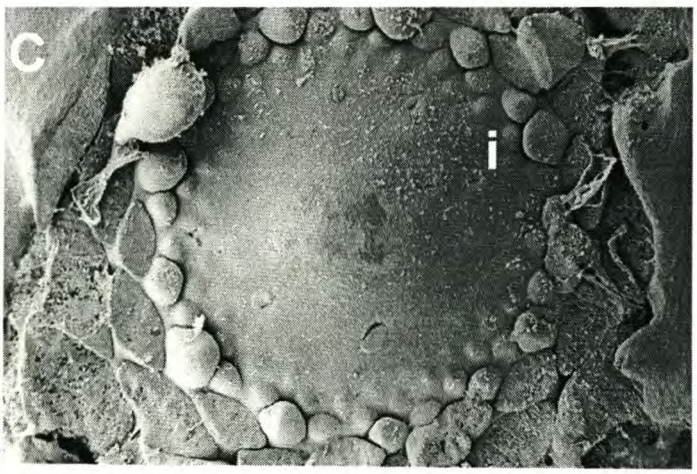

Fig. 3. Basal diameter of inflorescence during development (mean of 10 shoots). In 1997 spring budbreak occurred on 18 August, and measurement of 'Carnival' and 'Sylvia' started on 22 October 1997. In 1996 spring budbreak occurred on 16 October, and measurement of 'Lady Di' started on 5 February 1997. SE values (n = 10) for inflorescence diameter of 'Carnival = 1.28, of 'Sylvia' = 1.12, and of 'Lady Di' = 2.06.



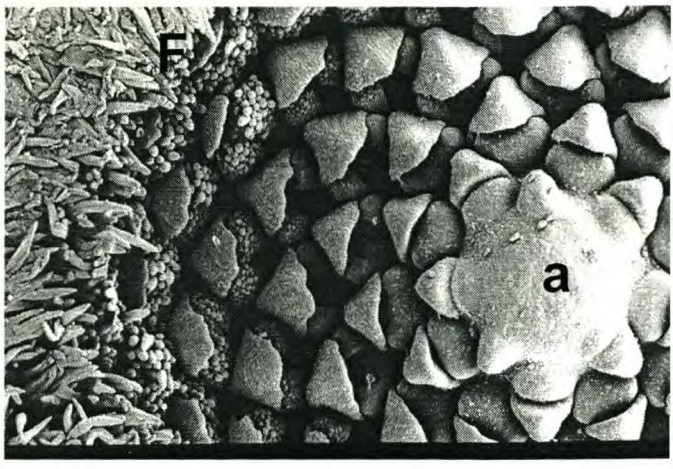




Fig. 4. Changes occurring during floral meristem development in *Protea* cv. Carnival. (A) The vegetativ meristem prior to flowering is domed and producing leaf primordia (x130). (B) The apical meristem is fla and expanded and producing involucral bracts (ib) ( x100). (C) The rate of meristem activity increases wit rapid production of floral bract initials (i) (x50). (D) Later stage of floral bract (fb) initiation with th involucral bracts removed (x27). (E) At the completion of floral bract initiation the meristem returns to th domed shape. Development of individual florets begins in the outer floral bracts first. (x20). (F) Detail o floret initiation showing gradual stages of development towards the apex (a). (x65)

# 3. PAPER II - Defoliation inhibits flowering and alters spring flush growth and inflorescence characteristics in *Protea* cv. Carnival.

## Defoliation inhibits flowering and alters spring flush growth and inflorescence characteristics in *Protea* cv. Carnival.

*Protea* cv. Carnival (*P. compacta* × *P. neriifolia*) initiates flowers terminally on the spring growth flush when subtended by one or more previous flushes (Greenfield et al., 1994). Inflorescence development occurs from mid-October and flowers are harvested from February to May. It rarely flowers on the first summer flush and never on the second summer or autumn flushes (Greenfield et al., 1994). On vigorous shoots apical dominance is lost following inflorescence initiation and lateral shoots develop below the inflorescence on the spring growth flush. Inflorescences may also develop on the first flush of these lateral shoots, but not on subsequent flushes. In commercial culture these inflorescences are worthless and side-shoots are removed when they develop below the inflorescence. The reason for restriction of flowering predominantly to the spring flush when it is subtended by one or more flushes in unknown. Daylength has been shown to control flower initiation in *Leucospermum* (Malan and Jacobs, 1990), *Serruria* (Malan and Brits, 1990), and may play a role in flower initiation of *Banksia*, although there is interaction with temperature (Rieger and Sedgley, 1996). Shoot quality has also been shown to be a determining factor in the shoots' ability to flower in *Banksia* (Sedgley, 1998) and *Protea* (de Swardt, 1989).

The spring flush is preformed (Paper I). Production of leaf primordia starts during elongation of the preceding autumn flush and continues during winter, but is complete before spring budbreak. Involucral bracts of the inflorescence differentiate during elongation of the spring growth flush. Shoots are, therefore, induced to produce flowers by the time of spring budbreak.

Defoliation and shading at critical times inhibit flower initiation in many plants. Shading of *Leucospermum* during summer, prior to the inductive phase, prevented flower initiation (Jacobs, 1983). In *Vaccinium ashei* (rabbiteye blueberry) (Lyrene, 1992) and *Solidago altisima* (goldenrod) (Meyer, 1998), defoliation causes a reduction in flower formation. The presence of mature leaves in mango was shown to be essential for flower induction which, therefore, was suggested to involve a labile floral stimulus (Nunez-Elisea and Davenport, 1992).

Defoliation of shoots of 'Carnival' at different times during winter and after spring budbreak was done to pinpoint the time when shoots became irreversibly induced to flower, and to quantify the effect of defoliation on the characteristics of the spring flush and the inflorescence subtended by the spring flush.

## Materials and Methods

**PLANT MATERIAL.** Experiments were done on *Protea* cv. Carnival plants grown in a commercial plantation in the Stellenbosch district (lat.33°15'S; long.19°07'E), South Africa. The climate is Mediterranean with an annual rainfall of 600 – 700 mm, falling mainly in winter. Summers are hot and dry. Plants were spaced 1 m in the row and 4 m between rows, clean cultivated, and were not irrigated or fertilised. Plants were managed and pruned for biennial bearing (Gerber et al., 1995)

**DEFOLIATION TREATMENTS.** In mid winter (early June) of 1996 shoots were tagged that were of similar size and consisted of three growth flushes, with the terminal flush being the autumn flush. Tagging shoots at the start of the experiment ensured that shoots used throughout the experiment had the same initial characteristics. Four-year-old plants were used and spring budbreak started on 4 September 1996. Defoliation treatments were

applied to individual shoots and were done at 2 weekly intervals (with one exception) from 29 July 1996, with the first date corresponding to 35 days before spring budbreak and the last date (4 November 1996) corresponding to 63 days after spring budbreak. The four degrees of severity of defoliation were: total defoliation, where all the leaves on the shoot were removed; severe partial defoliation, where all the leaves were removed except those on the uppermost, autumn, flush; mild partial defoliation, where only the leaves on the uppermost, autumn, flush were removed; and no defoliation, or control, where all the leaves remained on the shoot. Leaves were cut off with scissors at their point of inception.

With the onset of spring, vegetative growth continued on both defoliated and control shoots to produce a spring growth flush. On 10 February 1997, when flowers on control shoots were at the commercially harvestable stage, all shoots were picked and brought to our laboratory for analysis.

The experiment was repeated the following year on plants of the same age under the same cultivation conditions. Spring budbreak occurred on 20 August 1997. Defoliation was done on 3 June, 11 July, 29 July, 12 August, and 9 September 1997 (corresponding to 78, 40, 22 and 8 days before spring budbreak, and the last date corresponding to 20 days after spring budbreak.). All shoots were picked and brought to our laboratory for analysis on 11 February 1998.

**DATA RECORDED ON SHOOT AND INFLORESCENCE CHARACTERISTICS.** The characteristics of the current spring flush and the terminal inflorescence were recorded. The length of the spring flush was measured and the number of leaves was counted. The number of inflorescence bracts (including bracts and involucral bracts) and the number of florets in the inflorescence was counted. The diameter of the inflorescence was measured at the base using digital callipers. The internode length was calculated by dividing the final

length of the spring flush by the number of leaves on the spring flush. Inflorescence characteristics in 1997 were reported only for the dates when all shoots initiated flowers, i.e. 29 July, 12 August and 9 September 1997.

**STATISTICAL ANALYSIS.** The experiment was set out as a randomised design with 5 single shoot replicates in 1996 and 4 single shoot replicates in 1997. Analysis of variance was performed and orthogonal contrasts were fitted using the SAS programme (SAS Institute Inc., 1990).

## Results

**FLOWER INITIATION.** In 1996 when treatments started 35 days before spring budbreak (DBSB) none of the severities of defoliation, or the times at which they were applied had an effect on inflorescence initiation (data not presented). Total defoliation 40 DBSB or earlier completely prevented flower initiation in 1997 (Table 1). Partial defoliation performed at 78 DBSB prevented flower initiation in some shoots, but had no effect when delayed until 40 DBSB. Any defoliation treatment done later than 40 DBSB had no effect on inflorescence initiation.

**SHOOT CHARACTERISTICS.** The average length of the spring growth flush on non-defoliated shoots was 41.3cm (± 0.47) in 1996 and 36.4cm (± 0.64) in 1997, with 44 (± 0.5) and 45 (± 0.9) leaves respectively (data not presented).

Total defoliation in 1996 showed a quadratic trend with time in its effect on the number of leaves produced (Fig. 1a). The reduction was the greatest when shoots were defoliated early, and approximately 20 days after spring budbreak (DASB) total defoliation no longer affected the number of leaves produced on the spring flush. In 1997 a similar response to defoliation was seen (Fig. 1b), although, with total defoliation, the

trend over time was linear. The linear trend in 1997, as opposed to quadratic, was due to there being no further effect of defoliation at approximately 20 DASB (as in 1996) which was the last date used. The earliest time at which total defoliation was done in 1997 (78 DBSB) reduced the number of leaves to almost half of the control value. The number of leaves produced on the spring flush was not affected by partial defoliation in 1996 (Fig. 1a).

Total defoliation done before spring budbreak greatly reduced the final length of the spring growth flush (Fig. 2). The spring flush elongated to less than half the normal length when shoots were totally defoliated earlier than 40 DBSB in 1997. In both years the effect of total defoliation on the length of the spring flush decreased linearly with time of defoliation, until shortly after spring budbreak when defoliation had no further effect. Partial defoliation also decreased the final length of the spring growth flush in both years, and the effect was the same whether the defoliation was mild or severe (Fig. 2). When shoots were partially defoliated early in 1997 (78 DBSB) the length was reduced the most, and the effect decreased linearly with later defoliation.

The spacing of leaves, represented by the average internode length, was affected by defoliation in different manners in the two years (Fig. 3). In 1996 there was no interaction between time and severity of defoliation. The internode length was reduced on shoots which were subjected to total defoliation or severe partial defoliation. Shoots which were subjected to mild partial defoliation had the same internode length as the control. In 1997 the average internode length on shoots which were partially defoliated was not significantly different from the control. When shoots were totally defoliated early, the average internode length was reduced, indicating that leaves were more closely spaced on

the spring flush. With later defoliation the effect decreased linearly, until just before budbreak when defoliation no longer had an effect.

**INFLORESCENCE CHARACTERISTICS.** Flowers on control shoots were at the commercially harvestable stage on 10 February 1996 and 11 February 1997. At this stage in 1996 the inflorescence had a basal diameter of 38.27cm (± 0.42) and was composed of 99 (±0,5) inflorescence bracts and 232 (±2) florets. In 1997 the basal diameter of the inflorescence was 46.02cm (± 1.92) and had 100 (± 1) inflorescence bracts and 208 (± 4) florets (data not presented).

The number of inflorescence bracts (combining bracts and involucral bracts) was unchanged, regardless of the time or severity of defoliation applied (Fig. 4). The significance reported for time of treatment in 1996 was probably due to one or two outlying points. There was very little variation in the number of inflorescence bracts per flower in both years.

Both time and severity of defoliation affected the number of florets formed (Fig. 5). Shoots which had severe partial defoliation applied (where only the autumn flush remained) produced inflorescences with the same number of florets as the control. Mild partial defoliation (where only the autumn flush was removed) resulted in fewer florets being formed. In 1996 the effect decreased linearly with later defoliation, but there was no significant time effect in 1997. The significant interaction with time seen in 1996 was due to continuation of defoliation treatments long after spring budbreak. In 1997 treatments were ceased while there was still a marked effect on the number of florets produced - an effect which would have decreased with later defoliation. This is also apparent following total defoliation. In 1996 total defoliation, when done early, significantly decreased the number of florets (Fig. 5a). The effect diminished quadratically with time, compared with

the control, until, at approximately 40 DASB the number of florets was no longer affected by total defoliation. In 1997 treatments were discontinued long before the quadratic trend began, and only the significant treatment effect as a result of early total defoliation was noticed (Fig. 5b).

The basal diameter of the inflorescence on shoots which were subjected to either mild partial or total defoliation was smaller than non-defoliated shoots (Fig. 6). In 1996 the effect decreased linearly with time, but in 1997 treatments did not continue long enough to detect the trend. Severe partial defoliation did not affect the basal inflorescence diameter.

## Discussion

Defoliation only inhibited flower initiation when performed earlier than 40 DBSB (Table 1). Defoliation in 1996 was applied too late to inhibit flower initiation. The presence of mature leaves are, therefore, essential for flower initiation and these leaves must be retained on the shoot until 6-7 weeks before spring budbreak for the shoot to become induced for flower initiation to take place after spring budbreak. Leaf removal in many plants inhibits flowering, indicating that the ability to initiate flowers is probably related, at least partly, to supply of current photosynthates. In 'Carnival' this is possibly the explanation for the failure of a spring flush arising from an axillary position after pruning to form a flower. It is important, however, to note that it is only at this time of year that the shoot acquires the inductive state to initiate flowers. 'Carnival' rarely initiates flowers on flushes formed at other times of the year, despite an apparently adequate availability of carbohydrates (Greenfield et al., 1995).

An explanation other than carbohydrate availability should, therefore, be considered to explain why flower initiation is limited to the spring growth flush when subtended by one or more previous flushes.

The flowering spring flush had significantly more leaves than the non-flowering spring flush or other flushes (Paper I). Early complete defoliation produced shoots which had 23 leaves and no flowers, compared with later defoliation where flowering shoots had 34 or more leaves. Since defoliation decreased the number of leaves on the spring flush the possibility exists that defoliation inhibits flower initiation in an indirect way as a result of the reduced leaf number. In many plants the relationship between meristem activity and floral differentiation has been investigated. In grape a close correlation was found between the number of appendages in a bud and the likelihood of reproductive development (Buttrose, 1970) while in rose a minimum number of leaf primordia must be present before the transition to the reproductive state will occur (Marcelis-van Acker, 1994). The formation of a critical leaf number has previously been considered as an essential event prior to flower initiation in apple, but recent evidence has tended to contradict this (Verheij, 1996). It appears that in 'Carnival' a specific leaf number is not critical to flower initiation, rather the progression in leaf number of successive flushes allows a quantitative response to inductive conditions. The tendency to initiate a flower increases linearly with an increase in the number of preformed leaves, as was seen in the grape (Buttrose, 1970).

Alternatively, the inability of other flushes to flower, despite adequate supply of current photosynthates, may not be related to flush characteristics, such as the number of leaves, but may be due to the lack of other inductive conditions which only occur during winter, prior to spring flush growth. It appears that, for flower initiation in 'Carnival', mature leaves must have overwintered to allow induction for flowering to occur. When 'Carnival' plants were pruned in late autumn, but early enough for a shoot growth flush to occur before winter, flowers were initiated on the spring flush formed following winter

(Greenfield et al., 1994), but fewer shoots formed flowers compared with biennial production where shoots were long (Gerber et al., 1995). A lack of pre-winter growth when plants were pruned later, at the start of winter, resulted in non-flowering of the spring flush and subsequent summer and autumn flushes. The induced state, on shoots with mature overwintered leaves, is maintained after the first flowers are initiated, but is lost eventually. This is evident by the few flowers which are initiated on the first summer flush (Greenfield et al., 1994) and on lateral shoots arising below the inflorescence, and the inability for flowers to form on the second summer flush or the autumn.

The requirement for mature overwintering leaves to effect flower initiation suggests that environmental factors, such as low temperature and short daylength, may play an inductive role in flower initiation of 'Carnival'. In *Leucospermum,* short days are inductive for flowering (Malan and Jacobs, 1990). *Leucospermum* cv. Red Sunset requires a minimum of 42 days with daylength shorter than 12 hours for successful flower initiation to occur. Flower initiation in *Banksia* occurs in spring and the interaction of temperature and daylength has been investigated (Rieger and Sedgley, 1996). Their results showed flowering in *B. coccinea* to be influenced by daylength and in *B. hookeriana* to be influenced by temperature. Daylength was extended by supplementary light in the photosynthetically active radiation (PAR) frequency, and the positive effect of continued light on flowering could have been due to increased assimilate accumulation and not necessarily a photoperiodic response. In mango mature leaves are essential for flower initiation to occur, implying the presence of a labile floral stimulus (Nunez-Elisea & Davenport, 1992). However low night temperature is suggested to regulate the metabolism of the putative floral stimulus and is therefore also involved in the flowering process. Moss (1969) suggested that, in citrus, the plant was receptive to inductive

conditions in May and June in Australia, with flower initiation taking place in July. Environmental conditions prevailing in May and June are low temperatures and short daylength, but Moss's research indicated that low temperature had the greatest influence on flower initiation. Whatever the inductive conditions are, in all cases the induced state is gradually lost.

In conclusion, flower initiation in 'Carnival' is limited to a short period in spring, and the plants flower in February to May. For flower initiation to occur the presence of mature leaves on an overwintering shoot is required and the leaves must remain on the shoot until about 40 days before spring budbreak to effect flower initiation. The low temperatures and short days of winter may be environmental inductive conditions for flowering, but the role of intra-plant factors which control the number of leaves cannot be ruled out. The induced state is gradually lost, since flowers can form on the first summer flush, but never on the second summer or autumn flushes.

Involucral bracts differentiate concurrently with the elongation of the spring growth flush (Paper I). The number of involucral bracts that differentiated was not affected by the defoliation treatments, but elongation of the spring growth flush was greatly reduced by early, total defoliation. Differentiation of involucral bracts can, therefore, compete strongly for limited photosynthates whereas shoot elongation is compromised. Elongation of the spring growth flush is dependant on photosynthates supplied by the leaves on the overwintering shoot. This is evident when the supply of photosynthates was restored by partial defoliation and the length of the spring flush was comparable to a non-defoliated shoot. Furthermore, only when complete defoliation was delayed until about 20 days after spring budbreak was the length of the spring growth flush not affected by total defoliation.

This approximately corresponds with complete expansion of the first leaves on the spring flush, suggesting a degree of autonomy.

Differentiation of florets occurs after involucral bract differentiation and complete elongation of the spring flush. When defoliation was delayed to a date when spring flush characteristics were no longer affected the number of florets on defoliated shoots was comparable to non-defoliated shoots. Therefore, we conclude that floret initiation is essentially supported by new photosynthates from the spring flush. The same was found for flower development, where the increase in flower diameter was retarded by defoliation treatments affecting the spring flush.

Leaves on the overwintering shoot, therefore are essential for the formation of leaf primordia, shoot elongation, and flower initiation. Leaves located close to the shoot apex of the overwintering shoot are more efficient in supporting these events than leaves lower down on the flush. Differentiation of the involucral bracts is independent of new photosynthates, while floret differentiation and inflorescence growth are supported by the spring growth flush. Although early defoliation can inhibit flower initiation, once the shoot is committed to flowering inflorescence development can be delayed by defoliation and the shoot will flower later. Defoliation has potential merit as a technique for delaying flowering and extending the harvest period.

## Literature Cited

Buttrose, M.S. 1970. Fruitfulness in grapevines: Development of leaf primordia in buds in relation to bud fruitfulness. Bot. Gaz. 131:78-83.

De Swardt, D.C. 1989. Aspekte van die vegetatiewe en reproduktiewe ontwikkeling by geselekteerde *Proteaceae* cultivars. M.Sc. Thesis. University of Stellenbosch.

Gerber, A.I., K.I. Theron, and G. Jacobs. 1995. Pruning of *Protea* cv. Carnival to optimise economic biomass production. Acta Hort. 387:99-106.

Greenfield, E.J., K.I. Theron, and G. Jacobs. 1994. Effect of pruning on growth and flowering response of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 4(1): 42-46.

Greenfield, E.J., K.I. Theron, and G. Jacobs. 1995. Seasonal changes in carbohydrate and nitrogen levels in the bark and wood of pruned and unpruned plants of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 5(1):25-28.

Jacobs, G. 1983. Flower initiation and development in *Leucospermum* cv. Red Sunset. J. Amer. Soc. Hort. Sci. 108(1):32-35.

Lyrene, P.M. 1992. Early defoliation reduces flower bud counts on rabbiteye bluberry. HortScience. 27(7):783-785.

Malan, D.G. and G. Jacobs. 1990. Effect of photoperiod and shoot decapitation on flowering of *Leucospermum* 'Red Sunset'. J. Amer. Soc. Hort. Sci. 115(1):131-135.

Malan, D.G. and G.J. Brits. 1990. Flower structure and the influence of daylength on flower inititation of *Serruria florida* Knight (Proteaceae). Acta Hort. 264:87-92.

Marcelis-van Acker, C.A.M. 1994. Axillary bud development in the rose. Dissertation Agric. Univ. Wageningen, 131pp.

Meyer, G.A. 1998. Mechanisms promoting recovery from defoliation in goldenrod (*Solidago altissima*). Can. J. Bot. 76:450-459.

Moss, G.I. 1969. Influence of temperature and photoperiod on flower induction and inflorescence development in sweet orange (*Citrus sinensis* (L.) Osbeck). J. Hort. Sci. 44:311-320.

Nunez-Elisea, R., and T.L. Davenport. 1992. Requirement for mature leaves during floral induction and floral transition in developing shoots of mango. Acta Hort. 296:33-37.

Rieger, M.A. and M. Sedgley. 1996. Effect of daylength and temperature on flowering of the cut flower species *Banksia coccinea* and *Banksia hookeriana.* Aust. J. Exp. Agric. 36:747-753.

SAS Institute Inc. 1990. SAS/STAT User's guide, Version 6. 4th ed., Vol 1 and 2. Cary, NC.

Sedgley, M. 1998. *Banksia*: a new Proteaceous cut flower crop. Hort. Rev. 22:1-25.

Verheij, F.A. 1996. Morphological and physiological aspects of the early phases of flower bud formation in apple. Dissertation Agric. Univ. Wageningen 147pp.

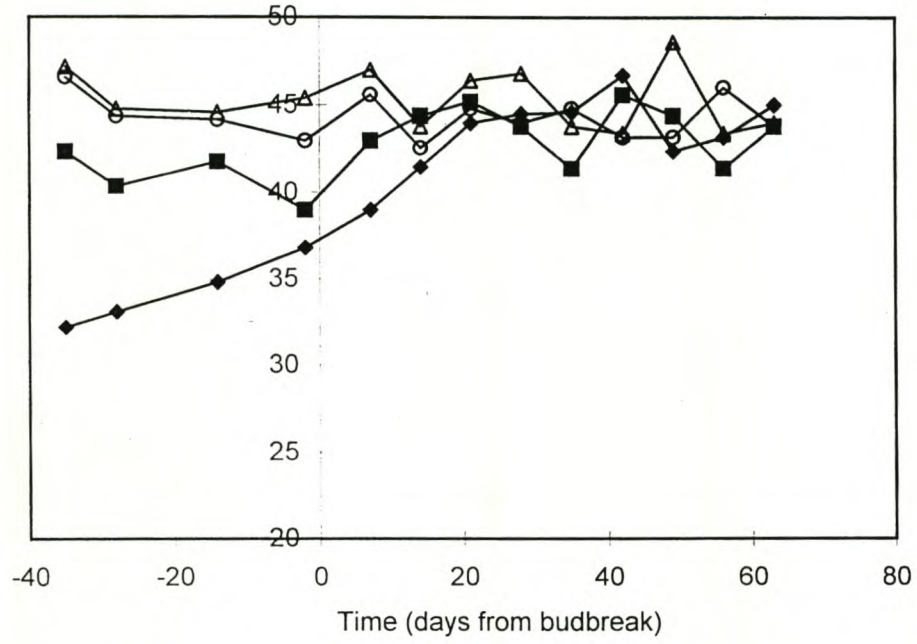
Table 1. Number out of five shoots which formed inflorescences after different severities of defoliation applied at different dates in 1997. Severities of defoliation applied were: total defoliation, severe partial defoliation, mild partial defoliation, and no defoliation, or control.

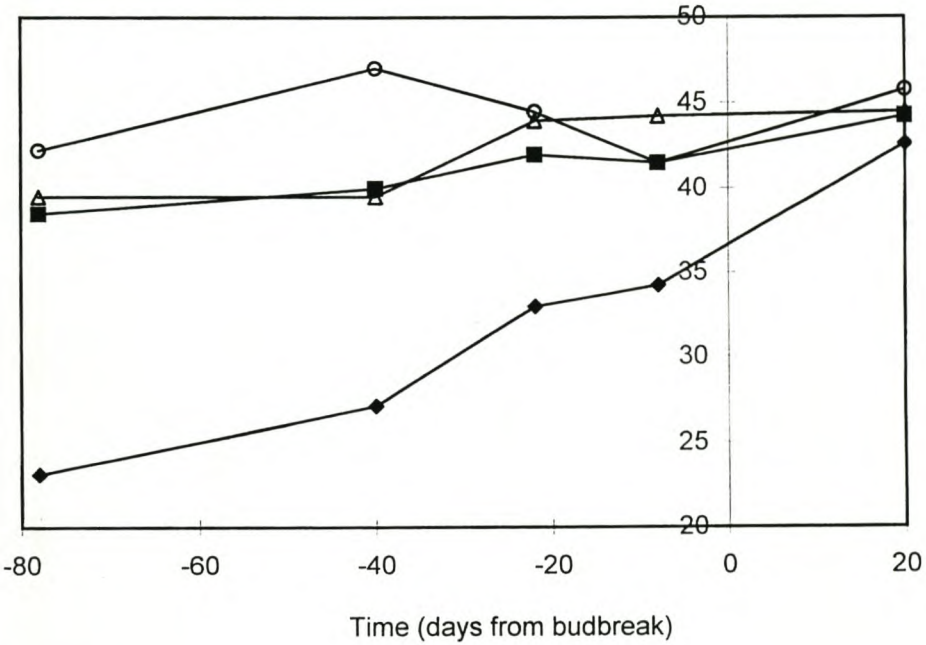
| | Date of defoliation (Days from spring budbreak) | | | | |
|---|---|---|---|---|---|
| Severity of defoliation | 3 June (-78) | 11 July (-40) | 29 July (-22) | 12 August (-8) | 9 September (20) |
| Total | 0/5 | 0/5 | 5/5 | 5/5 | 5/5 |
| Severe | 4/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Mild | 3/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Control | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation Δ




ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.0085 |
| Mild vs Control | 0.1984 | 0.1818 |
| Severe vs Control | 0.7141 | 0.2663 |

ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0022 |

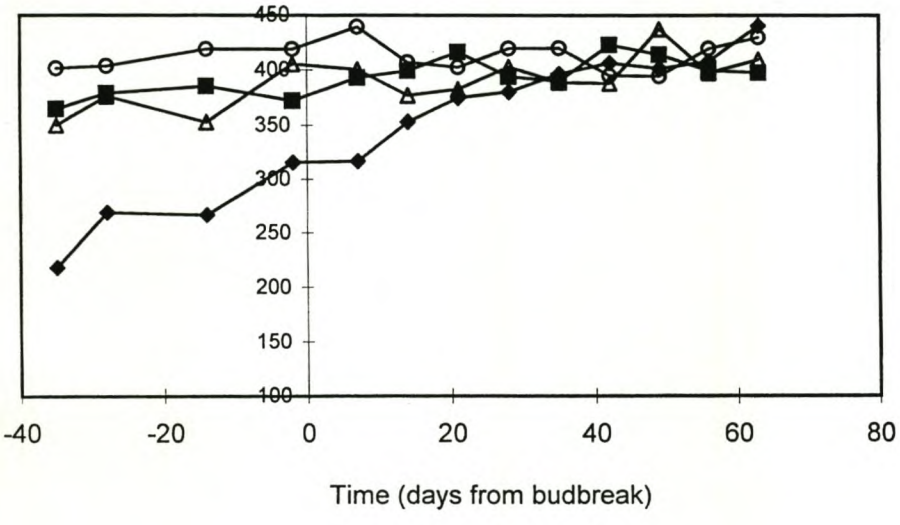
| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.5098 |
| Mild vs Control | 0.0724 | 0.9010 |
| Severe vs Control | 0.0579 | 0.7130 |

Fig. 1. Effect of severity and time of defoliation on number of leaves on the spring flush, compared with non-defoliated shoots. Time of application of defoliation treatments reported as days from spring budbreak.

Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △

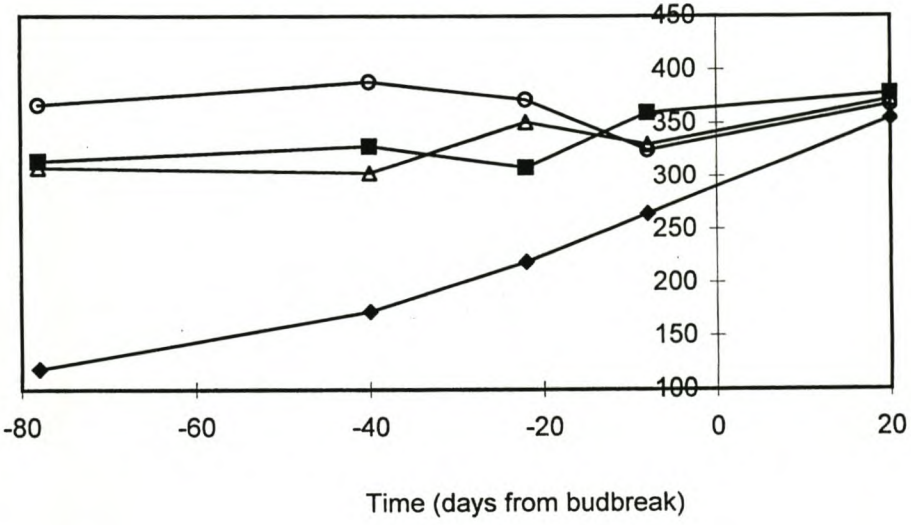



ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.4446 |
| Mild vs Control | 0.1255 | 0.6907 |
| Severe vs Control | 0.0304 | 0.9237 |

b) Shoots defoliated in 1997, picked in 1998




ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

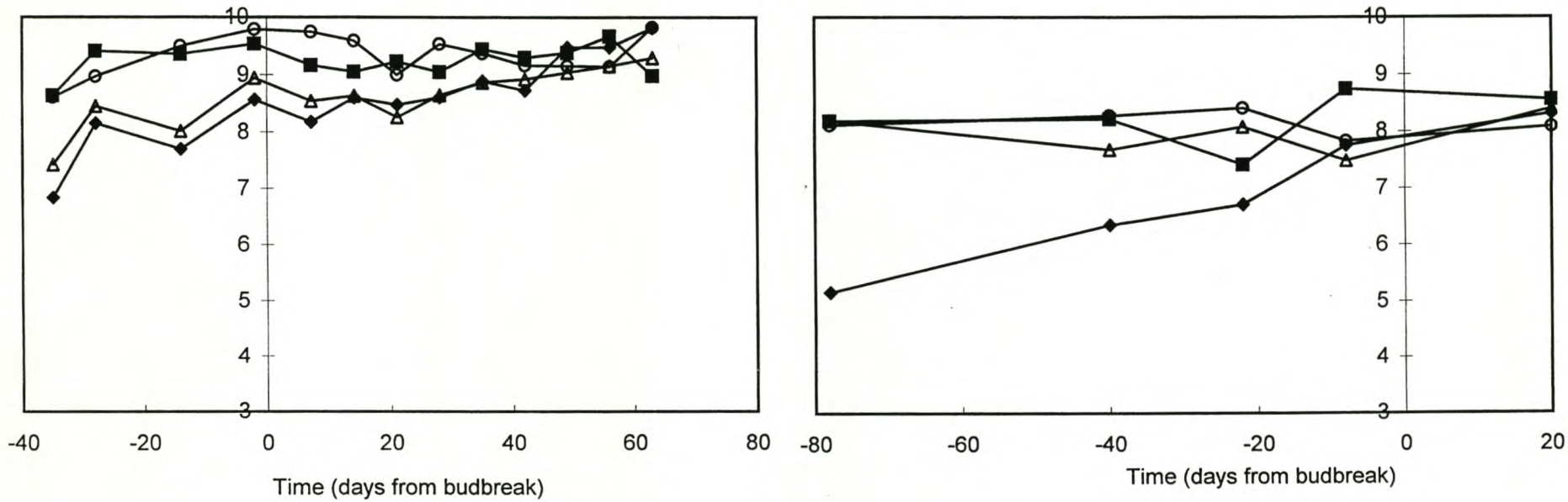
| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.0871 |
| Mild vs Control | 0.0043 | 0.2764 |
| Severe vs Control | 0.0038 | 0.4415 |

Fig. 2. Effect of severity and time of defoliation on length of the spring flush (mm), compared with non-defoliated shoots. Time of application of defoliation treatments reported as days from spring budbreak.
Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △




ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.1786 |
| Trt | 0.0001 |
| Time | 0.0007 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | |
| Mild vs Control | 0.1636 | |
| Severe vs Control | 0.0001 | |

ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0050 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.2064 |
| Mild vs Control | 0.1792 | 0.0718 |
| Severe vs Control | 0.5113 | 0.5670 |

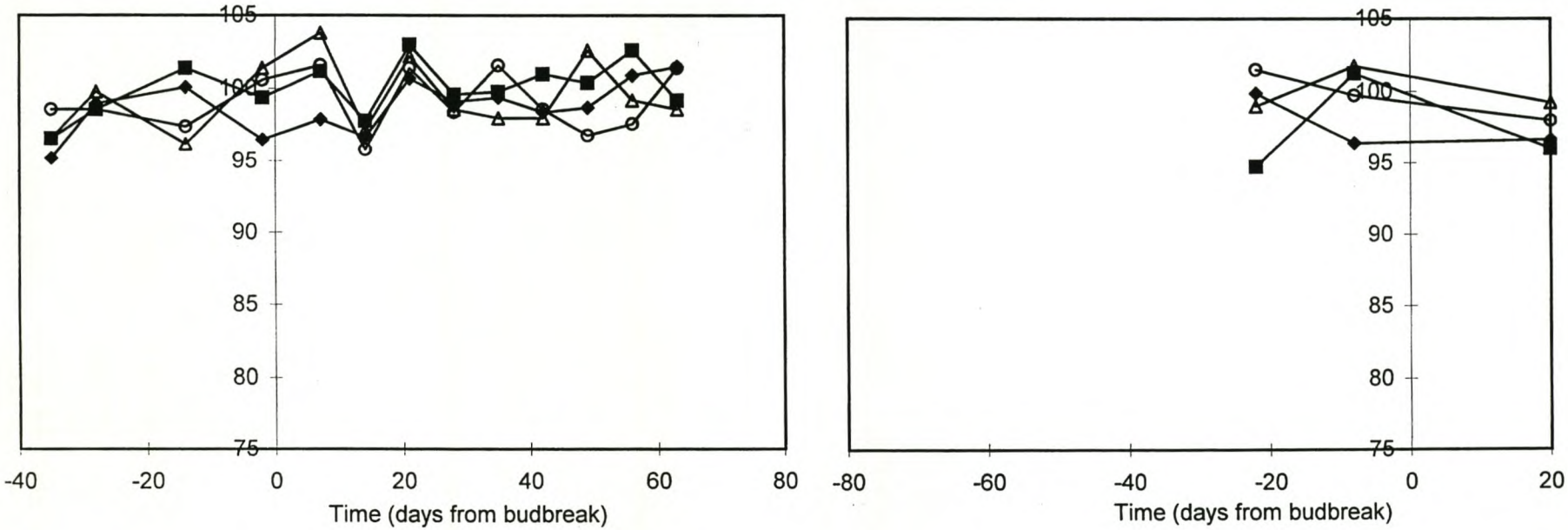
Fig. 3. Effect of severity and time of defoliation on average internode length (mm), compared with non-defoliated shoots. Time of application of defoliation treatments reported as days from spring budbreak.

Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △




| ANOVA | |
|---|---|
| Source | Significance |
| Trt*Time | 0.3897 |
| Trt | 0.2373 |
| Time | 0.0014 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.6054 | |
| Mild vs Control | 0.1439 | |
| Severe vs Control | 0.6479 | |

| ANOVA | |
|---|---|
| Source | Significance |
| Trt*Time | 0.1230 |
| Trt | 0.1269 |
| Time | 0.4224 |

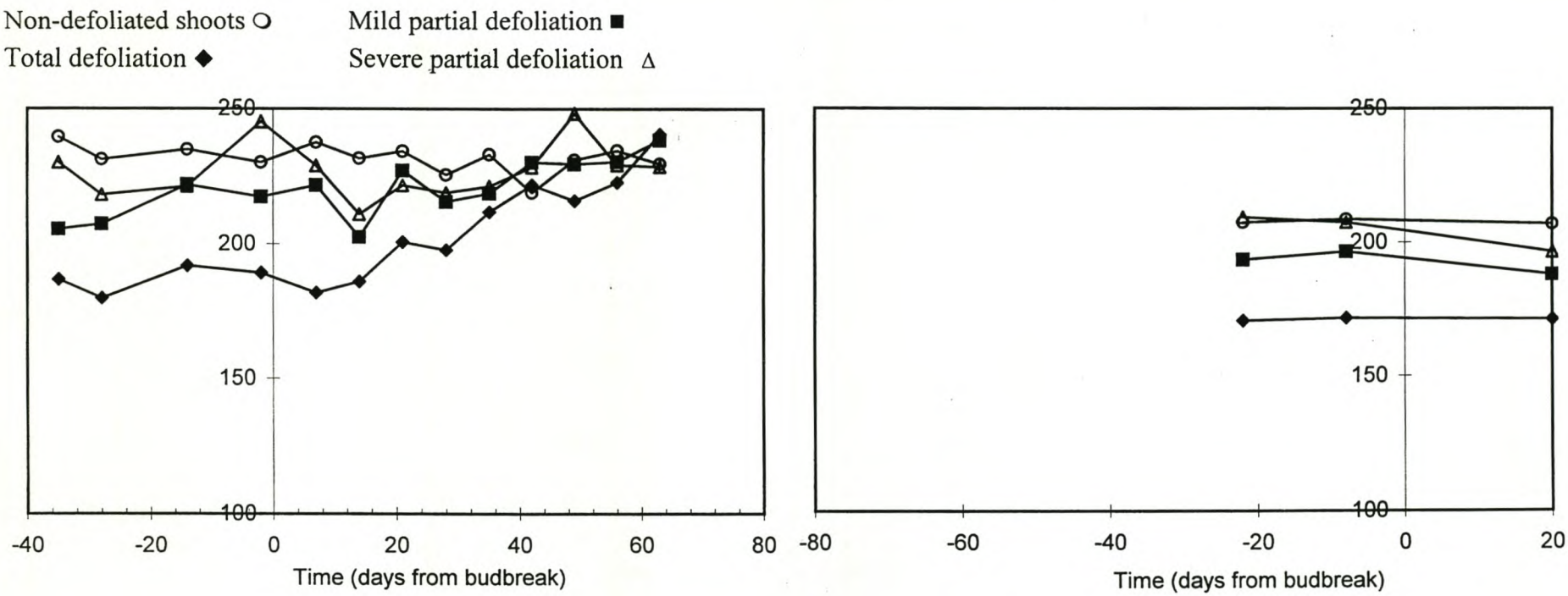
| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.1315 | |
| Mild vs Control | 0.0882 | |
| Severe vs Control | 0.8562 | |

Fig. 4. Effect of severity and time of defoliation on number of inflorescence bracts, compared with non-defoliated shoots. Time of application of defoliation treatments reported as days from spring budbreak.

Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998




| ANOVA | |
|---|---|
| Source | Significance |
| Trt*Time | 0.0001 |
| Trt | |
| Time | |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.0184 |
| Mild vs Control | 0.0001 | 0.7332 |
| Severe vs Control | 0.1009 | 0.6707 |

| ANOVA | |
|---|---|
| Source | Significance |
| Trt*Time | 0.9627 |
| Trt | 0.0001 |
| Time | 0.6037 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | |
| Mild vs Control | 0.0145 | |
| Severe vs Control | 0.5780 | |

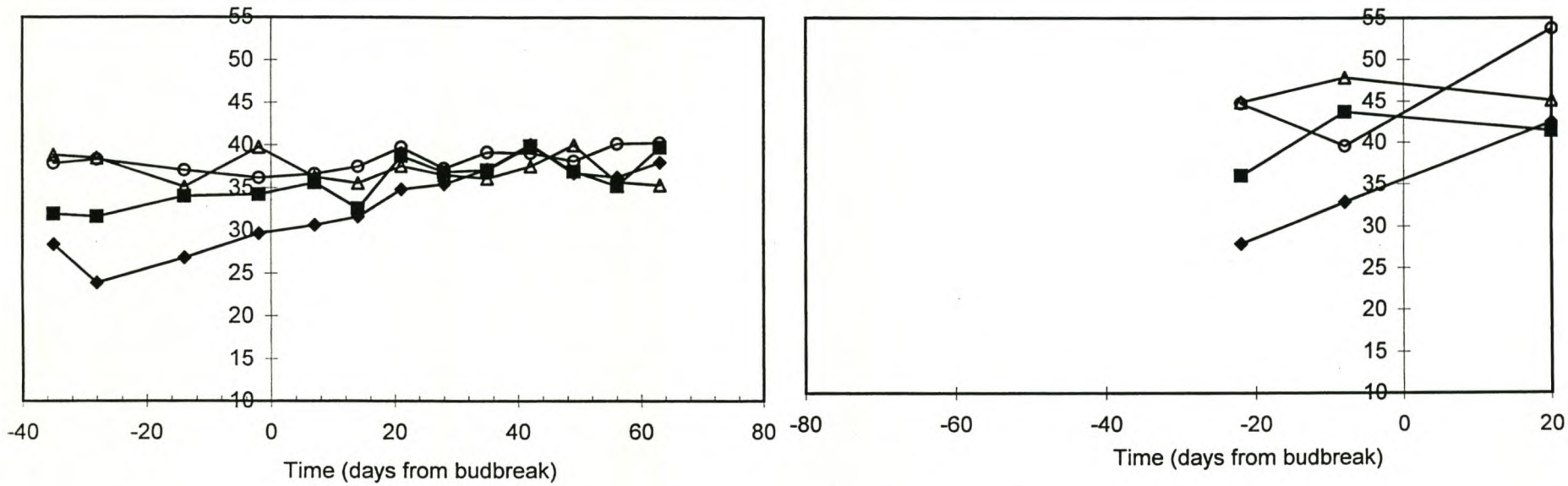
Fig. 5. Effect of severity and time of defoliation on number of florets per inflorescence, compared with non-defoliated shoots. Time of application of defoliation treatments reported as days from spring budbreak.

Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △




ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.1398 |
| Mild vs Control | 0.0253 | 0.1205 |
| Severe vs Control | 0.0381 | 0.4493 |

ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0856 |
| Trt | 0.0005 |
| Time | 0.1300 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0002 | |
| Mild vs Control | 0.0481 | |
| Severe vs Control | 0.9789 | |

Fig. 6. Effect of severity and time of defoliation on basal inflorescence diameter (mm), compared with non-defoliated shoots.
Time of application of defoliation treatments reported as days from spring budbreak.
Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

# 4. PAPER III - Changes in dry mass accumulation and allocation in response to source manipulation by defoliation in P*rotea* cv. Carnival.

## Changes in dry mass accumulation and allocation in response to source manipulation by defoliation in *Protea* cv. Carnival.

The accumulation and partitioning of carbohydrates is a function of source size and sink strength, and the relative allocation between organs determines growth rate and ultimate size. Manipulation of either the source or sink can result in a change in allocation and altered growth patterns. Fruit or blossom thinning is a standard horticultural practice to reduce the number of competing sinks and enhance growth of remaining sinks (Wibbe and Blanke, 1995; DelValle et al., 1985). Source modifications, such as shading or defoliation, necessitate a change in direction of translocation of assimilates from remaining sources to meet unaltered sink demands. The intensity of response of a plant to source manipulations, with respect to dry mass accumulation and allocation, is determined by the mechanisms available to overcome the decreased source size.

In comparing the response to defoliation by simulated herbivory of the deciduous bilberry (*Vaccinium myrtillus* L. ) and the evergreen lignonberry (*Vaccinium vitis-idaea* L.) Tolvanen and Laine (1997) found different recovery patterns. The deciduous shrub relied on reserves stored in the perennial structure and could recover quickly from the loss of leaves. To the evergreen shrub leaf loss was far more destructive as reserves were low and photosynthetic tissues were relied on to produce assimilates. A reduction in leaf area has been reported to result in an increase in photosynthetic rate of the remaining leaves (Patrick, 1988), although the compensation is seldom complete, and changes in dry mass accumulation and allocation can occur (Bhatt and Srinivasa Rao, 1993; Layne and Flore, 1995)

To investigate the role of leaves in supporting dry mass accumulation and partitioning during inflorescence initiation and development in *Protea* cv. Carnival shoots, different severities of defoliation were applied at different times. Starch concentrations were low in the bark and wood of two-year-old branches of 'Carnival', indicating a low reserve status (Greenfield et al., 1995), although reducing sugars were present and could supply short-term relief following defoliation.

## Materials and Methods

**PLANT MATERIAL.** Experiments were done on '*Protea* cv. Carnival (*P. compacta* × *P. neriifolia*). Plants were grown in a commercial plantation in the Stellenbosch district (lat.33°15'S; long.19°07'E), South Africa. The climate is Mediterranean with an annual rainfall of 600 - 700mm, falling mainly in winter. Summers are hot and dry. Plants were spaced 1 m in the row and 4 m between rows, clean cultivated, and were not irrigated or fertilised. Four-year-old plants were used. Plants were pruned and managed for biennial bearing (Gerber et al., 1995) and spring budbreak started on 20 August 1997.

**DEFOLIATION TREATMENTS.** In mid winter (early June, 1997) shoots were tagged that were of similar size and had three growth flushes, with the terminal flush being the autumn flush. Tagging shoots at the start of the experiment ensured that shoots used throughout the experiment had similar initial characteristics. Defoliation treatments were done on 3 June, 11 July, 29 July, 12 August, and 9 September 1997 (corresponding to 78, 40, 22 and 8 days before spring budbreak, with the last date corresponding to 20 days after spring budbreak.). The four degrees of severity of defoliation were: total defoliation, where all the leaves on the shoot were removed; severe partial defoliation, where all the leaves were removed except those on the uppermost, autumn, flush; mild

partial defoliation, where only the leaves on the uppermost, autumn, flush were removed; and no defoliation, or control, where all the leaves remained on the shoot. Leaves were cut off with scissors at their point of inception.

With the onset of spring, vegetative growth continued on both defoliated and control shoots to produce a spring growth flush. Flower development on 'Carnival' occurs once elongation of the spring growth flush is complete (Paper I). On 11 February 1998, when flowers on control shoots were at the commercially harvestable stage, all shoots were picked and brought to our laboratory for analysis.

**DRY MASS ANALYSIS.** Spring flush and inflorescence tissues were placed separately in brown paper bags and dried in a convection oven at 60°C for 4 to 7 days to determine the dry mass. The average leaf mass was calculated by dividing the number of leaves on the spring flush (Paper II) by the total leaf dry mass. Only shoots which initiated flowers will be discussed. This includes all shoots defoliated in 1996 and shoots defoliated on 29 July, 12 August and 9 September 1997 (Paper II).

**STATISTICAL ANALYSIS.** The experiment was set out as a randomised design with 5 single shoot replicates. Orthogonal contrasts were fitted and analysis of variance performed using the SAS program (SAS Institute Inc., 1990).

## Results

**DRY MASS ACCUMULATION.** When shoots were totally defoliated at or before spring budbreak the total dry mass accumulated was much lower than in the control shoots (Fig. 1a and b). This decrease in total dry mass was due to a reduction in dry mass accumulation in all components, viz. the leaf, the stem and the inflorescence (Table 1a and b). The lower dry mass of leaves was a function of fewer leaves (Paper II) and lower average leaf mass (Fig. 2a and b). The decrease in average leaf mass following

total defoliation was linear with time in 1996 and showed a quadratic trend in 1997 (Fig. 2a and b). These trends were also shown by the total dry mass accumulation of the leaves (Fig. 3a and b). The decrease in stem dry mass accumulation was less marked the later total defoliation was applied, also showing a linear trend with time in 1996 (Fig. 4a and b). The effect of total defoliation on dry mass accumulation of the inflorescence showed a quadratic trend over time (Fig. 5a and b). Early total defoliation markedly reduced the inflorescence dry mass, compared with non-defoliated shoots, but the effect was no longer apparent when total defoliation was applied approximately 40 days after spring budbreak in 1996 (Fig. 5a).

Following severe partial defoliation at any of the times applied the total dry mass of the spring flush and inflorescence was unchanged (Fig. 1a and b ). Neither the dry mass of the inflorescence (Fig. 5a and b), the stem (Fig. 4a and b) nor the leaves (Fig. 3a and b) on the spring growth flush were significantly different from the control.

Mild partial defoliation applied at or before spring budbreak decreased the total dry mass accumulated by the spring flush and inflorescence (Fig. 1a and b). When defoliation was done before spring budbreak the decrease was the most marked. The effect was similar to that described for total defoliation, with decreases in dry mass of leaves (Fig. 3a and b), stem (Fig. 4a and b) and inflorescence (Fig. 5a and b), but all components were affected to a lesser extent by mild partial defoliation. The decrease in leaf dry mass was due both to a decrease in the number of leaves on the spring flush (Paper II) and a decrease in the average leaf mass (Fig. 2a and b).

The effect of mild partial defoliation in 1996 on the dry mass of leaves and stem showed a linear trend, the reduction being the greatest the earlier the shoots were defoliated (Fig. 3a and 4a). The reduction in dry mass of the inflorescence after mild

partial defoliation was also the most marked when shoots were defoliated early, but the effect decreased quadratically and was no longer apparent when shoots were defoliated approximately 20 days after spring budbreak (Fig. 5a). The decline in effect occurred earlier than following total defoliation, where inflorescence mass was still affected by defoliation up to 40 days after spring budbreak. Mild partial defoliation in 1997 showed similar effects as mild partial defoliation applied in 1996, but the trends were more difficult to assess due to fewer dates on which defoliation was performed (Fig. 1-5b).

**DRY MASS ALLOCATION.** The total dry mass of the spring flush and terminal inflorescence on control/ non-defoliated shoots harvested on 10 February 1996 was approximately 55g (Table 1a). Of this 43% was allocated to the spring flush leaves, 23% to the stem and 34% to the inflorescence (data not presented). Shoots harvested on 11 February 1998 had a similar total dry mass of 53g (Table 1b) and a similar pattern of allocation, with 38% allocated to the leaves, 19% to the stem and 43% to the flower (data not presented ).

When shoots were totally defoliated before spring budbreak both dry mass accumulation and allocation were affected. The total dry mass accumulated in the spring flush and inflorescence on totally defoliated shoots in 1996 was 34 % of the dry mass accumulated in non-defoliated shoots (Table 1a). The dry mass of the stem and inflorescence was 28% and 26% of the non-defoliated value, respectively, indicating allocation of a lower proportion of the total dry mass than in a non-defoliated shoot. If the dry mass had been allocated in the same proportion as on non-defoliated shoots all components should reflect the same percentage as the total dry mass. A higher proportion of dry mass was allocated to the leaves, since accumulation was less compromised by defoliation, reaching 45% of the non-defoliated value. A similar

pattern was seen following total defoliation before spring budbreak in 1997 (Table 1b). These effects were no longer apparent when defoliation was done after spring budbreak. (Table 1a and b)

Neither dry mass accumulation nor allocation was affected by severe partial defoliation at the times at which it was applied. The proportion allocated to the stem, leaves, or inflorescence did not differ significantly whether severe partial defoliation was applied before, at or after spring budbreak (Table 1a and b).

Mild partial defoliation significantly reduced the total dry mass produced when applied before spring budbreak (Fig. 1) and also resulted in a redirection of assimilates. The proportion of the total dry mass assigned to the stem was unchanged when mild partial defoliation was applied to shoots 35 days before spring budbreak in 1996 (Table 1a). The total dry mass accumulated in the defoliated shoot was 74% of the non-defoliated shoot, and the dry mass accumulated by the stem alone was 72% of that accumulated by the stem of the non-defoliated shoot. The change in allocation which did occur following mild partial defoliation at this time was a decrease in allocation to the inflorescence and an increase in allocation to the leaves. The dry mass accumulated by the inflorescence following mild partial defoliation was only 57% of that accumulated by the non-defoliated shoot. The dry mass of the leaves was as much enhanced as the inflorescence was compromised. This promotion of leaf growth to the detriment of the inflorescence was not seen with later defoliation in 1996 nor in 1997.

## Discussion

During winter there is no shoot growth in 'Carnival', although the apical meristem is active and produces the appendages necessary for the spring flush. Elongation of the spring flush is complete approximately 50-60 days after spring budbreak (Paper I) with

the first new leaves having expanded and unfolded by approximately 20-30 days after spring budbreak (Gerber, field observation). Differentiation of involucral bracts occurs concurrently with spring flush growth. After completion of the spring growth flush differentiation of floral bracts and florets takes place. Flower development continues through summer and flowers reach anthesis in February/March (Greenfield et al., 1994).

Total defoliation markedly reduced dry mass accumulation of the spring flush, indicating a reduced source. Greenfield et al. (1995) found that, unlike deciduous fruit trees, reserve carbohydrates in permanent aerial parts of 'Carnival' remain low throughout the year. New growth and dry mass accumulation in proteas are afforded either by provision of newly synthesised photosynthates or reserves in the current season's growth. In citrus the high sugar content in older leaves can serve as a reserve source for the growth of new leaves in spring (Smith et al., 1952). Napier (1985) reported leaf sugar concentrations of 7% of dry mass in late winter in *Leucospermum* cv. Red Sunset, and studies on the dynamics of carbohydrate movement during the season indicate that the same may be true in proteas (Hettasch, 1999). Total defoliation, therefore, removed not only the source of new photosynthates, but a potential reserve source as well. In grape, translocation of photosynthates can occur between adjacent shoots, as seen when the source of carbohydrates on a shoot was reduced, either by shading or defoliation (Kramer and Koslowski, 1979), although in olive, shoots were not influenced by adjacent, non-treated branches and were considered independent units (Proietti and Tombesi, 1996). In 'Carnival' initiation and growth of the spring flush on the totally defoliated shoot must have been supported either by translocation of assimilates from neighbouring shoots or by small amount of carbohydrates in the stem. Carbohydrate reserves in the stems of decapitated *Populus* trees were mobilised to

buffer short term changes in sink demand (Tschaplinski and Blake, 1989). Layne and Flore (1995) concluded that, if the same applied in sour cherry, the supply of reserves was small and soon depleted. Whatever the mechanism in 'Carnival' the alternative source was significantly weaker.

The pattern of assimilate distribution in plants is such that lower leaves on the shoot export assimilates basipetally and support root growth (Wardlaw, 1968; Kriedemann, 1970). New leaves on the shoot, when they become autonomous and begin net export, support growth of leaves forming at the shoot apex. When these leaves in turn mature, the direction of translocation of assimilates from the lower leaves changes from acropetal to basipetal. Leaves at an intermediate position on the shoot can export in either or both directions.

'Carnival' shoots used in these defoliation trials consisted of three growth flushes, with the uppermost flush having been formed in the previous autumn. According to the pattern of assimilate distribution discussed above the autumn flush leaves, by virtue of their close proximity to the shoot apex, would be expected to be the prime source of assimilates for growth of the spring flush. This theory is supported by the effect of partial defoliation on dry mass accumulation.

Normal dry mass accumulation was supported when only the autumn flush leaves remained on the shoot following severe partial defoliation. Thus, the position of the leaves relative to the active sink is more important than the number of leaves on the shoot. When only the autumn flush leaves were removed (mild partial defoliation) the dry mass of the spring flush was reduced, despite the continued presence of many leaves on the lower portion of the shoot. Removal of the autumn flush leaves in mild partial defoliation necessitated a change in direction of translocation of photosynthates from the

remaining, lower leaves on the shoot, forcing photosynthates to move acropetally toward the developing apical organs. That the change did indeed take place is apparent by the continued growth of the spring flush, but the lower dry mass indicated that the new, substitute source was, however, an inferior source. After defoliation photosynthesis in the remaining leaves tends to increase to compensate for the decreased surface area (Bhatt and Srinivasa Rao, 1993; Gifford and Evans, 1981; Meyer, 1998; Layne and Flore, 1995; Ronzhina and Mokronosov, 1994), although Ovaska et al. (1992) suggested that the increase may be due to removal of competition between leaves rather than a decreased source to sink ratio. The decreased source as a result of mild partial defoliation was either due to the inability of the older leaves to restore the same level of photosynthesis as younger leaves, or was only due to a delay in supply as the direction and distance of translocation changed.

The allocation of dry mass between the spring flush components and the inflorescence is a measure of relative sink strength, the priorities of which become apparent under source limiting conditions imposed by defoliation.

Leaf expansion, stem extension, and involucral bract differentiation occur concurrently and, therefore compete for available assimilates. Differentiation of involucral bracts has sink priority, as the number formed was unchanged by time or severity of defoliation (Paper II). Defoliation decreased leaf dry mass, and this was due to both a decrease in the number of leaves (Paper II) and the average leaf mass (Fig. 2), the latter indicating that expansion was limited by defoliation. Elongation of the stem, however, was most severely affected by defoliation (Paper II) indicating a low sink priority. During elongation of the spring flush sink priority can be illustrated: involucral bract differentiation > leaf expansion > stem extension. These parameters were

complete by 50 - 60 days after spring budbreak, therefore defoliation after this date had no further effect.

The effect of defoliation on the inflorescence dry mass can be attributed largely to the source strength of the spring flush and, as such, is a secondary effect. Where the spring flush was not compromised by either time or severity of defoliation the accumulation of dry mass of the inflorescence continued as normal. More accurately, this is a function of the leaves on the spring flush which serve as a source of assimilates for inflorescence growth. In *Leucospermum* cv. Red Sunset defoliation shortly before flower initiation decreased the dry mass of the inflorescence (Jacobs, 1983) as did heavy shading (Jacobs and Minaar, 1980)

The effect of defoliation was, in many instances, no longer apparent at approximately 20 to 30 days after spring budbreak. This coincides with completed expansion of the first leaves on the spring flush and suggests the emergence of a new, local source of photo-assimilates. The autumn leaves in citrus are reported to resume photosynthesis in order to support post-winter growth and flowering, until the new spring flush leaves acquire photosynthetic competence (Ruan, 1993). The same is true in kiwi-fruit, although initial growth is supported by reserves stored in the perennial structure, due to the deciduous nature of the plant (Piller et al., 1998). In grapes new leaves were capable of export when they had expanded to about one half of their final size (Kramer and Koslowski, 1979), and in apple new shoot growth became fully autonomous about six weeks after budbreak (Priestley, 1962).

In conclusion, the change in source size and position subsequent to different severities of defoliation lead to reduced dry mass accumulation and altered partitioning.

It is obvious, from continued growth, that mechanisms exist in 'Carnival' to aid recovery after defoliation.

## Literature Cited

Bhatt, R.M., and N.K. Srinivasa Rao. 1993. Response of bell-pepper (*Capsicum annuum* L.) to defoliation. Photosynthesis, dry matter and fruit growth. Photosynthetica 28(3):377-382.

DelValle, T.B.G., J.A. Barden, and R.E. Byers. 1985. Thinning of peaches by temporary inhibition of photosynthesis with Terbacil. J. Amer. Soc. Hort. Sci. 110(6):804-807.

Gerber, A.I., K.I. Theron, and G. Jacobs. 1995. Pruning of *Protea* cv. Carnival to optimise economic biomass production. Acta Hort. 387:99-106.

Gifford, R. M., and L. T. Evans. 1981. Photosynthesis, carbon partitioning and yield. Ann. Rev. Plant Physiol. 32:485-509.

Greenfield, E.J., K.I. Theron, and G. Jacobs. 1994. Effect of pruning on growth and flowering response of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 4(1):42-46.

Greenfield, E.J., K.I. Theron, and G. Jacobs. 1995. Seasonal changes in carbohydrate and nitrogen levels in the bark and wood of pruned and unpruned plants of *Protea* cv. Carnival. J. S. Afr. Soc. Hort. Sci. 5(1):25-28.

Hettasch, H.B. 1999. Studies of the vegetative development of *Protea* cvv. Sylvia and Cardinal. M.Sc. Thesis. University of Stellenbosch, 63pp.

Jacobs, G. 1983. Flower initiation and development in *Leucospermum* cv. Red Sunset. J. Amer. Soc. Hort. Sci. 108(1):32-35.

Jacobs, G. and H.R. Minnaar. 1980. Light intensity and flower development of *Leucospermum cordifolium.* HortScience. 15(5):644-645.

Kramer, P.J., and T.T. Koslowski. 1979. Physiology of woody plants. Academic Press, New York.

Kriedemann, P.E. 1970. The distribution of 14C-labelled assimilates in mature lemon trees. Austr. J. Agric. Res. 21:623-632.

Layne, D.R., and J.A. Flore. 1995. End-product inhibition of photosynthesis in *Prunus cerasus* L. in response to whole-plant source-sink manipulation. J. Amer. Soc. Hort. Sci. 120(4):583-599.

Meyer, G.A. 1998. Mechanisms promoting recovery from defoliation in goldenrod (*Solidago altissima*). Can. J. Bot. 76:450-459.

Napier, D.R. 1985. Initiation, growth and development of *Leucospermum* cv. Red Sunset inflorescences. M. Sc. Thesis. University of Stellenbosch.

Ovaska, J., M. Walls, and P. Mutikainen. 1992. Changes in leaf gas exchange properties of cloned *Betula pendula* saplings after partial defoliation. J. Exp. Bot. 43:1301-1307.

Patrick, J. W. 1988. Assimilate partitioning in relation to crop productivity. HortScience. 23:33-40.

Piller, G.J., A.J. Greaves and J.S. Meekings. 1998. Sensitivity of floral shoot growth, fruit set and early fruit size in *Actinidia deliciosa* to local carbon supply. Ann. Bot. 81:723-728.

Priestley, C.A. 1962. Carbohydrate resources within the perennial plant. Technical Comm #27. Commonwealth Bureau of Horticulture and Plantation crops.

Proietti, P. and A. Tombesi. 1996. Translocation of assimilates and source-sink influences on productive characteristics of the olive tree. Adv. Hort. Sci. 10:11-14.

Ronzhina, E.S., and A.T. Mokronosov. 1994. Source-sink relations and the role of cytokinins in the regulation of transport and partitioning of organic substances in plants. Russ. J. Plant Physiol. 41(3):396-406.

Ruan, Y-L. 1993. Fruit set, young fruit and leaf growth of *Citrus unshiu* in relation to assimilate supply. Scientia Hort. 53:99-107.

SAS Institute Inc. 1990. SAS/STAT User's guide, Version 6. 4th ed., Vol 1 and 2. Cary, NC.

Smith, P.F., W. Reuther and A.W. Specht. 1952. Seasonal changes in Valencia orange trees. II. Changes in micro-elements, sodium, and carbohydrates in leaves. HortScience. 59:31-35.

Tolvanen, A., and K. Laine. 1997. Effects of reproduction and artificial herbivory on vegetative growth and resource levels in deciduous and evergreen dwarf shrubs. Can. J. Bot. 75:656-666.

Tschaplinski, T. and T.J. Blake. 1989. Photosynthetic reinvigoration of leaves following shoot decapitation and accelerated growth of coppice shoots. Physiol. Plant. 75:157-165.

Wardlaw, I.F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-105.

Wibbe, M.L. and M.M. Blanke. 1995. Effects of defruiting on source-sink relationship, carbon budget, leaf carbohydrate content and water use efficiency of apple trees. Physiol. Plant. 94:529-533.

Table 1. Dry mass changes of the inflorescence and subtending spring flush of *Protea* cv. Carnival in response to different times and severities of defoliation. Values expressed are a percentage of the average value of non-defoliated shoots. Means (n = 5) within columns followed by the same letter are not significantly different according to LSD values (0.5%)

Non-defoliated shoot values for 1996 data.

Leaves: 23.3g; Stem: 12.9g; Inflorescence: 18.8g; Total: 54.8g

a) Shoots defoliated 35 days before spring budbreak in 1996

| Defoliation severity | Leaves | Stem | Inflorescence | Total |
|---|---|---|---|---|
| Total defoliation | 45 $^{b}$ | 28 $^{c}$ | 26 $^{c}$ | 34 $^{c}$ |
| Partial, mild | 89$^{a}$ | 72 $^{b}$ | 57 $^{b}$ | 74 $^{b}$ |
| Partial, severe | 107$^{a}$ | 83$^{ab}$ | 105$^{a}$ | 101$^{a}$ |
| No defoliation | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ |

b) Shoots defoliated at spring budbreak (2 September) in 1996

| Defoliation severity | Leaves | Stem | Inflorescence | Total |
|---|---|---|---|---|
| Total defoliation | 60 $^{d}$ | 52 $^{c}$ | 50 $^{d}$ | 55 $^{d}$ |
| Partial, mild | 82 $^{c}$ | 77 $^{b}$ | 80 $^{c}$ | 80 $^{c}$ |
| Partial, severe | 115$^{a}$ | 111$^{a}$ | 122$^{a}$ | 116$^{a}$ |
| No defoliation | 100 $^{b}$ | 100$^{a}$ | 100 $^{b}$ | 100 $^{b}$ |

c) Shoots defoliated 35 days after spring budbreak in 1996

| Defoliation severity | Leaves | Stem | Inflorescence | Total |
|---|---|---|---|---|
| Total defoliation | 96$^{a}$ | 84$^{a}$ | 90$^{a}$ | 91$^{a}$ |
| Partial, mild | 91$^{a}$ | 85$^{a}$ | 95$^{a}$ | 91$^{a}$ |
| Partial, severe | 93$^{a}$ | 87$^{a}$ | 101$^{a}$ | 94$^{a}$ |
| No defoliation | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ |

Table 1 (continued). Dry mass changes of the inflorescence and subtending spring flush of *Protea* cv. Carnival in response to different times and severities of defoliation. Values expressed are a percentage of the average value of non-defoliated shoots. Means (n = 4) within columns followed by the same letter are not significantly different according to LSD values (0.5%)

Non-defoliated shoot values for 1997 data.
Leaves: 20.3g; Stem: 10.0g; Inflorescence: 22.5g; Total: 52.8g

d) Shoots defoliated 22 days before spring budbreak in 1997

| Defoliation severity | Leaves | Stem | Inflorescence | Total |
|---|---|---|---|---|
| Total defoliation | 47 $^{c}$ | 32 $^{c}$ | 33 $^{c}$ | 39 $^{c}$ |
| Partial, mild | 77 $^{b}$ | 66 $^{b}$ | 65 $^{b}$ | 70 $^{b}$ |
| Partial, severe | 99$^{a}$ | 92$^{a}$ | 98$^{a}$ | 97 $^{a}$ |
| No defoliation | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100 $^{a}$ |

e) Shoots defoliated 8 days before spring budbreak in 1997

| Defoliation severity | Leaves | Stem | Inflorescence | Total |
|---|---|---|---|---|
| Total defoliation | 53 $^{c}$ | 45 $^{c}$ | 48 $^{b}$ | 49 $^{c}$ |
| Partial, mild | 83 $^{b}$ | 84 $^{b}$ | 69 $^{b}$ | 77 $^{b}$ |
| Partial, severe | 95$^{ab}$ | 92$^{ab}$ | 97$^{a}$ | 95$^{a}$ |
| No defoliation | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ |

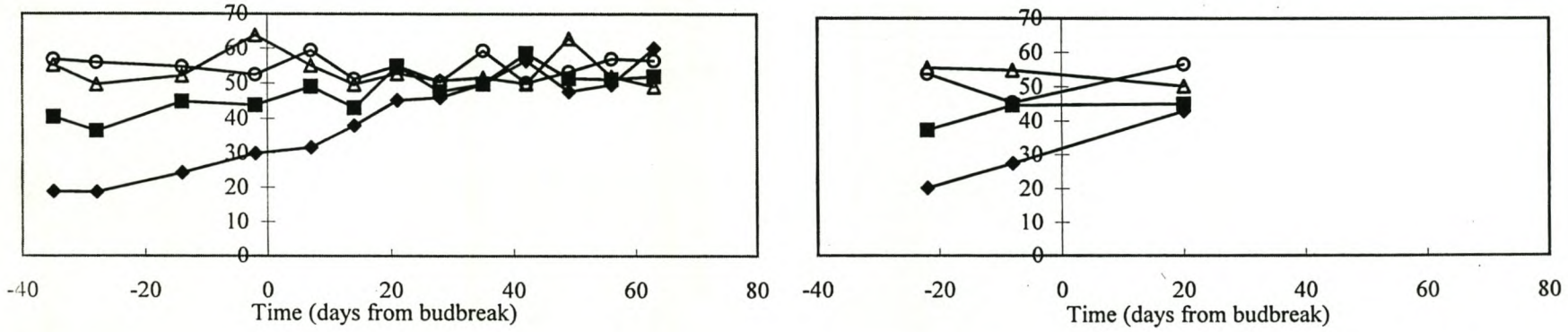
f) Shoots defoliated 20 days after spring budbreak in 1997

| Defoliation severity | Leaves | Stem | Inflorescence | Total |
|---|---|---|---|---|
| Total defoliation | 81 $^{b}$ | 74 $^{b}$ | 73 $^{b}$ | 76 $^{b}$ |
| Partial, mild | 79 $^{b}$ | 85$^{ab}$ | 66 $^{b}$ | 74 $^{b}$ |
| Partial, severe | 92$^{ab}$ | 95$^{ab}$ | 83$^{ab}$ | 87$^{ab}$ |
| No defoliation | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ |

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation Δ




| ANOVA | |
|---|---|
| Source | Significance |
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.2495 |
| Mild vs Control | 0.0002 | 0.1231 |
| Severe vs Control | 0.9145 | 0.2457 |

| ANOVA | |
|---|---|
| Source | Significance |
| Trt*Time | 0.0025 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0077 | 0.1277 |
| Mild vs Control | 0.7629 | 0.0200 |
| Severe vs Control | 0.1121 | 0.0787 |

Fig. 1. Total dry mass accumulation (g) of spring flush components on defoliated and non-defoliated shoots. Time of application of defoliation treatments reported as days from spring budbreak.
Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

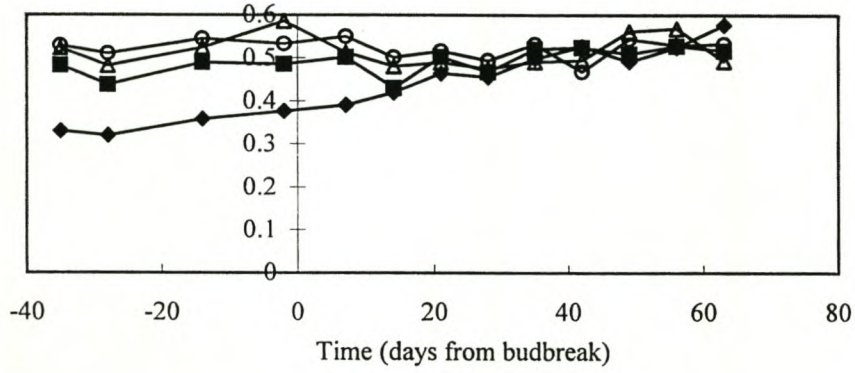
a) Shoots defoliated in 1996, picked in 1997

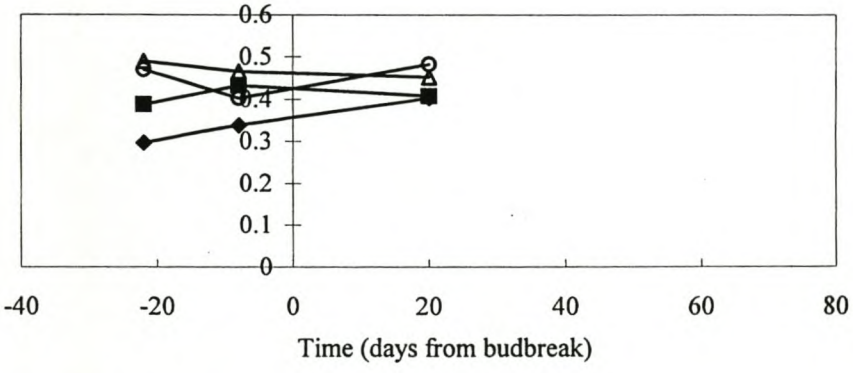
b) Shoots defoliated in 1997, picked in 1998

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998




ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.7625 |
| Mild vs Control | 0.0182 | 0.4447 |
| Severe vs Control | 0.6110 | 0.4419 |

ANOVA

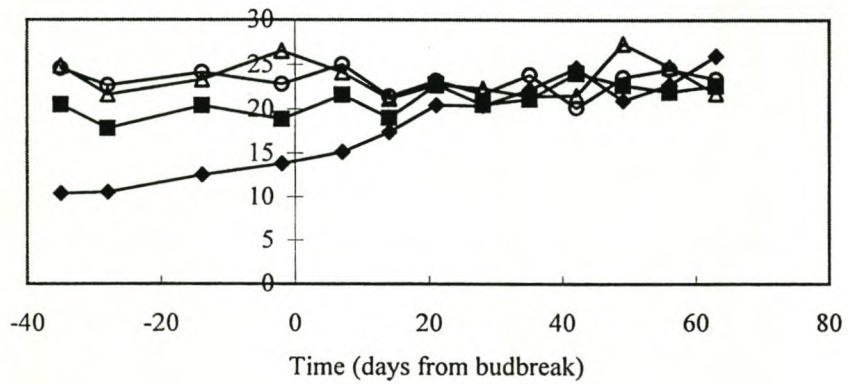
| Source | Significance |
|---|---|
| Trt*Time | 0.0012 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0325 | 0.0178 |
| Mild vs Control | 0.6240 | 0.0014 |
| Severe vs Control | 0.0744 | 0.0638 |

Fig. 2. Average leaf mass (g) of the spring flush on defoliated and non-defoliated shoots.
Time of application of defoliation treatments reported as days from spring budbreak.
Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △

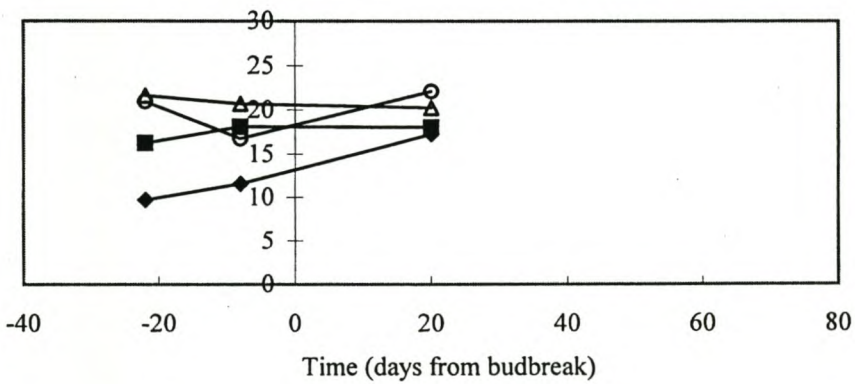



ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.3889 |
| Mild vs Control | 0.0087 | 0.6434 |
| Severe vs Control | 0.8091 | 0.7344 |

b) Shoots defoliated in 1997, picked in 1998




ANOVA

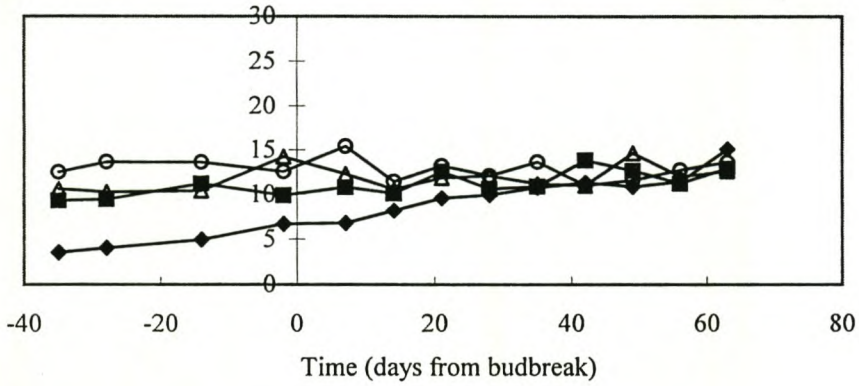
| Source | Significance |
|---|---|
| Trt*Time | 0.0052 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0246 | 0.0711 |
| Mild vs Control | 0.7800 | 0.0090 |
| Severe vs Control | 0.1474 | 0.0586 |

Fig. 3. Dry mass accumulation (g) of leaves on defoliated and non-defoliated shoots.
Time of application of defoliation treatments reported as days from spring budbreak.
Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △

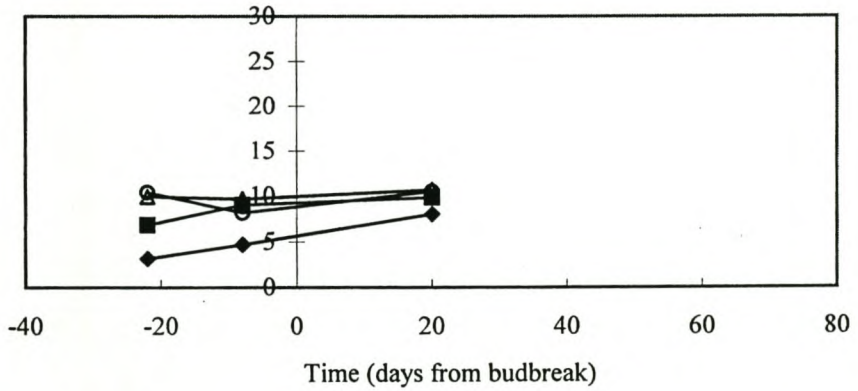



ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.6749 |
| Mild vs Control | 0.0012 | 0.9936 |
| Severe vs Control | 0.0240 | 0.7209 |

b) Shoots defoliated in 1997, picked in 1998




ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0077 |

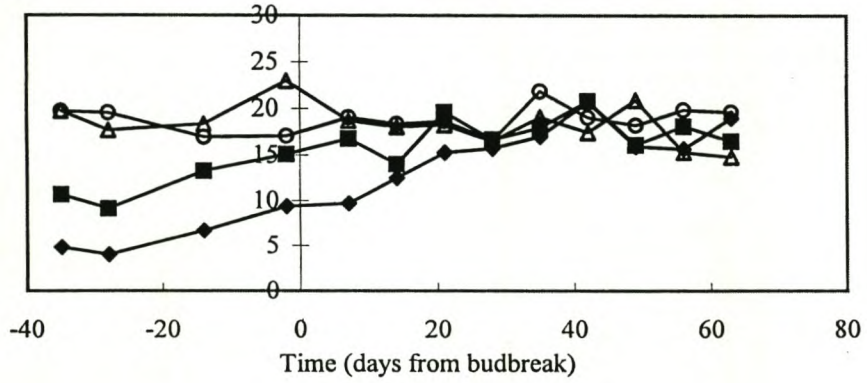
| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.00331 | 0.0751 |
| Mild vs Control | 0.1139 | 0.0066 |
| Severe vs Control | 0.1182 | 0.2848 |

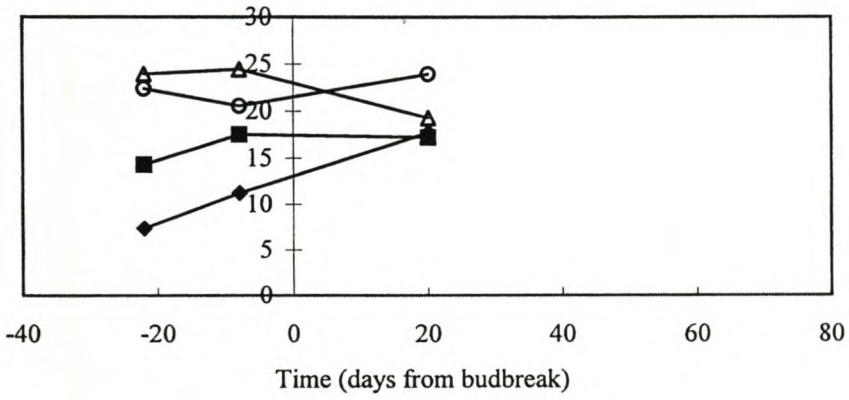
Fig. 4. Dry mass accumulation (g) of spring flush stem on defoliated and non-defoliated shoots.
Time of application of defoliation treatments reported as days from spring budbreak.
Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

a) Shoots defoliated in 1996, picked in 1997

b) Shoots defoliated in 1997, picked in 1998

Non-defoliated shoots ○ Mild partial defoliation ■
Total defoliation ◆ Severe partial defoliation △




ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0001 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0001 | 0.0516 |
| Mild vs Control | 0.0005 | 0.0045 |
| Severe vs Control | 0.4013 | 0.0528 |

ANOVA

| Source | Significance |
|---|---|
| Trt*Time | 0.0102 |

| CONTRASTS | Linear | Quadratic |
|---|---|---|
| Total vs Control | 0.0284 | 0.0770 |
| Mild vs Control | 0.9089 | 0.7629 |
| Severe vs Control | 0.0562 | 0.1121 |

Fig. 5. Dry mass accumulation (g) of the inflorescence on defoliated and non-defoliated shoots. Time of application of defoliation treatments reported as days from spring budbreak.
Data represented are averages of 5 shoots picked on 10 February 1997 and 4 shoots picked on 11 February 1998.

# 5. PAPER IV - The role of leaves and carbohydrates in flowering of P*rotea* cv. Lady Di.

## The role of leaves and carbohydrates in flowering of *Protea* cv. Lady Di.

Mature leaves on an overwintering shoot are essential for induction of flowering in *Protea* cv. Carnival (Paper II). Induction is complete in late winter, 6-7 weeks before budbreak in spring, and from this time the shoot is committed to flowering although initiation only commences with spring budbreak. Removal of leaves by defoliation before the shoot is induced results in failure to flower.

Initiation of inflorescences in 'Carnival' and *P.* cv. Lady Di occurs predominantly on the spring flush, when subtended by one or more previous flushes. The spring flush is preformed in the apical bud before the start of spring budbreak (Paper I). Spring flush growth occurs by elongation of preformed internodes together with differentiation and growth of leaves. During this phase of rapid growth the apical meristem on an induced shoot produces involucral bracts. Floral bracts and florets are produced after elongation of the spring flush is complete, followed by growth and development of the inflorescence.

Production of new tissues in spring in deciduous plants is supported by the mobilisation of reserve carbohydrates stored in the permanent, woody structures until new leaves have developed sufficiently and become net exporters of photosynthates (Priestley, 1962). Carbohydrates in the two-year-old branches of 'Carnival' were low throughout the year, indicating a poor supply of stored reserves (Greenfield et al., 1995). A decrease in carbohydrate content of current season's growth of *Protea* cv. Sylvia occurred in early spring, although both starch and sugars were present in low concentrations at all times (Hettasch, 1999). In both *Leucospermum* cv. Red Sunset (Napier, 1985) and *Brunia albiflora* (Poole, 1999) carbohydrate levels were generally low

with an increase in starch prior to inflorescence initiation. Shading of 'Red Sunset' during shoot growth reduced flowering, suggesting that current photosynthates were necessary for successful inflorescence initiation. Inflorescence development in 'Carnival' was delayed by a reduction in leaf area as a result of defoliation (Paper II). The delay was most obvious when defoliation was applied before spring budbreak, but was still apparent when defoliation was applied after inflorescence initiation had occurred, but before expansion of leaves on the spring flush was complete. Leaf area was reduced by both a decrease in the number of mature leaves on the shoot (removed by defoliation) and a decrease in the average mass of new leaves produced on the spring flush (an effect of defoliation).

Shoots of 'Lady Di' were defoliated at different times, starting prior to spring budbreak, to determine the dependence of spring flush growth on stored carbohydrates in the overwintering shoot and to ascertain the importance of leaves in inflorescence initiation and development.

## Materials and Methods

**PLANT MATERIAL.** Experiments were done on 6-year-old Protea cv. Lady Di plants (*P. compacta* × *P. magnifica* ). Plants were grown in a commercial plantation spaced 1 m in the row and 4 m between rows, clean cultivated, and were not irrigated or fertilised. The climate in the Stellenbosch district (lat.33°15'S; long.19°07'E), South Africa, is Mediterranean with an annual rainfall of 600 – 700 mm, falling mainly in winter. Summers are hot and dry.

'Lady Di' plants were pruned in September 1997 for biennial bearing (Gerber et al., 1995) to improve stem length. Two growth flushes were produced in a growing season: a spring flush produced shortly after pruning in September, followed by a late summer

flush. Vegetative growth ceased during winter and continued from the terminal position in spring. The spring flush arising from the terminal bud was preformed during winter (Paper I). Inflorescence initiation occurred terminally during elongation of the spring flush, followed immediately by development.

**DRY MASS AND CARBOHYDRATE CHANGES DURING PRODUCTION OF THE SPRING FLUSH AND INFLORESCENCE.** From late winter until inflorescence maturity, non-defoliated shoots were picked at phenologically determined intervals, viz, pre-spring budbreak (20 August 1998); at spring budbreak (1 October 1998) (this coincides with the start of production of involucral bracts (Paper I)); when elongation of the spring flush was approximately half the final length (29 October 1998); when elongation of the spring flush was complete (19 November 1998); when the inflorescence was approximately 30 mm in basal diameter (8 April 1999); and when the inflorescence was ready for commercial harvest (14 July 1999).

Three shoots were harvested on each date and brought to our laboratory. The length of the spring flush was measured and the shoot was separated into leaf and stem portions for each flush, and the developing inflorescence. Samples were lyophilised and the dry mass measured before being milled to a fine powder.

**DEFOLIATION TREATMENTS.** Shoots consisting of 2 growth flushes were defoliated starting before spring budbreak (20 August 1998), at spring budbreak (1 October 1998) and when the spring flush was half of the final length (29 October 1998). These shoots had all the leaves removed from the two mature flushes. From the completion of spring flush elongation individual shoots were defoliated at two-weekly intervals until inflorescences were approximately 30mm in diameter (8 April 1999). Once growth of the spring flush was complete (19 November 1998) defoliation treatments removed the leaves on the mature flushes as well as newly formed leaves on the spring flush, but

leaving the 10 - 12 uppermost leaves. These leaves were left on the shoot to enable the mature inflorescence to be harvested and marketed. In commercial practice protea shoots are stripped of their leaves in the packshed before being packed for marketing. The lower two-thirds of the leaves on the shoot are removed. Five shoots were defoliated on each date. Defoliation entailed cutting off leaves at their point of inception using scissors.

The diameter of developing inflorescences was measured at two-weekly intervals until they reached commercial picking stage. The date on which defoliated shoots reached this stage was recorded. Non-defoliated shoots were used for comparison.

When inflorescences were at the commercial picking stage, on 14 July 1999, 3 shoots which were defoliated in February or March were harvested and brought to our laboratory. They were separated into leaf and stem portions for each flush, and the inflorescence. Samples were freeze-dried and the dry mass measured before being milled to a fine powder for carbohydrate analysis. Results were compared with non-defoliated shoots.

**CARBOHYDRATE ANALYSIS.** A 0.5 g sample of the dried tissue described above was taken for carbohydrate analysis. Samples were extracted overnight in 1% acetic acid by shaking and then centrifuged. The supernatant was filtered and brought to volume. Thereafter the pellet was dissolved in an acetate buffer (pH 4,8) and gelatinised in a boiling steambath for two hours. After cooling to 60°C, the starch fraction was hydrolyzed to glucose with the enzyme amyloglucosidase in an incubator for 18 hours.

Further analyses for reducing sugars and starch were done on a Sanplus Segmented Flow Analysis System from Skalar, using Method number 551-965w/r issue 070798/MH and number 356-001w/r issue 012998/MH97203066.

## Results

**DRY MASS AND CARBOHYDRATE CHANGES.** Carbohydrate analysis was started approximately 6 weeks before spring budbreak. Shoots consisted of 2 mature growth flushes. Analysis continued during development of new tissues i.e. the spring flush and subtended inflorescence.

The dry mass of mature tissues did not vary much during growth of new tissues (1 October 1998 – 19 November 1998) (Fig. 1). There was a significant increase from 20 August 1998 to 29 October 1998, which was due to an increase in the dry mass of the stem of the mature flushes. Total carbohydrate levels (reducing sugars and starch) remained constant, showing a significant drop only during the last 3 weeks of growth of the spring flush (Fig. 2). This drop was due to a decrease in total sugar content in all mature tissues (Fig. 3). After growth of the spring flush was complete the carbohydrate content of mature tissues increased, until the same level as before spring budbreak was attained. Carbohydrate levels in the mature tissues remained constant during inflorescence development. No significant differences were seen in the total starch levels of the mature tissues, despite seemingly obvious fluctuations (Fig. 4), and this was probably due to large variability between samples.

The dry mass and carbohydrate content of developing tissues increased gradually during elongation of the spring flush (Fig. 1 and 2). The major portion of dry mass accumulation occurred after elongation was complete and was accompanied by an increase in carbohydrate content, with both starch and sugar levels showing significant increases (Fig. 3 and 4). The overall increase in dry mass was largely due to an increase in the leaves of the spring flush, and the contribution of carbohydrate to this increase was small.

Inflorescence development during elongation of the spring flush occurred at the microscopic level (Paper I), and increases in dry mass and carbohydrate content were only apparent after completion of the spring flush (Fig. 1 and 2). Significant increases in starch, sugar and total carbohydrate content occurred together with a large increase in dry mass during the last 3 months of inflorescence development.

Newly formed tissue, comprising the spring flush and inflorescence, had a larger dry mass at the time of flower harvest (14 July 1999) than tissues which were mature prior to growth of the spring flush and inflorescence (Fig. 1). The carbohydrate content of newly formed tissues was also greater than mature tissues, and this was due to both starch and sugar levels (Fig. 3 and 4).

**DEFOLIATION.** Shoots defoliated before or at spring budbreak did not produce an inflorescence. These shoots either produced a terminal vegetative flush, a flower bud which aborted early, or ceased further growth entirely (Table 1). When shoots were defoliated before elongation of the spring flush was complete (before 19 November 1998), flowering was still negatively affected, but some shoots produced inflorescences which developed through to anthesis.

When applied after completion of spring flush growth (after 19 November 1998) defoliation had no effect on flowering. All shoots initiated inflorescences and development occurred at a similar rate, with all inflorescences reaching commercial picking stage within a 6 week period (Fig. 5).

When shoots were defoliated after completion of the spring flush only the top 20-25% of the leaves on the spring flush remained (10-12 out of the normal complement of approximately 47 leaves (Paper I)). At harvest these leaves had an average dry mass of less than 30% of non-defoliated shoots which had a complete set of leaves

(approximately 40 leaves). Analysis of defoliated shoots compared with non-defoliated shoots showed that the leaves which remained following defoliation in February and March supported normal dry mass accumulation of the inflorescence (Fig. 6a). Carbohydrate content was also unaffected (Fig. 6b). Neither the dry mass nor total carbohydrate content of the stem of all three flushes was significantly affected by defoliation, and levels were similar to those of non-defoliated shoots.

**Discussion**

The increase in dry mass of the overwintering shoot during spring (20 August to 1 October 1998) when budbreak occurred is largely due to an increase in the stem dry mass of the two mature flushes. Although an increase in non-structural carbohydrates also occurred at this time the increase in dry mass is attributable to production of structural tissue due to cambial activity. The dry mass of the overwintering shoot did not change during the remainder of the growth period studied and the major portion of the overwintering shoot dry mass was located in the leaves of the uppermost mature flush (summer flush).

Only during the period of rapid new shoot elongation did starch decline in mature tissues which is evidence that carbohydrate reserves are mobilised from the overwintering shoot to support new growth. Greenfield et al. (1995) reported low levels of carbohydrate in the two-year-old bearers of 'Carnival', so it is unlikely that new growth was supported by reserves stored in the permanent parts of the plant, as in deciduous trees. In citrus, carbohydrate produced by old leaves in spring was used for development of the new spring flush and developing flower (Akao et al., 1981)

The dry mass of the leaves and stem of the new growth (spring flush) continued to increase after shoot extension growth and leaf expansion was complete on 19 November

1998. This increase was not due to reserve carbohydrates. Although the levels of starch and sugars increased during this period the mass accumulated did not contribute significantly to the dry mass. After expansion, protea leaves are soft and later harden and develop a thick, waxy appearance, accompanied by an increase in mass. During the period of rapid dry mass accumulation in the spring flush and inflorescence, carbohydrate levels in mature tissues did not change, indicating that dry mass accumulation was supported by current photosynthates. At anthesis the inflorescence contributed approximately 40% of the dry mass of new growth, of which starch and sugars make up a small part.

In conclusion, prior to spring budbreak the dry mass of the overwintering shoot increases due to cambial activity. During rapid elongation of the new flush following budbreak carbohydrates are mobilised from the overwintering shoot for new growth. After elongation the dry mass of new growth increases, but carbohydrates contribute only a small portion of this increase. Development of the inflorescence and new spring flush is, therefore, mainly dependant on current photosynthates.

Inflorescence initiation did not occur in 'Lady Di' on shoots which were defoliated before or at spring budbreak. In 'Carnival' defoliation prevented flowering only when done earlier than 6-7 weeks before spring budbreak (Paper II). The presence of mature leaves which had overwintered was considered essential for inflorescence initiation in 'Carnival'. It was concluded that environmental factors probably play an inductive role and that the carbohydrate status of the shoot, while contributing to tissue growth, did not play a definitive role in inflorescence initiation. Mature leaves which had overwintered appear essential for inflorescence initiation in 'Lady Di', as seen by the failure to initiate on the late summer flush, and the inductive factors may be the same as in 'Carnival'.

Unlike 'Carnival', however, where inflorescence initiation (once induced) can occur in the absence of mature leaves on a shoot, successful inflorescence initiation in 'Lady Di' requires newly synthesised carbohydrates. Defoliation of 'Lady Di' no longer affected flowering only when applied after the leaves on the spring flush were expanded and, presumably, exporting new photosynthates. The involvement of an increase in carbohydrate levels, particularly sugars, during flower initiation has been reported (Bernier et al., 1993; Kinet, 1993), but the early stages of flower development do not require a large energy input and the role of sugars is thought to be as a messenger. Napier (1985) reported an increase in leaf starch during the period of acquisition of the induced state for flowering in *Leucospermum* cv. Red Sunset. In 'Lady Di' an increase in leaf starch and sugars was observed at spring budbreak, coinciding with the start of inflorescence initiation, but the increase was not statistically significant.

Unlike 'Carnival', defoliation of 'Lady Di' shoots caused flower abortion in some shoots. Defoliation before or at spring budbreak either prevented inflorescence initiation or caused reversion of the meristem to the vegetative state. Defoliation after inflorescence initiation but before differentiation of the involucral bracts was complete (29 October 1998) caused flower bud abortion at an early stage of development in some shoots, indicating that carbohydrates from current photosynthesis on the overwintering shoot are necessary for sustained differentiation of the inflorescence.

This effect was also seen when shoots were totally defoliated after completion of spring flush elongation (unpublished results). All leaves on the shoot, including the newly formed spring flush leaves, were removed, starting 4 weeks after completion of spring flush elongation in 1996 and continuing until the inflorescence was macroscopically visible. All shoots had initiated inflorescences and undergone a degree

of differentiation before defoliation, and all aborted. Current photosynthates are, therefore, essential for inflorescence development.

Inflorescence development continued unimpeded to anthesis when shoots were defoliated after spring flush growth, but leaving the uppermost 10-12 leaves of the newly formed spring flush. Development must have been supported by photosynthesis occurring in the few leaves which remained after defoliation, since carbohydrate levels in the stem portion of the defoliated shoot remained unchanged. A drop in carbohydrate levels would have suggested mobilisation of reserves to compensate for the loss of photosynthetic apparatus. An increase in photosynthesis in the leaves remaining after defoliation has been described as a compensatory mechanism to overcome the decreased leaf surface area (Bhatt and Srinivasa Rao, 1993; Meyer, 1998)

The rate of inflorescence development of 'Lady Di' was not affected by defoliation. Inflorescence development was delayed in 'Carnival' by defoliation, but only when defoliation was applied before completion of the spring growth flush. Defoliation applied at this stage in 'Lady Di' resulted in non-flowering. Later defoliation in 'Lady Di', which resulted in flowering was more severe than defoliation applied to 'Carnival', yet failed to delay flowering.

In conclusion, the presence of mature leaves which have overwintered is essential for inflorescence initiation in 'Lady Di', and also for successful inflorescence differentiation and development. Low reserve carbohydrate status in mature tissues indicates that new growth starting in spring is supported by new photosynthates. If the leaf surface area is reduced by defoliation the remaining leaves possess the ability to compensate and support normal growth. This is presumably achieved by an increase in photosynthetic efficiency.

## Literature Cited

Akao, S., S. Tsukahara, H. Hisada, S. and Ono. 1981. Contribution of photosynthetic assimilates to development of flower and spring flush in *Citrus unshiu* Marc. J. Japan. Soc. Hort. Sci. 50(1):1-9.

Bernier, G., A. Havelange, C. Houssa, A. Petitjean, and P. Lejuene. 1993. Physiological signals that induce flowering. Plant Cell 5:1147-1155.

Bhatt, R.M., and N.K. Srinivasa Rao. 1993. Response of bell-pepper (*Capsicum annuum* L.) to defoliation. Photosynthesis, dry matter and fruit growth. Photosynthetica 28(3):377-382.

Gerber, A.I., K.I. Theron, and G. Jacobs. 1995. Pruning of *Protea* cv. Carnival to optimise economic biomass production. Acta Hort. 387:99-106.

Greenfield, E.J., K.I. Theron, and G. Jacobs. 1995. Seasonal changes in carbohydrate and nitrogen levels in the bark and wood of pruned and unpruned plants of *Protea* cv. Carnival. J. S . Afr. Soc. Hort. Sci. 5(1): 25-28.

Hettasch, H.B. 1999. Studies of the vegetative development of *Protea* cvv. Sylvia and Cardinal. M.Sc. Thesis. University of Stellenbosch, 65pp.

Kinet, J.-M. 1993. Environmental, chemical, and genetic control of flowering. Hort. Revs. 15: 279-334.

Meyer, G.A. 1998. Mechanisms promoting recovery from defoliation in goldenrod (*Solidago altissima*). Can. J. Bot. 76:450-459.

Napier, D.R. 1985. Initiation, growth and development of *Leucospermum* cv. Red Sunset inflorescences. M. Sc. Thesis. University of Stellenbosch.

Poole, C.J. 1999. Growth and nutrition of *Bruniaceae*. M.Sc. Thesis. University of Stellenbosch, 103pp.

Priestley, C.A. 1962. Carbohydrate resources within the perennial plant. Technical Comm #27. Commonwealth Bureau of Horticulture and Plantation crops.

Table 1 : Effect of defoliation on flowering on *Protea* cv. Lady Di. Spring budbreak occurred on 1 October 1998. Elongation of the spring flush was complete on 19 November 1998.

| | Defoliation applied [z] | | Vegetative meristem | | Reproductive meristem | |
|---|---|---|---|---|---|---|
| Date of defoliation | overwintering shoot | new spring flush | Quiescent | Growth flush | Aborted | Anthesis |
| 20 August 1998 | + | - | 3/3 | | | |
| 1 October 1998 | + | - | 2/4 | 2/4 | | |
| 29 October 1998 | + | - | 2/4 | | 1/4 | 1/4 |
| 19 November 1998 | + | + | | | | 4/4 |
| 3 December 1998 | + | + | | | | 3/3 |
| 17 December 1998 | + | + | | | | 4/4 |
| 31 December 1998 | + | + | | | | 5/5 |
| 13 January 1999 | + | + | | | | 5/5 |
| 27 January 1999 | + | + | | | | 4/4 |
| 11 February 1999 | + | + | | | | 5/5 |
| 25 February 1999 | + | + | | | | 5/5 |
| 11 March 1999 | + | + | | | | 5/5 |
| 25 March 1999 | + | + | | | | 5/5 |
| 8 April 1999 | + | + | | | | 5/5 |
| Non-defoliated | - | - | | | | 5/5 |

[z] 5 shoots were defoliated on each date. Where less than 5 shoots are reported shoots were mechanically damaged during normal farming practices.

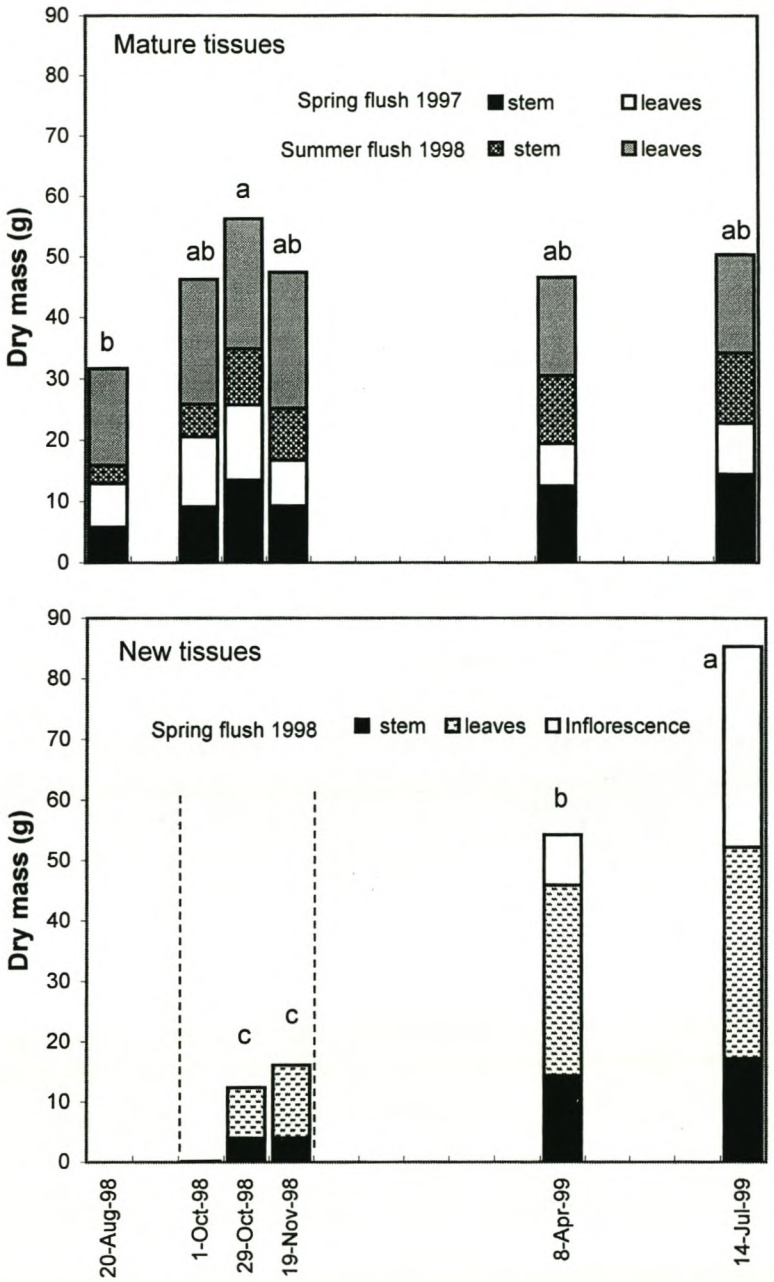



Fig. 1. Dry mass changes in mature and new tissues during development of new tissues. Spring budbreak occurred on 1 October 1998, and elongation growth of the spring flush occurred until 19 November 1998 (indicated by dotted lines).

Letters above columns indicate significant differences according to LSD values (5%).

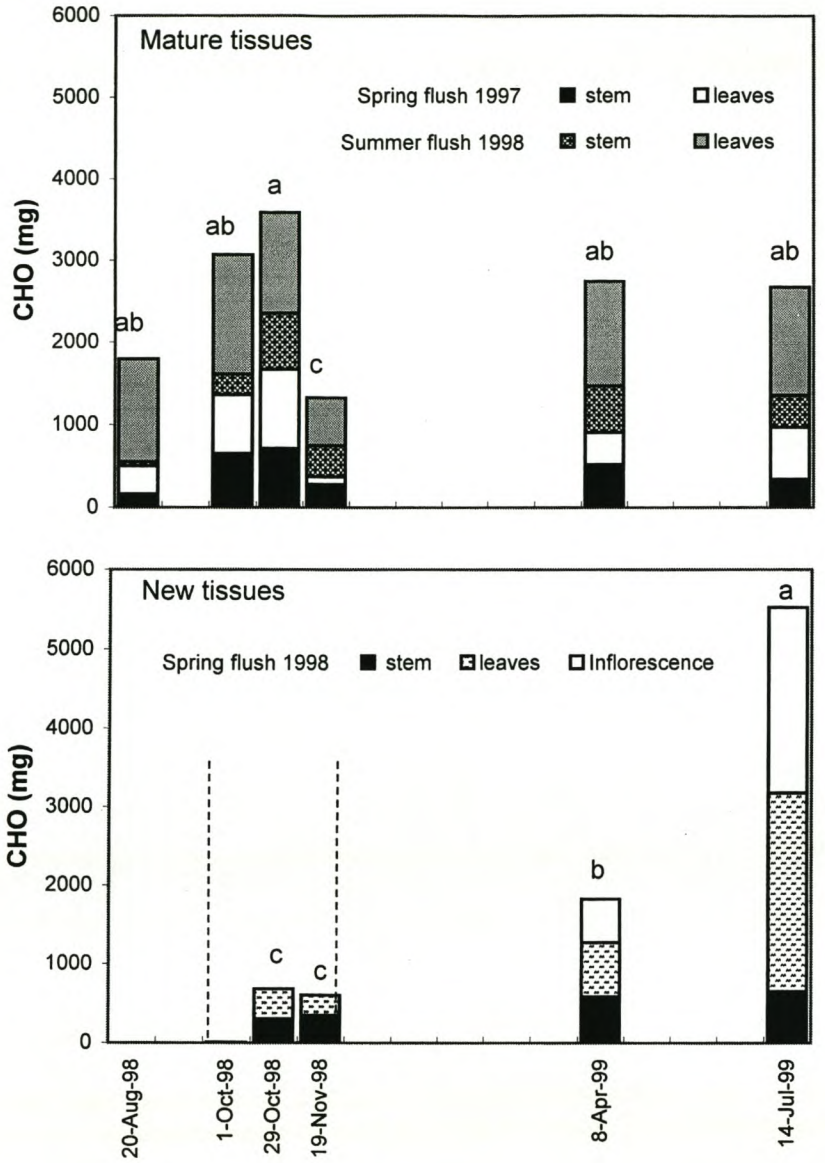



Fig. 2. Total carbohydrate (CHO) content (starch and reducing sugars) in mature and new tissues during development of new tissues.
Spring budbreak occurred on 1 October 1998, and elongation growth of the spring flush occurred until 19 November 1998 (indicated by dotted lines).
Letters above columns indicate significant differences according to LSD values (5%).

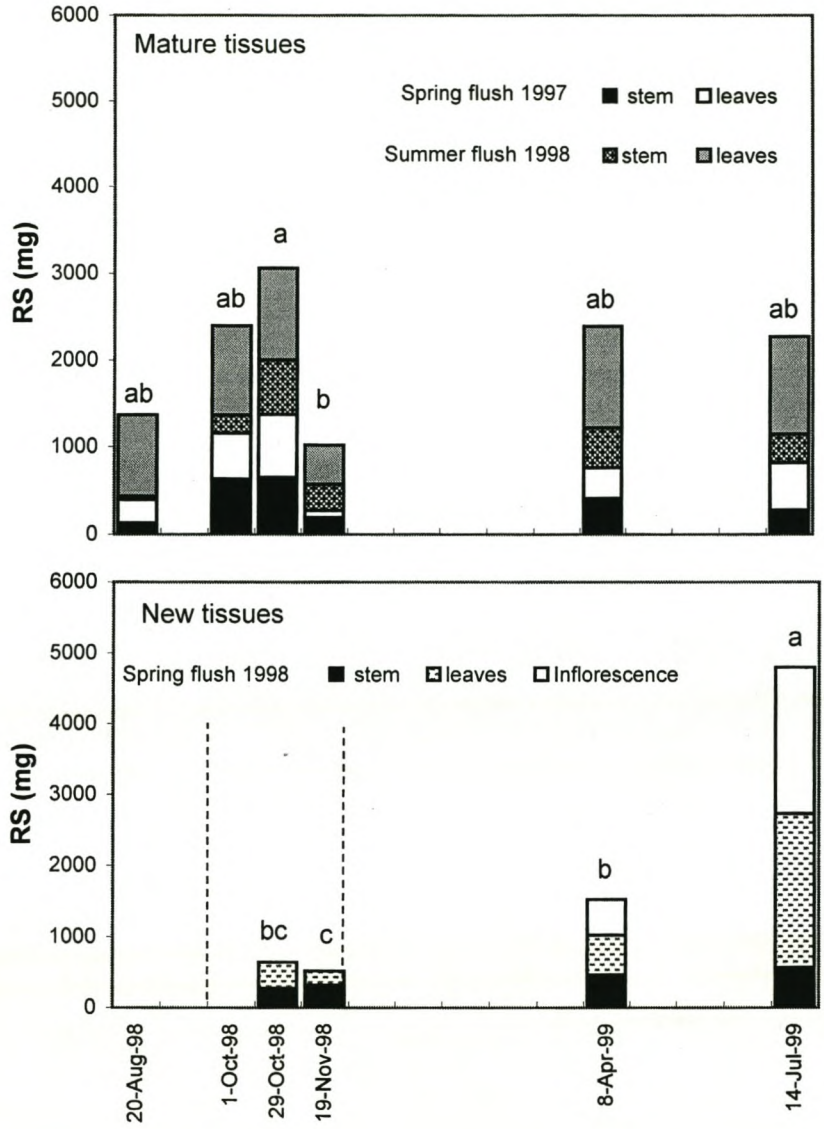



Fig. 3. Total reducing sugars (RS) content in mature and new tissues during development of new tissues.
Spring budbreak occurred on 1 October 1998, and elongation growth of the spring flush occurred until 19 November 1998 (indicated by dotted lines).
Letters above columns indicate significant differences according to LSD values (5%).

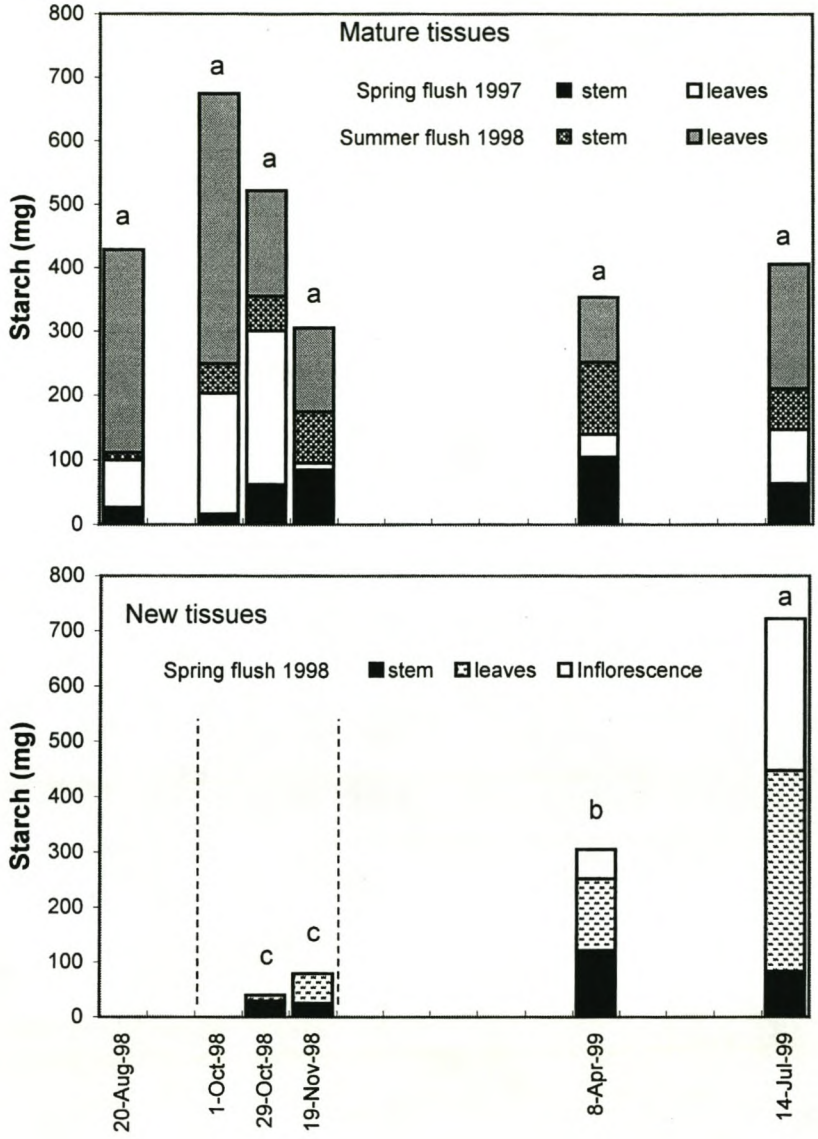



Fig. 4. Total starch content in mature and new tissues during development of new tissues. Spring budbreak occurred on 1 October 1998, and elongation growth of the spring flush occurred until 19 November 1998 (indicated by dotted lines).

Letters above columns indicate significant differences according to LSD values (5%).

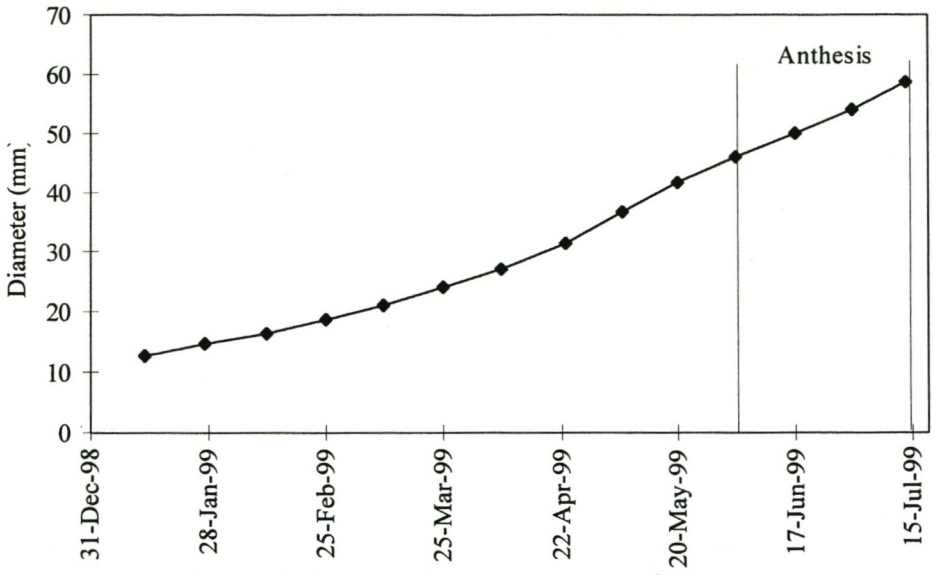
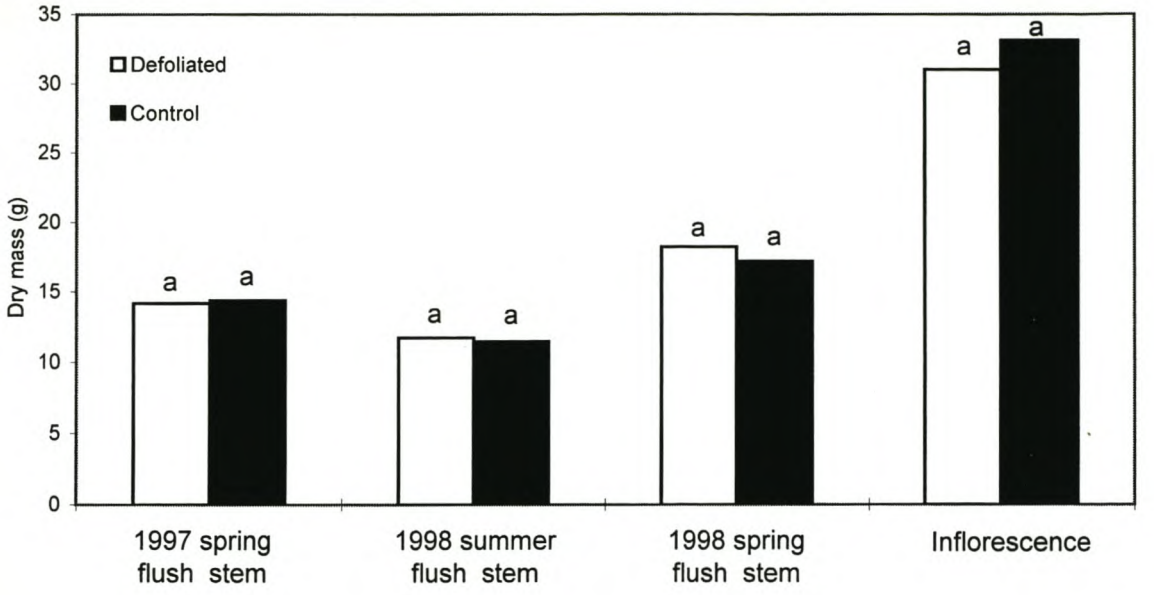



Fig. 5. Basal diameter (mm) of 'Lady Di' inflorescences during development. (Mean of 50 shoots, including defoliated and non-defoliated shoots, which developed at the same rate and reached anthesis within the 6 week period marked.)

a) Dry mass of tissues (g)




b) Total carbohydrate (CHO) content (starch and reducing sugars (TRS)) per tissue (mg)

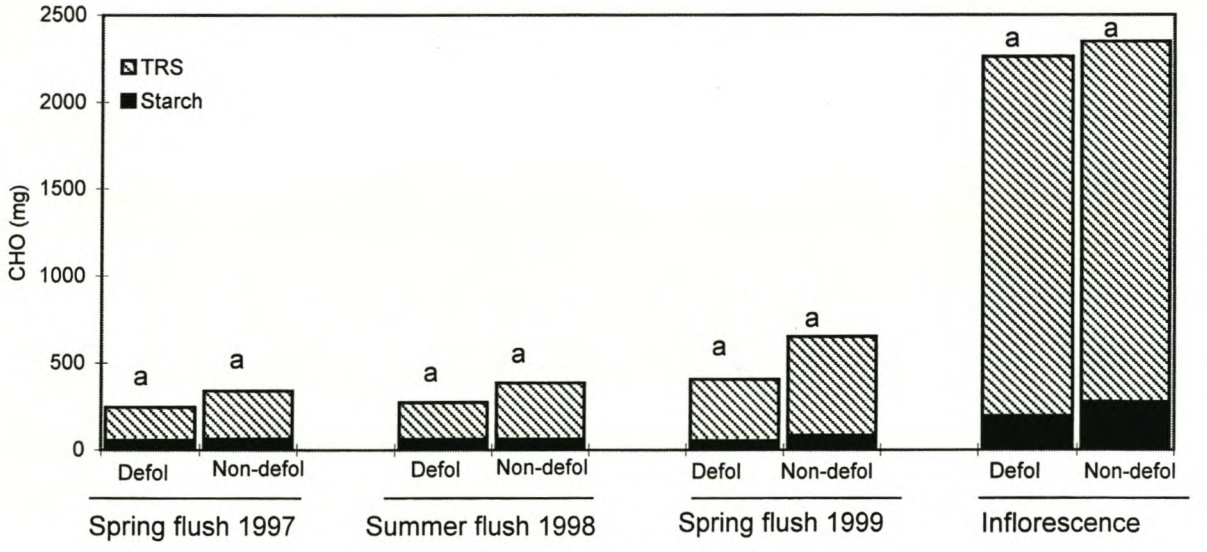



Fig. 6. Comparative analysis of defoliated (defol) and non-defoliated (non-defol) shoots harvested at the commercial picking stage (14 July 1999).

Letters above columns indicate significant differences between adjacent columns according to LSD values (5%).

## 6. PAPER V - Manipulation of flowering time by pruning of *Protea* cv. Sylvia.

## Manipulation of flowering time by pruning of *Protea* cv. Sylvia (*P. eximia* × *P. susannae*).

The commercial production of *Proteaceae* products is increasing world-wide. To optimise this expansion of the industry it is important both to ensure an even, year round distribution of product availability as well as to target peaks of harvest for times of high market demand. This can be done by manipulating and extending the natural flowering times.

The flowering windows of Southern African proteas can be classified broadly into three categories. The first category contains the summer to autumn flowering types. Flowers develop terminally on the spring shoot growth flush. Development continues during spring and summer and flowers reach anthesis during the period January to May (Paper I). Examples are *P. neriifolia* selections and hybrids (e.g. 'Satin Pink', 'Kouga', and 'Carnival'), 'Pink Ice' (*P. compacta* × *P. susannae)*, and *Protea* cv. Ivy *( P. lacticolor* hybrid)

Proteas in the second category also initiate flowers on the spring growth flush, but visual development does not start until January (Paper I). Inflorescence development continues during late summer and winter and anthesis is reached from late winter to early summer, depending on the time required for inflorescence development. *P. magnifica* selections and hybrids (e.g. 'Susara', 'Lady Di' and 'Sheila' ) belong in this category.

The third category encompasses those proteas which can initiate flowers at any time of year. Inflorescence development directly follows initiation and the time of anthesis depends on the time of inflorescence initiation. *P. eximia* selections and hybrids (e.g. 'Sylvia' and 'Cardinal') have the ability to produce flowers throughout the year. These

types have, in effect, an open window for inflorescence initiation whereas the former two categories have a limited window for inflorescence initiation.

The open window characteristic offers the opportunity to manipulate plants to flower at a specific time of year and to direct the harvest to meet periods of high demand. Pruning of plants at different times of the year may be an effective method to manipulate time of flowering.

## Materials and Methods

Plants of *Protea* cv. Sylvia (*P. eximia* × *P. susannae*) were grown in a commercial plantation near Stellenbosch, Western Cape, South Africa (lat.33°15'S; long.19°07'E). The area receives an annual rainfall of 600 - 700 mm, with most of the rain falling in winter between April and August. The plants were grown at a spacing of 1 m within the row and 4 m between rows and were not fertilised or irrigated. Pest and disease management practices necessary to produce export quality flowers were applied.

**PRUNING EXPERIMENTS.** Pruning entailed heading both flowering and non- flowering shoots, leaving a 15 cm portion of the stem to serve as a bearer for the subsequent growth. Spindly shoots were removed by thinning cuts. Pruning released lateral buds from apical dominance, resulting in growth of vegetative shoots in flushes, elongation of which stopped when inflorescence initiation occurred terminally. For 24 months following pruning flowering shoots were harvested when the flowers were commercially mature, leaving a 15 cm portion of stem to serve as a bearer, as is done in commercial practice. The flowering shoots were brought to our laboratory where stem length was measured and date of harvest was recorded.

**EXPERIMENT 1.** Plants were pruned on 7 dates at four-weekly intervals from 23 September 1993 to 10 March 1994. The experiment was laid out as a randomised complete block with seven single plant replicates. A total of 49 plants were used.

**EXPERIMENT 2.** A second experiment was executed starting in April 1996, where plants were pruned at four-weekly intervals from 26 April 1996 to 13 September 1996. Seven single plant replicates were used per treatment, laid out in a randomised complete block design. In total 42 plants were used and pruning was performed in the same manner as for the first experiment.

**RATE OF INFLORESCENCE DEVELOPMENT.** The basal diameter of the inflorescence was measured when subtended by different growth flushes. Ten shoots were tagged with inflorescences on the spring flush, first summer flush, second summer flush and autumn flush, when the inflorescence was approximately 10 mm in diameter. At two-weekly intervals the basal diameter of the inflorescence was measured until anthesis. Measurements were started in spring 1997 and continued until mid-summer 1998.

**STATISTICAL ANALYSIS.** Analysis of variance was performed using the SAS program (SAS Institute Inc., 1990). Logit transformation of the data was performed on values expressed as percentages. In Experiment 1, one plant died during the experiment and, due to these missing data, LSD values were calculated using harmonic means.

## Results

Shoot elongation of 'Sylvia' occurs by successive growth flushes. The most vigorous shoot growth flush on 'Sylvia' plants occurs in spring after a period of winter dormancy. In summer one or two growth flushes occur, termed the first and second summer flushes, which are followed by a short, less vigorous autumn flush (Malan and le Roux, 1995).

**RATE OF INFLORESCENCE DEVELOPMENT.** Visual development of the inflorescence subtended by the spring growth flush started on 22 October 1997 and the inflorescence reached anthesis on 28 January 1998 (Fig. 1). The inflorescence subtended by the first summer flush developed from 14 January to 26 April 1998; the second summer flush from 14 April to 25 August 1998; and the autumn flush from 17 June to 13 November 1998. A similar rate of development (over a period of 3 months) was seen for flowers borne on the spring, and first and second summer flushes, reaching anthesis in January, May and August, respectively. Flowers borne on the autumn flush, which developed through winter, had a slower rate of development and reached anthesis in mid-November.

**PRUNING EXPERIMENTS.** The time of pruning influenced the pattern of distribution of flowers reaching harvest maturity in both experiments (Fig. 2a and b). When plants were pruned in September or October 1993 the main portion of the harvest was picked from November 1994 to January 1995 (Fig. 2a). The main peak shifted to January and February 1995 when plants were pruned in November 1993, December 1993 or January 1994. The harvest was spread more or less evenly over 10-11 months when pruning was delayed until February or March 1994. When plants were pruned in April 1996 a harvest peak was seen during October to December 1997 (Fig. 2b). The peak shifted to include January 1998 when plants were pruned later, in May to August 1997, and the shift in harvest continued with later pruning until the main peak of harvest occurred only in December 1997 and January 1998 following pruning in September 1996.

The time taken from pruning to flower harvest differed with time of pruning. The shortest time taken from pruning to harvest was 10 months, following pruning in March 1994 (Fig. 2). Flowers were initiated on the spring flush of the same year, and were harvested on short stems in January 1995. In contrast the harvest peak occurred 20

months following pruning in April 1996, although the first few flowers were harvested 5 months earlier (Fig. 2b).

Despite the differences in time and distribution of harvest there was no difference in the total number of flowers picked from plants pruned in September 1993 to March 1994 (Table 1). An average of 33±4 flowers was harvested per plant grown under these conditions. In the second pruning experiment total yield was affected by time of pruning (Table 2). Yield differed significantly when plants were pruned in different months during winter. The lowest yield was obtained from plants pruned in September 1996.

The yield can be considered with regard to marketing criteria, specifically stem length and time of harvest. Stems of a minimum of 40 cm long are considered to be of export quality, and the demand for proteas is highest, as are prices, from September to February, during the European winter. The marketable portion of the harvest is, therefore, the number of stems which are longer than 40 cm and harvested between September and February (Tables 1 and 2). Although approximately 80% of the stems harvested from plants pruned in September and October 1993 had long stems, and more than 90% of the total yield was harvested in the desired time period, only 77 and 74% respectively fulfilled both requirements (Table 1). Delaying pruning until November or December reduced the marketable yield to 60% despite a high percentage being harvested in the relevant time period. Only 29% of the total yield harvested from plants pruned in January 1994 was marketable, and this was mostly due to short stems since 81% were harvested from September to February. The low percentage of marketable stems from plants pruned in February and March 1994 was due to both short stems and unsuitable flowering time.

Pruning on all dates in 1996, except April, resulted in a marketable yield of more than 70% of the total yield (Table 2). The highest marketable yield of 82% was attained when plants were pruned in June 1996. Only 61% of the yield from plants pruned in April 1996 had ideal marketing characteristics. The low percentage was due mainly to unsuitable flowering time.

## Discussion

The time of harvest of 'Sylvia' flowers is dependant on the flush on which inflorescence initiation occurs. The rate of inflorescence development during spring and summer occurred at a similar rate. Inflorescences subtended by the autumn flush developed at a slower rate, probably due to cooler weather in winter. Inflorescences initiated on the spring flush reach anthesis in January and February; on the first summer flush predominantly in April and May; on the second summer flush in July and August; and on the autumn flush in November and December. The inflorescences harvested in the ideal marketing period from September to February, therefore, were borne on the autumn and spring flushes.

Despite the fact that 'Sylvia' has an 'open' flowering window, and can initiate inflorescences on any flush, they are initiated more readily on the spring growth flush, if subtended by one or more previous growth flush. The peak of harvest occurring in January 1995 following pruning in September 1993 to March 1994 is evidence of enhanced inflorescence initiation on the spring flush of 1994. Pruning on these dates resulted in production of at least one flush before winter. The presence of mature leaves which have overwintered is essential for inflorescence initiation in *Protea* cvs. Carnival and Lady Di which only initiate on the spring flush, when subtended by one or more previous flushes (Paper I). The predominant initiation of flowers on the spring flush of an

overwintering shoot in 'Sylvia' is perhaps the expression of a facultative response to inductive conditions for which 'Carnival' and 'Lady Di' have an obligate requirement. The strong 'signal' for initiation on the spring flush, together with strong synchrony of shoot growth in spring, resulted in a sharp peak of harvest of flowers which were initiated on the spring flush.

Initiation on the autumn flush, although less prevalent than on the spring flush, occurred more frequently than initiation on either summer flush. When plants were pruned in April 1996 a shoot flush was not produced before winter (data not presented) and no shoots initiated flowers on the spring growth flush of the same year. This is in agreement with Paper I which reports that shoots originating from an axillary bud following pruning do not flower. Shoot growth continued and the majority of flowers were initiated on the autumn flush of the following year. Flowers were harvested from October to December 1997. A similar pattern was seen when plants were pruned in May to August 1996. Only the strongest shoots initiated flowers on summer flushes, resulting in a long spread of harvest over March to August.

According to De Swardt (1989) and Sedgley and Fuss (1992), working on *Protea* and *Banksia* respectively, a shoot must attain a critical minimum stem length or diameter before inflorescence initiation can occur. Following pruning, 'Sylvia' shoots elongate by successive growth flushes until they have developed the necessary characteristics, at which time inflorescence initiation will occur. If this pattern was rigidly adhered to by the plant all shoots would be a similar length and the harvest would be uniformly distributed over time following pruning at different times. The strong 'signal' for initiation on the spring flush decreased the time from pruning to harvest and resulted in inflorescence initiation occurring on short shoots when plants were pruned in January to

March to permit growth of a flush before winter. This was the reason for many flowers being unmarketable despite being harvested during the peak marketing period.

The shortest time from pruning to flowering, seen following pruning in March 1994, was due to flush growth before winter (data not presented), followed by initiation on the spring of the same year. Plants pruned in April 1996 did not initiate on the spring flush and vegetative growth continued, hence a longer time from pruning to flowering.

In general, pruning in spring and early summer produced a high percentage of the total yield as long stemmed flowers which reached picking maturity during the period of high market demand from September and February. Plants pruned in mid-summer also produced most of the yield during the high demand period, but a very low percentage of these flowers had stems longer than 40 cm. Pruning in late summer resulted in short-stemmed flowers being produced in January and February and long stemmed flowers later in the year from September to November. Plants pruned in winter produced a high percentage of marketable stems, but the yield appeared to fluctuate in response to climatic conditions. Inclement weather conditions following pruning could result in poor budbreak, leading to a lower yield. The high yield from plants pruned in June indicates that pruning in winter *per se* does not result in low yields. The reason for the low yield following pruning in September 1996 compared with September 1993 is unclear.

Since the vegetative and reproductive cycles necessary to produce a long flowering stem span more than one year (12 - 17 months) annual cropping is not possible. Under South African conditions the best yield will be obtained when 'Sylvia' plants are pruned in June or July. Shoot growth will start in spring of the same year and continue until autumn of the following year when inflorescence initiation will occur. Flowers will have long stems and be ready for harvest during the peak marketing time of October to

December. For commercial production a system similar to that described for 'Carnival' (Gerber et al., 1995; Hettasch et al., 1997) with two blocks out of phase with one another is recommended.

## Literature Cited

De Swardt, D.C., 1989. Aspects of the vegetative and reproductive development of selected *Proteaceae* cultivars. M.Sc. Agric. Thesis. Univ. of Stellenbosch.

Gerber, A.I., E.J. Greenfield, K.I. Theron, and G. Jacobs. 1995. Pruning of *Protea* cv Carnival to optimise economic biomass production. Acta Hort. 387:99-106

Hettasch, H.B., A.I. Gerber, K.I. Theron, and G. Jacobs. 1997. Pruning *Protea* cv. Carnival for biennial crops of improved yield and quality. Acta Hort. 453:127-133.

Malan, D.G., and R.D., Le Roux. 1995. Preliminary investigation into the effect of time of pruning on shoot growth and flowering time of *Protea.* Acta Hort. 387:91-97

SAS Institute Inc., 1990. SAS/STAT User's guide, Version 6. 4th ed., Vol. 1 and 2. Cary, NC.

Sedgley, M. and A. Fuss. 1992. Correct pruning lifts *Banksia* yields. Aust. Hort. 90 (7):50-53

Table 1. Number of stems harvested from 'Sylvia' plants pruned on different dates from September 1993 to March 1994. The total yield is further divided according to marketing criteria, viz, stem length and time of harvest. Means (n = 7) within columns followed by the same letter are not significantly different according to LSD values (5%).

| | Number of stems, or percentage, harvested per plant | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Longer than 40 cm | | September to February | | September to February >40 cm | |
| Pruning date | Total | Number | %[z] | Number | %[z] | Number | %[z] |
| September 23, 1993 | 30 [a] | 24 [ab] | 80 | 28 [ab] | 93 | 23 [a] | 77 |
| October 21, 1993 | 38 [a] | 30 [a] | 79 | 36 [a] | 95 | 28 [a] | 74 |
| November 18, 1993 | 35 [a] | 24 [ab] | 69 | 33 [ab] | 94 | 22 [a] | 63 |
| December 16, 1993 | 35 [a] | 25 [ab] | 71 | 31 [ab] | 89 | 21 [a] | 60 |
| January 13, 1994 | 31 [a] | 15 [c] | 48 | 25 [bc] | 81 | 9 [b] | 29 |
| February 10, 1994 | 26 [a] | 17 [bc] | 65 | 16 [c] | 62 | 8 [b] | 31 |
| March 10, 1994 | 34 [a] | 16 [bc] | 47 | 17 [c] | 50 | 8 [b] | 24 |
| Average | 33 ± 4 | | | | | | |

[z] expressed as a percentage of total yield

Table 2. Number of stems harvested from 'Sylvia' plants pruned on different dates from April 1996 to September 1996. The total yield is further divided according to marketing criteria, viz, stem length and time of harvest. Means (n = 7) within columns followed by the same letter are not significantly different according to LSD values (5%).

| | Number of stems, or percentage, harvested per plant | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Longer than 40cm | | September to February | | September to February >40cm | |
| Pruning date | Total | Number | %[z] | Number | %[z] | Number | %[z] |
| April 26, 1996 | 30 [ab] | 25 [ab] | 84 | 23 [ab] | 77 | 18 [ab] | 61 |
| May 24, 1996 | 23 [bc] | 20 [b] | 84 | 20 [b] | 87 | 17 [b] | 73 |
| June 21, 1996 | 33 [a] | 30 [a] | 92 | 29 [a] | 89 | 27 [a] | 82 |
| July 19, 1996 | 21 [bc] | 18 [b] | 90 | 18 [b] | 85 | 15 [b] | 75 |
| August 16, 1996 | 27 [bc] | 24 [ab] | 88 | 24 [b] | 88 | 21 [ab] | 77 |
| September 13, 1996 | 19 [c] | 19 [b] | 98 | 16 [b] | 79 | 16 [b] | 77 |
| Average | 26 ± 3 | | | | | | |

[z] expressed as a percentage of total yield

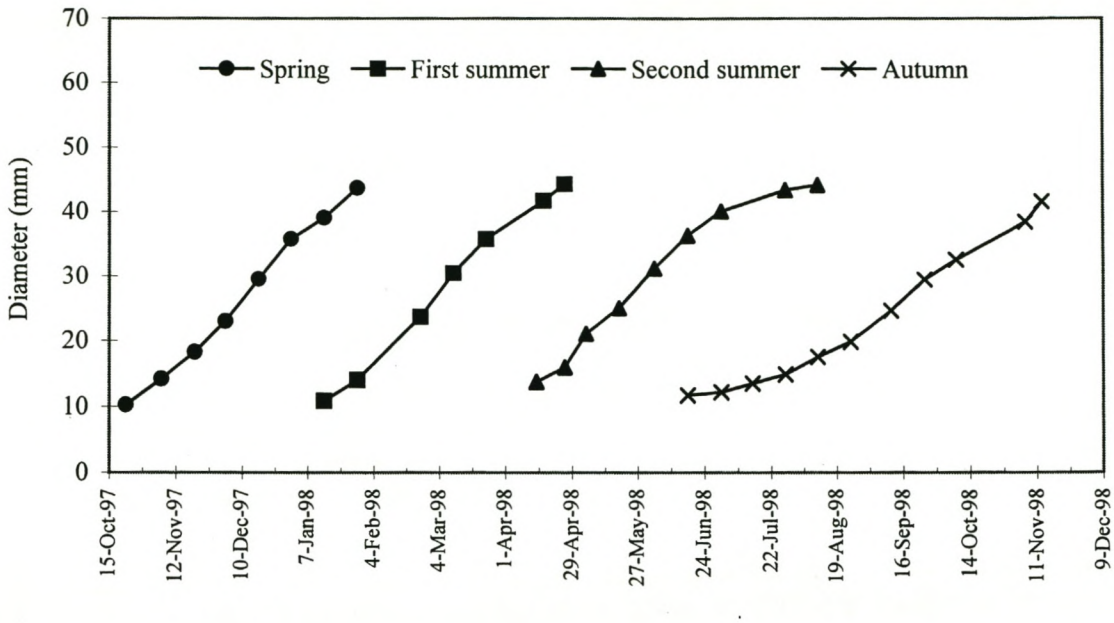



Fig. 1. Basal diameter of 'Sylvia' inflorescences when subtended by different growth flushes. SE values (n = 10) for inflorescence diameter on the spring flush = 1.12, on the first summer flush = 0.67, on the second summer flush = 0.92, and on the autumn flush = 0.87.

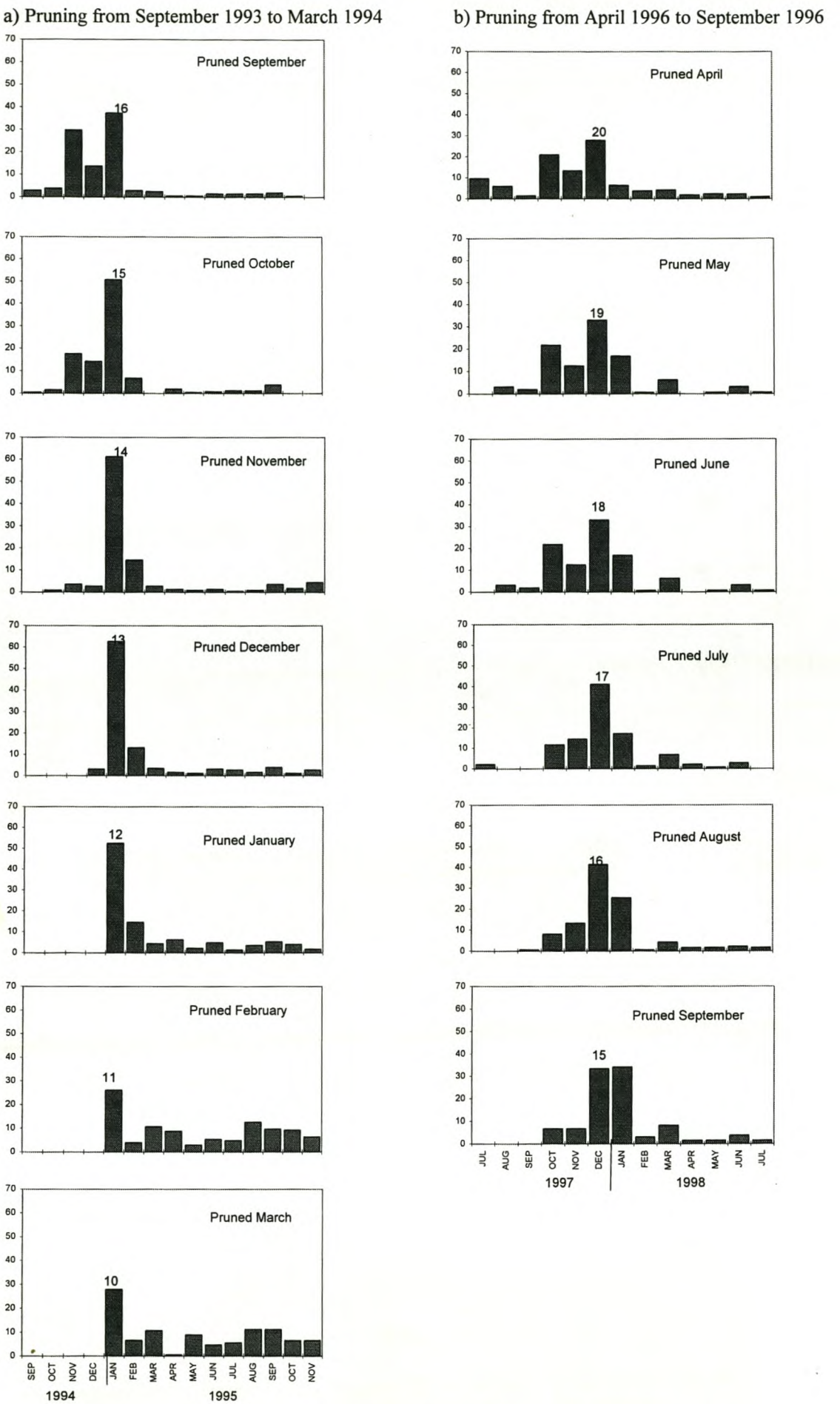



Fig. 2. Distribution of yield from Sylvia plants pruned on different dates, expressed as a percentage of total yield (month of pruning indicated on each graph ). Numbers above columns indicate time from pruning to harvest in months.

# 7. GENERAL CONCLUSION.

## General Conclusion.

There is little information available regarding flower initiation and development in *Protea*. Information on these aspects in more sophisticated floriculture crops, e.g. Chrysanthemum and Carnation, has been essential for the development of strategies to provide a continuous supply of high quality product. In *Proteaceae*, the factors controlling flower initiation have been described in *Leucospermum* and *Serruria*. This knowledge of flowering in *Leucospermum* has lead to the development of disbudding techniques which enable manipulation of flowering time. The wide variety of flowering times in *Protea* suggests that no single mechanism will explain flowering in all species.

South African *Protea* species can be classified broadly into three categories according to flowering times. The first category contains the summer to autumn flowering types, and includes *P.* cv. Carnival (*P. compacta* × *P. neriifolia*). Flowers develop terminally on the spring shoot growth flush. Development continues during spring and summer, and flowers reach anthesis from January to May.

Proteas in the second category also initiate flowers on the spring flush, but development does not start until January. Inflorescence development occurs during summer and winter and anthesis is reached from late winter to early summer, depending on the rate of flower development. *P.* cv. Lady Di (*P. magnifica* × *P. compacta*) belongs in this category.

The third category, to which *P.* cv. Sylvia (*P. eximia* × *P. susannae*) belongs, includes those proteas which can initiate inflorescences at any time of year. Inflorescence development directly follows initiation and the time of anthesis is dependant on the time of initiation. 'Sylvia' has, in effect, an open window for

inflorescence initiation, whereas 'Carnival' and 'Lady Di' have a limited window for inflorescence initiation.

In all three cultivars flower initiation on the spring growth flush occurs at the time of spring budbreak. The spring flush subtending the inflorescence is preformed and the full complement of leaf primordia for the spring flush is present in the apical bud before spring budbreak. Growth of the spring flush is due to elongation of preformed internodes together with differentiation and development of leaves. Meristematic activity does not cease once the spring flush appendages are formed. Production of involucral bract primordia occurs during elongation of the spring flush. When elongation is complete the developing inflorescence contains all the involucral bracts, and initiation of floral bracts and florets begins. The rate of involucral bract formation relative to extension growth of the spring flush is initially rapid in 'Carnival' and 'Sylvia', and decreases toward the end of elongation of the spring flush. The rate of involucral bract formation in 'Lady Di' occurs at a constant rate relative to spring flush elongation.

Despite having different windows for inflorescence initiation all three cultivars show the same synchrony of inflorescence initiation relative to spring flush growth. Inflorescence development occurs at the same rate in 'Carnival' and 'Sylvia' when inflorescences are borne on the spring flush, and inflorescences reach anthesis at approximately the same time. The later time of anthesis in 'Lady Di' is due to later spring budbreak (and, therefore, start of inflorescence initiation), a longer period required for flush elongation and a slower rate of inflorescence development.

The preformed nature of the spring flush is also apparent for other flushes of 'Carnival'. During elongation of a flush the meristem produces the appendages

necessary for the subsequent flush. This is only the case when the is flush formed on the terminal position. The first flush to form following pruning arises from an axillary bud on the bearer. Meristematic activity proceeds following release from apical dominance by pruning and leaf primordia are produced. Buds swell and enlarge, but elongation of the flush only starts once the full complement of appendages has formed.

Flower initiation on the spring flush in 'Carnival' and 'Lady Di' requires the presence of overwintering leaves. Induction has occurred in 'Carnival' and the shoot is committed to flowering six to seven weeks before spring budbreak. Shoot defoliation applied to 'Carnival' earlier than six to seven weeks before spring budbreak prevents flowering, indicating that induction is incomplete at this time. Inflorescence initiation only starts at spring budbreak, so the induced state is retained for a period. How long the induced state is maintained is unclear from these studies, but formation of inflorescences on the first summer flush and on lateral buds arising below the inflorescence is evidence that it is maintained past spring budbreak when inflorescence initiation normally occurs.

Although 'Sylvia' can initiate inflorescences on any flush they are more readily formed on the spring flush, provided it is subtended by one or more overwintering flushes. This is probably an expression of a facultative response to inductive conditions for which 'Carnival' and 'Lady Di' have an obligate requirement.

Mature overwintering leaves are essential in 'Lady Di' to achieve the induced state, but are also crucial to the early stages of inflorescence initiation. Production of inflorescences in 'Lady Di' is prevented by defoliation applied before elongation of the spring flush is complete. Current photosynthates from the overwintering leaves, therefore, support early growth and development of both the spring flush and

inflorescence. Further growth is supported by the spring leaves once they are mature. Reserve carbohydrates in the leaves and stem are insufficient to account for the increase in dry mass during the major portion of growth of the spring flush and inflorescence. Growth must, therefore, be supported by current photosynthates from the spring flush leaves. Inflorescence development continues normally following removal of the lower two thirds of the leaves on the spring flush, and the remaining leaves presumably increase their rate of photosynthesis to overcome the decrease in leaf area due to defoliation. Flowering time of 'Lady Di' was not delayed by defoliation.

A hierarchy of sink priorities is revealed by defoliation during inflorescence development in 'Carnival'. Involucral bract formation, together with elongation of the spring flush and leaf growth, occur concurrently and are dependant on photosynthates from the overwintering leaves. Under source-limiting conditions following defoliation, meristem activity has priority over tissue growth and the number of involucral bracts formed is not compromised. By the same reasoning, leaf growth has priority over stem elongation. As with 'Lady Di', dry mass accumulation in the spring flush and inflorescence in 'Carnival' is supported by current photosynthates from the spring flush leaves. However, unlike 'Lady Di', the rate of inflorescence development in 'Carnival' is decreased by early defoliation and inflorescences reach anthesis later than on non-defoliated shoots. Early defoliation in 'Lady Di' results in shoots remaining vegetative or inflorescence abortion.

The rate of flower development in 'Sylvia' is slower when it occurs through winter, probably due to lower winter temperatures. Inflorescences borne on the spring, first summer and second summer flushes develop at the same rate. Inflorescences initiated

on the spring flush reach anthesis in January and February; on the first summer flush predominantly in April and May; on the second summer flush in July and August; and on the autumn flush in November and December.

Pruning can be used to manipulate flowering in 'Sylvia' to fall in the optimum marketing period for export to Europe (September to February). Under South African conditions pruning in June or July results in a yield of long stemmed inflorescences reaching anthesis from October to December, and is, therefore, to be recommended. Since the vegetative and reproductive cycles needed to produce flowers borne on long stems span more than a year, annual cropping is not possible and implementation of a biennial cropping system is recommended.

The ultimate solution to achieve production in the optimum marketing period is breeding of cultivars which naturally produce flowers between September and February. Incorporation of the open window characteristic of *P. eximia* by breeding is another alternative which would enable manipulation of flowering time. However, breeding programmes have yet to make progress of this nature, and production of flowers in the optimum marketing period will depend on our understanding of the factors which control inflorescence initiation and development, and techniques devised to manipulate these processes.

For those cultivars which have a narrow window for inflorescence initiation and produce inflorescences in March and April, just outside the optimum marketing period, the following approach should be considered. To advance the start of spring budbreak and hasten the onset of flower initiation by one or two months daylength extension could be applied, or terminal buds could be treated with exogenous cytokinins.

Treatments to delay time of anthesis are necessary for those cultivars with a narrow window for inflorescence initiation and which produce inflorescences which develop through winter. Anthesis is normally reached in July and August, before the optimum marketing period. To delay the start of spring budbreak, and concomitant inflorescence initiation, would be difficult, however, evaporative cooling by overhead irrigation may have potential. Attempts to slow inflorescence development by defoliation to limit source size have not been successful. Alternative treatments to limit source size, such as foliar sprays with chemical photosynthesis inhibitors, should be considered.

Further elucidation of factors, environmental and intra-plant, that control inflorescence initiation in overwintering shoots is essential. This would require experiments under controlled environmental conditions. There is also a need to know the period for which the induced state is retained in those cultivars with a narrow window for inflorescence initiation. Once the shoot is in the induced state for flowering the terminal bud should be removed by pinching at different times. Whether the lateral shoots formed following pinching produce an inflorescence or not will reveal retention or loss of the induced state.

In order to extrapolate these and future findings to all commercially cultivated proteas the basic biology of inflorescence initiation and development needs to be studied for different cultivars.