FLOWER INITIATION AND DEVELOPMENT

OF *PROTEA* CV. CARNIVAL

BY

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PROMOTER

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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.

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Signature Date

Flower initiation and development of *Protea* cv. Carnival

SUMMARY

Advancement of the flowering time of *Protea* cv. Carnival by approximately three months, without compromising the product quality, was achieved by the application of 6-benzyladenine-containing plant growth regulators to three-flush shoots in autumn. This earlier flowering time coincides favourably with the prime European marketing period (November-January). The percentage three-flush shoots initiating an inflorescence following the brush application of the 6-benzyladenine (BA)-containing regulators, ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) and Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) on dormant terminal buds, increased with later application dates and flowering percentages as high as 90% was achieved. No inflorescences were initiated on flushes induced by Promalin® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 1.8% $^{w}/_{w}$).

Phenological phase progression of green point, flush expansion and inflorescence development of 'Carnival' shoots as induced by BA was calculated to have base temperatures of 8°C, 6°C and 1°C respectively.

The days required from application of the BA-containing growth regulator until green point stage increased progressively over the six consecutive treatment dates in autumn (14 March - 22 May 2003). In contrast, the days required to complete inflorescence development decreased with each successive treatment date. The days required between the respective stages were mostly negatively correlated with temperature, except for the phase 'green point to flush expansion', where the relationship was unclear. For three-flush shoots of eight-year old plants, between 13-57, 39-65 and 121-177 days were required to reach green point, to

achieve full flush expansion following green point and to complete inflorescence after flush expansion respectively.

BA application enhanced budbreak in most dormant shoots, irrespective of plant age, BA concentration, decreasing temperature over time or shoot characteristics. However, two-flush shoots treated in late May had low budbreak and hence low flowering percentages.

Shoots varied considerably in their responsiveness to BA treatments. BA application ($500 mg \cdot L^{-1}$) as MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) to terminal buds alone of mature three-flush shoots from less vigorous growing plants resulted in the highest flowering percentages. Applications were most effective when applied to the terminal bud in the dormant state or up to the 'green point' stage. Shoot characteristics such as flush length, leaf area, shoot dry mass, number and proximity of the leaves to the terminal bud were all positively correlated with the propensity of shoots to initiate inflorescence under BA induction. Terminal flush intercalation shoot diameter (>7mm) was identified as the most important variable influencing the likeliness of flowering and can effectively serve as a non-destructive estimation of a shoot's propensity to flower.

The presence of developing inflorescences or possible floral inhibiting factors derived from the previous flowering season is suggested to be inhibitory to inflorescence initiation following BA application. Synchronisation of shoot growth by pruning plants in late winter appears to be an essential step to ensure high percentages inflorescence initiation with BA treatment the following autumn**.** The use of BA as a management tool to control flowering times in *Protea* for better market opportunities is shown to hold considerable commercial potential.

Bloeiwyse-inisiasie en-ontwikkeling van *Protea* cv. Carnival

OPSOMMING

Protea cv. Carnival se blomtyd is met ongeveer drie maande vervroeg sonder om produkkwaliteit prys te gee. Hierdie vervroegde blomtyd wat gunstig saam val met die optimale Europese bemarkingstyd van November-Januarie is bewerkstelling deur die herfs-toediening van 6-bensieladenien-bevattende plantgroei-reguleerders op lote bestaande uit drie groeistuwings. Die persentasie lote met drie groeistuwings wat 'n bloeiwyse geïniseer het na 'n kwas-aanwending met die 6-bensieladenien (BA)-bevattende groeireguleerders, ABG-3062 (aktiewe bestandeel: BA 2% $^{w}/_{w}$) en Accel® (aktiewe bestandele: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$), het toegeneem met latere behandelingsdatums en blompersentasies so hoog as 90% is behaal. Geen bloeiwyses is geïnisieer op groeistuwings wat deur Promalin® (aktiewe bestandeel: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 1.8% $^{w}/_{w}$) teweeggebring is nie.

Basis temperature van 8°C, 6°C en 1°C respektiewelik is bereken vir fenologiese fase-vordering vanaf groeireguleerder toediening tot by groenpunt, groeistuwing-voltooing en bloeiwyse-ontwikkeling van 'Carnival' lote soos geïnduseer deur BA. Die dae wat benodig was vanaf toediening van die BA-toediening totdat groenpunt stadium bereik is, het progressief toegeneem oor die ses opeenvolgende herfsbehandelingsdatums (14 Maart-22 Mei 2003). In teenstelling met bostaande, het die vereiste aantal dae om bloeiwyse-ontwikkeling te voltooi afgeneem met elke opeenvolgende behandelingsdatum. Die aantal dae wat benodig was vir die onderskeie fases was meestal negatief gekorreleer met temperatuur, behalwe vir die fase 'groenpunt tot groeistuwing-voltooing', waar die verhouding onduidelik was. Vir lote van agt-jaar-oue plante met drie groeistuwings was tussen 13-57, 39-65 en 121-177 dae respektiewelik benodig om groenpunt te bereik,

volledige groeistuwingverlenging te bewerkstellig en om bloeiwyse-ontwikkeling wat volg na groeistuwing verlenging, te voltooi.

BA-toediening het knoprusbreking bevorder in die meeste dormante lote, ongeag plant ouderdom, BA konsentrasie, afname in temperatuur met tyd of loot eienskappe. Lote met twee groeistuwings wat laat in die herfs behandel is, het egter lae rusbreking en dus gevolglik ook lae blompersentasies getoon.

Lote varieer aansienlik in hul reaksie op BA behandeling. BA toediening ($500 mg \cdot L^{-1}$) as MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) op die terminale knop van afgeharde lote met drie groeistuwings en afkomstig van minder groeikragtige plante het tot die hoogste blompersentasies gelei. Die effektiwiteit van die behandeling was die hoogste met toedienings aan dormante terminale knoppe tot en met groenpuntstadium. Loot eienskappe soos groeistuwinglengte, blaaroppervlakte, loot droë massa, asook die aantal en nabyheid van die blare relatief tot die terminal knop was almal positief gekorreleerd met die vermoë van die loot om 'n blom te inisisieer in reaksie op BA induksie. Terminale groeiverstuwing interkalasie-lootdikte (>7mm) is geïdentifiseer as die belangrikste veranderlike wat die vermoë om te kan blom kan beïnvloed en kan gebruik word as 'n nie-destruktiewe voorspeller vir blom-inisiasie.

Die teenwoordigheid van ontwikkelende bloeiwyses of potensiële blom-inhiberende faktore aanwesig in die loot na die vorige blomperiode, word moontlik beskou om inhiberend te wees vir BA-geïnduseerde blom-inisiasie. Sinchronisering van lootgroei deur die snoei van plante in laat-winter blyk krities te wees om 'n hoë blompersentasie met BA behandeling te verseker in die daaropvolgende herfs. Die aanwending van BA as 'n bestuurstegniek om die blomtyd van *Protea* te posisioneer vir beter bemarkingsgeleenthede toon aansienlike kommersiële potensiaal.

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1. INTRODUCTION AND LITERATURE REVIEW.

Control of flowering time in woody perennials with specific reference to the genus *Protea.*

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Importance of flowering time in Protea.

The South African fynbos industry has grown from a localized small scale enterprise based on collection from natural stands, which primarily supported the dried flower market, to an international floricultural commerce serving a niche export market for exotic flowers. The industry faces competition from other southern hemisphere countries such as Australia, New Zealand, Chile and Zimbabwe. Northern hemisphere countries such as Israel, the U.S.A. (California and Hawaii), Portugal and Spain (Madeira and Canary Islands) and more recently Colombia and China are also aggressive competitors. Northern hemisphere producers have the advantage of being closer to European markets resulting in lower freight costs and have an alternate flowering season to South Africa.

The Kaiser Associates Floriculture study (2000) envisaged "enormous opportunity for the South African floriculture industry to become a significant player in the world market". However, to ensure sustainable growth in a current depressed global floriculture market the following key areas need to be addressed: freight availability and affordable freight rates; securing the gene bank for the provision of new cultivars; producer training and developing a focused marketing strategy where supply meets demand. Factors influencing flowering time, insect control for international shipping, root disease, nutrition and plant management have also been identified as research priorities to meet the challenges facing the international *Protea* industry (Parvin *et al.*, 2003).

The genera *Protea*, *Leucospermum* and *Leucadendron* of the family Proteaceace yield the highest returns in South African Fynbos exports (SAPPEX, 2004). The commercial value of *Protea* lies in their attractive, large involucral bracts that vary in colour from pink to

red, yellow, white and occasionally green. These bracts may be pubescent or smooth and enclose many individual florets to form an inflorescence. Floral bracts are small and inconspicuous, but florets may be tipped with attractive black or white hair.

An increasing local awareness and pride in indigenous natural resources and a world-wide trend towards exotic flowers have created a specific market for proteas. The marketing strategy for cultivated proteas in southern Africa concentrates primarily on the European buyer. Peak demand periods where the highest prices are obtained are centred round the European autumn and winter (September to February), a time when competition from European grown flowers is low.

Proteas for the fresh cut flower market are increasingly being produced under cultivation as opposed to harvesting from natural stands. The latter resulted in over-utilization and extensive quality variations which impacted negatively both on the environment and market. Cultivation allows for the advantageous implementation of various management practices, for example, pruning, to manipulate growth and development of the crop in order to enhance yield and align flowering times with market demands (Hettasch *et al.*, 1997; Gerber *et al.*, 2001a). An understanding of the bearing habit of *Leucospermum* led to the development of disbudding techniques as a procedure for delaying natural harvest times and smoothing out periods of oversupply (Jacobs and Honeyborne, 1978). Day length requirements reported for *Serruria* (Malan and Brits, 1990), *Leucospermum* (Malan and Jacobs, 1990) and *Leucadendron* (Hettasch and Jacobs, 2006) offer additional management tools for manipulation of flowering times.

Approximately 10-12 species of the genus *Protea* and a growing number of interspecific hybrids are commercially grown as cut flowers (Coetzee and Littlejohn, 2001). The most commonly produced *Protea* cut flowers are from pure species selections or hybrids of *P.*

compacta, *P. cynaroides*, *P. eximia*, *P. grandiceps*, *P. magnifica*, *P. neriifolia* and *P. repens* (Gerber, 2000). When pure species selections of some seedling origin are grown in commercial plantations, natural genetic variation ensures that production occurs over an extended period of which a large percentage may fall within the optimum marketing window. *Protea magnifica* displays such a flowering window (Gerber, 2000). Selection criteria in cultivar development favouring visual characteristics and high production potential has unfortunately led to the use of selections and hybrids with a flowering time, that for southern Africa, falls almost entirely outside the premium price bracket for export (Gerber, 2000). The inability of the South African *Protea* industry to meet this period of high market demand or produce a continuous supply of high quality products, places it at a distinct disadvantage to other established consumer floral favourites such as roses and chrysanthemums.

The great variation that exists in *Protea* flowering times and apparent flowering prerequisites, suggests multi-factorial control of the *Protea* flowering cycle. Manipulation of flowering time to fall within the desired October to December window has been successful for two *P. eximia* hybrids ('Syliva' and 'Cardinal') by synchronizing shoot growth through pruning (Hettasch, 1999; Gerber *et al.,* 2001a). However, application of this technique has not been successful with the majority of the commercial hybrid cultivars that flower almost exclusively on the spring flush. Mechanisms controlling *Protea* floral initiation remain largely unclear and fragmented, hampering the development of management strategies for continuous supply of a high quality product.

***Vegetative growth and inflorescence initiation in* Protea.**

Phenology. Shoot extension growth in *Protea* is expressed in distinct flushes within loose defined growth periods during the year. After pruning, growth occurs successively

from a distal axillary bud with active growing shoots exhibiting strong apical dominance (Malan and Le Roux, 1995). Within the climatic conditions of the Western Cape, South Africa, the predominant growth periods are Spring (September to November), Summer (December to February) and Autumn (March to April). The number of flushes produced per growth period is largely determined by genetic identity. Cultivars such as 'Sylvia', 'Cardinal' and 'Carnival' may produce up to four flushes a year, whilst 'Pink Ice' can produce as many as seven flushes and *P. magnifica* no more than two flushes per year. In addition to genetic identity, Coetzee and Littlejohn (2001) speculated that intra-plant factors such age and complexity may also appear to play a determining role as young, vigorous plants produce more shoot growth flushes per year than older plants.

Comparative bud morphological studies of 'Syliva', 'Lady Di' and 'Carnival' by Gerber *et al.* (2001b) showed leaf primordia of the succeeding flush to be differentiated during the extension growth of the current flush. At completion of a growth flush, the terminal bud of each flush contains the preformed shoot with its full complement of leaves for the next flush. Involucral bract differentiation also occurs during elongation of the flush subtending the inflorescence. In contrast, floral bract and floret formation commences on completion of the elongation of the subtending flush (Gerber *et al*., 2001b). Since the phyllotaxis of involucral bracts differs from that of leaves, Gerber *et al.* (2001b) concluded that flower initiation occurred at onset of the elongation of the flush subtending the inflorescence.

Variation in Protea *flowering time*. Proteas cultivated in South Africa can be broadly classified into three categories according to flowering and subsequent harvesting times (Gerber *et al.*, 2001a).

The first category, containing the majority of cultivars and hybrids such as 'Carnival' (*P. neriifolia* R.Br. × *P. compacta* R.Br.), 'Pink Ice' (*P. compacta* R.Br. × *P. susannae* Phill.) and 'Ivy' (a *P. lacticolor* Salisb. hybrid) initiate inflorescences preferentially on the spring

flush where after development continues during spring and summer, reaching anthesis from January to May. In the second category are *P. magnifica* Link selections and hybrids (eg. 'Susara', 'Lady Di' and 'Sheila') which also initiate inflorescences on the spring flush. However, a delayed spring budbreak, combined with a longer developmental period required for the large inflorescence, results in anthesis for these cultivars to peak in May to November (Gerber *et al.*, 2001c).

Most species have a flowering time restricted to a particular time of year. However, *Protea eximia* (Salisb. ex Knight) Fourc. selections and hybrids (eg. 'Sylvia' and 'Cardinal') are exceptions as they can initiate inflorescences terminally at any time of the year, except winter when no shoot growth occurs. Proteas with an open window for inflorescence initiation therefore make up the third flowering type category. Coetzee and Littlejohn (2001) viewed the ability of 'Sylvia' to initiate an inflorescence to be independent of environmental cues, but having a prerequisite of two or more vegetative flushes subtending to the terminal bud which can act as a supply of sufficient carbohydrates to the developing inflorescence. Despite the flexibility of this open flowering window characteristic, 'Sylvia' and 'Cardinal', when not subjected to a pruning regime (Gerber *et al.*, 2001b), preferentially flower on the spring flush, the most dominant growth flush produced after a period of winter dormancy. The unique ability of 'Sylvia' and 'Cardinal' to initiate flowers on any flush is possibly carried over from the shared *P. eximia* parentage which exhibits the same characteristic. In contrast to these hybrids, *P.eximia* has a greater propensity to flower on shoot growth flushes initiated during late summer and autumn. This flowering habit leads to *P. eximia* inflorescences reaching anthesis from July to November, as compared to January to March for inflorescences of 'Sylvia' and 'Cardinal' (Gerber, 2000).

Although great variation exists in *Protea* flowering time, inflorescence initiation appears to predominantly occur on spring flushes of over-wintering shoots containing at least two or more flushes (Gerber *et al.*, 2002).

Plasticity in bearing habit. Cultivars such as 'Carnival' show some degree of plasticity in flowering habit as demonstrated in experiments designed to develop a biennial pruning system for increased stem length and productivity (Gerber *et al.,* 1995). 'Carnival' plants pruned back to bearers in the autumn months of March and April caused axillary buds to sprout before winter. Spring flushes that developed on these pre-winter formed shoots initiated flowers (Greenfield *et al.*, 1994). In addition, shoots that failed to initiate flowers on the spring flush, flowered on the 1st summer flush. However, when plants were pruned June to August (mid-winter), spring flushes that developed from an axillary bud rarely initiated a flower (Greenfield *et al*., 1994). Furthermore, no flowers initiated on the following 2nd summer or autumn flushes of this pruning experiment. This inability to flower on the above-mentioned flushes is also generally true for shoots not subject to any pruning manipulation as flower initiation on the autumn flush has been observed only on rare occasions in 'Carnival' and 'Pink Ice' (Jacobs, pers. comm., 2001).

***Control of flowering time in* Protea.**

The exact environmental and/or endogenous factors and mechanisms that trigger and control floral initiation in *Protea* are poorly understood and remain largely unresolved.

Environmental control of flowering. A defoliation trial of four-flush 'Carnival' shoots starting approximately 80 days before spring budbreak indicated mature, over-wintering leaves to be essential for shoots to become induced for flower initiation. Furthermore, these leaves should be present for at least six weeks before budbreak for successful floral initiation (Gerber *et al.*, 2002). Similarly, Greenfield *et al.* (1994) observed that when 'Carnival' was pruned at the start of winter so that budbreak only occurred in the following spring, the

absence of a pre-winter flush resulted in a non-flowering spring flush. It is unclear whether environmental factors such as low temperatures or short days, prevailing through winter or alternatively intra-plant factors, such as increased cytokinin production during spring resumption of root growth as observed in lychee (O'Hare and Turnbill, 2004), are responsible for flower initiation.

Short days have been found to induce flowering in *Serruria florida* (Malan and Brits, 1990), *Leucospermum* cv. Red Sunset (Malan and Jacobs, 1990) and *Leucadendron* (Hettasch and Jacobs, 2006). In contrast to this short day requirement, De Swardt (1989) reported that BA applications during winter were not effective in promoting the release of correlative inhibition of axillary buds nor did it increase secondary thickening. Instead, shoot growth continued in winter when long days were simulated with artificial lightning (Jacobs, unpublished data). Similar to 'Ivy', Gerber (2000) speculated that the delay in spring budbreak that has been recorded for 'Lady Di' proteas may be linked to a possible photoperiodic requirement correlating with the long days that follow after the September equinox.

An indication that *Protea* flowering may not be strictly dependant on photoperiod was derived from observations on a number South African species selections and hybrids planted in Hawaii (Leonhardt and Criley, 1999; Coetzee and Littlejohn, 2001). The absence of clear environmental cues in Hawaii compared to the Mediterranean climate of the Western Cape resulted in markedly different flowering times and number of inflorescences per plant. It would appear that in the absence of changes in photoperiod, many *Protea* produce a flower on a stem when the stem attains a certain threshold quality such as a sufficient carbohydrate status (Coetzee and Littlejohn, 2001). Therefore, apparently, a facultative response rather than an obligate requirement to inductive conditions is expressed in the absence of environmental cues.

When *P. neriifolia* cv. Salmon Pink was evaluated at four different locations varying in altitude and temperature, flowering time differed up to six months between extreme sites (Dupee and Goodwin, 1990a; 1992). However, it was not clearly indicated whether these observed differences could be ascribed to different floral induction and/or initiation patterns at the different sites or whether a slower inflorescence development reflected the effect of colder temperatures at higher altitudes.

Although interaction of photoperiod and temperature has been investigated for *Banksia* (Rieger and Sedgley, 1996), an Australian member of the Proteaceace, little is known about the role of temperature in *Protea* floral initiation. Dupee and Goodwin (1990b) reported apical meristem reversion within a limited time following floral initiation in *P. neriifolia* cv. Salmon Pink. This phenomenon was linked to possibly higher than normal temperatures coinciding with the early stages of floral differentiation and consequently attributed to a devernalizing effect on the induced apical meristem.

The duration of the induced state in 'Carnival' is unknown. Floral initiation on a 1st summer flush (after specific pruning) or the flowering ability of axillary shoots that develop beneath an inflorescence on a spring flush is indicative that induction may last for some time after the spring flush is completed. Bud sprouting in spring for 'Carnival' (Western Cape, South Africa) occurs during the last week of August with flush elongation being completed by October. Temperatures are still low and days relatively short during this development stage. The observation that no flowers developed on the 2nd summer flush of winter-pruned 'Carnival' (Greenfield *et al.*, 1994) suggests that the induced state for flowering had dissipated by the time of bud sprouting of the 2nd summer flush in mid summer.

Endogenous control of flowering. An earlier report from Vogt (1977) where the flowering period of *P. cynaroides* variants was found to be genetically stable raises the question of a primary endogenous control of flowering in *Protea*. When four or more

variants with different flowering times were grown together in the same environment the characteristic flowering time of each variant was retained. The open window for flowering of *P. eximia* hybrids, such as 'Sylvia' and 'Cardinal', lends support to the existence of an endogenous control mechanism as primary regulator of floral initiation in *Protea*.

The importance of leaves as a source of current photosynthates for both floral initiation and development and as possible signal perceptive tissue was investigated in 'Carnival' and 'Lady Di' (Gerber *et al.*, 2001c; Gerber *et al.*, 2002).

Defoliation of 'Carnival' inhibited flower initiation on the spring flush when performed at a critical time (earlier than six weeks before budbreak). The defoliation treatment indicated that leaves play a role in the floral transition process. Defoliation treatments on the over-wintering shoot before spring budbreak not only removed a current source of photosynthates, but also resulted in a weakened spring flush with fewer leaves (Gerber *et al.*, 2002). This treatment retarded inflorescence development and resulted in delayed anthesis (Gerber, 2000). The delay was most obvious following defoliation before spring budbreak and was still apparent when defoliation was applied after inflorescence initiation had occurred, but before completion of spring flush leaf expansion. Proteas are thought to be reliant on current photosynthates for floral initiation and development, as carbohydrate reserves throughout the year are generally considered to be low (Greenfield *et al.*, 1995).

Defoliation of 'Lady Di' shoots, prior to completion of spring growth flush elongation either completely inhibited flowering or the inflorescence aborted at an early stage (Gerber *et al.*, 2001c). Available carbohydrates reserves in the flowering shoot were insufficient to account for the rapid increase in dry weight of both the spring flush and developing inflorescence after elongation of the spring flush was completed. Gerber *et al.* (2001c) concluded that current photosynthates from the leaves regulated floral initiation and inflorescence development in 'Lady Di'. The efficiency of a mature spring flush to sustain

growth and development of the inflorescence can be deduced from the observation that defoliation treatments that did not prevent inflorescence initiation in 'Lady Di' had no influence on inflorescence development or flowering time (Gerber *et al.*, 2001c).

Use of traceable stable isotopes confirmed current photosynthates to be the main carbohydrate source driving the flowering process (Smart, 2005). In addition, Smart (2005) suggested supplementation of current photosynthates by possible translocation of carbon from vegetative to flowering shoots as well as the utilization of stored reserves to support sinks such as flowers and young developing leaves.

A morphological study of 'Carnival', 'Lady Di' and 'Sylvia' vegetative shoots, reported an increase in the number of bud scales and transitional leaves with each successive growth flush as a common feature between those cultivars (Gerber *et al.*, 2001b). A large increase in the number of leaves on the flush subtending the inflorescence was also observed compared to other non-flowering flushes. It is unclear whether a specific leaf number or the increase in appendage number is a pre-requisite for flowering (Gerber *et al.*, 2001b). However, the progressive increase in leaf number with successive flushes would clearly be advantageous to floral initiation, enabling an enhanced quantitative response for the perception of inductive cues or as an increase in resources to support floral initiation and development.

The importance of dry mass accumulation and carbon allocation in 'Sylvia' and 'Cardinal' vegetative shoots leading up to flowering was assessed by Hettasch (1999). After pruning, whole shoots were sampled at the termination of each successive growth flush over a period of one growing season. Dry mass, total sugars and starch concentrations of leaves and stems were then measured for each respective flush. The total dry mass of both stem and leaves remained low during the growth of the spring and 1st summer flushes as available photosynthates were used to support the growth of developing flushes. At completion of the

2nd summer flush, the shoot had developed enough photosynthetic capacity to produce the autumn flush and to accumulate dry mass in the subtending flush. Leaf starch content similarly increased through the summer period, but was significantly reduced after winter, implicating leaves are used as a main source of stored carbohydrates to mobilize spring budbreak and flush elongation. Total sugar content of both stem and leaves increased significantly between sampling of the two-flush shoot (end of 1st summer flush) and the three-flush shoot (end of 2nd summer flush). A clear pattern of capacity building in the vegetative shoot leading up to flowering can be deduced from above results.

The superior characteristics of floral shoots above that of shoots that remained vegetative were demonstrated in *P. neriifolia* cultivated in a summer rainfall area (Heinsohn and Pammenter, 1988). Initial vegetative extension growth of shoots that became floral was greater than that of the shoots that remained vegetative, although maximum growth rates occurred in the same month. Reproductive shoots reached higher growth rates a month prior to shoots that remained vegetative.

Flowers rarely initiate on the 2nd summer or autumn flushes in 'Carnival' (Greenfield *et al.*, 1994; Gerber *et al.*, 2002). However, it has been observed that in those few exceptions where individual 'Carnival' shoots terminated in an inflorescence on flushes not exposed to winter conditions, the stem diameter was noticeably greater than the comparative non-flowering shoots (G. Jacobs, pers. comm., 2001). Similarly, the length and thickness of over-wintering 'Ivy' shoots were found to significantly affect the ability of the stem to produce an inflorescence (De Swardt, 1989). In addition, Coetzee and Littlejohn (2001) commented that flowering on secondary shoots formed from axillary buds below a developing inflorescence on the spring flush, only occurred on stems with a large diameter.

In *Banksia*, a minimum shoot diameter measured at the uppermost intercalation has been identified as critical for inflorescence initiation (Sedgley, 1998). Such a minimum

diameter requirement of the flush subtending the inflorescence has been recognized (De Swardt, 1989), but has not been determined for any members of the genus *Protea*.

***Inflorescence development of* Protea.**

Inflorescence development in *Protea* may proceed directly after floral initiation as in 'Carnival', 'Lady Di' and 'Sylvia' or be delayed up to a year in *P. aristata* (Le Maitre and Midgley, 1991) or to a lesser extent in *P. repens* and *P. lanceolata* (Gerber *et al*., 2001b). During the time that the inflorescence development is interrupted, axillary bud sprouting and elongation may proceed.

In systems where inflorescence development immediately follows floral initiation, most of the involucral appendages were formed in a linear relationship with time during the early stages of extension growth, with fewer appendages being formed towards the end of the elongation period of the spring flush (Gerber *et al.*, 2001b). Inflorescence development then advanced to the stage where all involucral bract initiation is completed by the time of cessation of spring flush elongation. Subsequently a phase of floral bract and floret initiation immediately followed. The number of florets per inflorescence appears to be species or cultivar dependent as floret numbers of 205±15.3, 232±10.4 and 240±18.4 were recorded for 'Sylvia', 'Carnival', and 'Lady Di' respectively (Gerber *et al*., 2001b).

Inflorescence enlargement occurred along side continued floret differentiation (Gerber *et al.*, 2001b). Post-floret differentiation, inflorescence development continued at a similar rate for 'Carnival' and 'Sylvia' exhibiting comparable inflorescence sizes of 40.5±5.5 and 43.8±2.6 mm respectively. The larger 'Lady Di' inflorescence (60.5±3.3 mm) required a longer period to complete its development (Gerber *et al.*, 2001b). The rate of inflorescence development appears to be source dependent (Gerber, 2000). Defoliation of over-wintering shoots significantly extended the development period of 'Carnival' inflorescences. When defoliation of both the over-wintering shoot and newly produced spring flush leaves in 'Lady

Di' surpassed a threshold value, flower abortion or arrestment of any further development of the inflorescence followed (Gerber *et al.*, 2001c, Gerber *et al*., 2002). Furthermore, the observation that flowers borne on long, thick shoots have shorter developmental periods until anthesis than flowers borne on thinner and shorter shoots (G. Jacobs, pers. comm., 2001), supports the idea of inflorescence development being related to source availability.

Environmental factors affect the rate of flower development. This is particularly evident for cultivars that can initiate an inflorescence at any time of year. The development of 'Sylvia' inflorescences from being macroscopically visible (±10mm) to a final size of approximately 43.8±2.6mm varied from 98 days to 149 days for those borne on the spring and autumn flush respectively (Gerber *et al.*, 2001a). Inflorescences borne on the autumn flush developed at a slower rate, possibly because of the lower winter temperatures experienced (Gerber *et al.*, 2001a). When the flowering time of *P. neriifolia* cv. Salmon Pink was compared between different planting locations, flowering time and percentages were significantly higher at sites with elevated temperatures (Dupee and Goodwin, 1990a). The time of flowering of *Leucospermum cordifolium* has also been shown to have a linear relationship with daily mean temperature (Jacobs and Honeyborne, 1979). Heat unit accumulation of 925 units with a base temperature of 5.8°C in spring resulted in maturation of approximately 90% of buds. Criley *et al.* (1990) found degree day accumulation above 6°C as base temperature, together with solar radiation accumulation, a requirement for continued flower development of *Leucospermum cordifolium* cv. Vlam.

***Management and cultural practices for manipulation of flowering time in* Protea.**

To date, three pruning regimes have been successful in manipulating the flowering time of proteas (Greenfield *et al.*, 1994; Gerber *et al.*, 1995; Gerber *et al.* 2001a; Nieuwoudt, 2006).

In 'Carnival', two principles were exploited to achieve earlier flowering. Firstly, pruning of the 'Carnival' plant to bearers in July-August was performed to prevent flowering on the subsequent spring and 1st summer flushes (Greenfield *et al.*, 1994). Furthermore, as no flowers initiate on the 2nd summer flush, shoots proceed to elongate by the formation of additional successive growth flushes. At the onset of winter, plants consist predominantly of four-flush shoots, which allows for the second principle to be exploited.

This second principle is based on observations that inflorescences that initiate on long and thick shoots have a shorter developmental period to anthesis. Four-flush over-wintering shoots initiate inflorescences on the spring flush. These inflorescences then develop terminally on over-wintering shoots consisting of five flushes and on average reach anthesis two months earlier than flowers on two-flush shoots. Sixty percent of five-flush shoots were harvested in February compared with four percent of two-flush shoots (Gerber *et al.*, 1995).

This procedure implies that a flowering crop is harvested only every second year. The first year is dedicated to vegetative development of a four-flush shoot with development of a second spring flush and inflorescence in the following year. Growers therefore manage this pruning regime by dividing the crop into two blocks. The 'off' year will represent the shoots in the vegetative stage, with the 'on' year when shoots turn reproductive. The economic return of high yields of flowers with longer stems combined with an earlier flowering time more than compensate for the loss of having a marketable crop only every second year.

In the case of 'Sylvia', plants are also pruned to bearers in July-August (Gerber *et al.*, 2001a). The subsequent spring or 1st summer flush does not initiate flowers. A need for dry mass and carbohydrate accumulation in these flushes initially retards shoot extension (Hettasch, 1999). The lag in carbohydrate accumulation is significantly reduced in the 2nd summer and autumn flushes due to the presence of functional leaves on the first two flushes serving as a source of photosynthates. Since 'Sylvia' can initiate an inflorescence on any

flush, the shoot characteristics in May subsequent to pruning, are conducive to flower initiation on the autumn flush. These flowers can then be harvested from September-December when higher prices can be obtained compared to January-March when flowers that initiated on the spring flush reach anthesis.

The strategy followed by 'Pink Ice' of initiating flowers predominantly on the spring flush is similar to that of 'Carnival'. In contrast to 'Carnival', but similar to 'Sylvia', 'Pink Ice' has the ability to initiate flowers on an autumn flush, provided shoot growth was synchronized by pruning (June-July) and shoots had acquired a length of at least 70 to 80cm by the following autumn (May). Without the preceding pruning to synchronize shoots, the presence of developing inflorescences both inhibited the return bloom by ca. 50% and inflorescences failed to initiate on the autumn flush, with initiation consequently only taking place on the spring flush.

Although, pruning of 'Sylvia' (Gerber *et al*. 2001a) and 'Pink Ice' (Nieuwoudt, 2006) proved effective to advance flowering time to such an extent as to allow harvests to fall in December-January, cultivars such as 'Carnival' which almost exclusively initiates inflorescence on the spring flush will require a different or additional approach to allow harvest time to fall within the Christmas holiday period.

To briefly summarise: it would appear that conditions inductive to inflorescence initiation for most *Protea* cultivars and in particular for 'Carnival' entail:

- Over-wintering shoots with a minimum, but unspecified quality in terms of stem diameter- and length, number of leaves or leaf area subtending the terminal bud, and/or
- Shoots with an adequate carbohydrate status and a sufficient current supply of photosynthates, and/or

- Environmental factors or intra-plant dynamics or a combination of both as prevailing in winter.

Role of cytokinin in floral initiation.

Current concepts of the angiosperm flowering model. The model of multi-factorial control of flowering which envisages transition to flowering as a complex system of interacting factors has largely replaced the search for the single, elusive 'florigen' (Bernier, 1988; Bernier *et al.*, 1993). Leaf-generated transmissible signals are believed to be required for determination of the shoot apex in both autonomously regulated and photoperiodic species (Thomas and Vince-Prue, 1997). Genetic studies have established four genetically distinct developmental pathways that control flowering in the LDP *Arabidiopsis* (Levy and Dean, 1998; Blazquez, 2000; Colasanti and Sundaresan, 2000). These pathways include: a photoperiodic pathway where photoreceptors interact with a circadian clock; an autonomous/vernalization pathway where flowering occurs either in response to internal signals such as number of leaves or low temperatures; a carbohydrate (sucrose) pathway that reflects the metabolic state of a plant relative to flowering and a gibberellin pathway which can facilitate early flowering and flowering under non-inductive conditions. All four pathways converge by increasing the expression of the key floral meristem identity gene AGAMOUS-LIKE 20 (AGL20). AGL20, a MADS box-containing transcription factor, integrates the signals into a unitary output activating the LEAFY (LFY) gene which, in turn, activates the floral homeotic genes required for floral organ development.

The existence of multiple flowering pathways is thought to be universal amongst angiosperms allowing for maximum reproductive flexibility in an often unpredictable environment.

Cytokinin: a component of multi-factorial control of flowering. Cytokinin has frequently been implicated as part of the multi-factorial model for flowering, although this model was primarily developed on experimental data obtained from day-length sensitive, herbaceous plants (Bernier *et al*., 1993). In addition, cytokinins have also been known to affect vegetative characteristics considered promotive of flowering in plants, such as increased leaf area (Nielsen and Ulvskov, 1992), accelerated cambium activity leading to increase secondary thickening (Katsumi, 1962; Rupp *et al.*, 1999) and altered carbohydrate mobilization partitioning (Ogawa and King, 1979; Abou-Haidar *et al.*, 1985; Fetene and Beck, 1993).

Changes in endogenous cytokinin concentration have been closely correlated with the early events of floral transition in numerous herbaceous plants such as *Begonia* (Hansen *et al.*, 1988), *Chenopodium* (Macháčková *et al.*, 1993), *Perilla* (Grayling and Hanke, 1992), and the day-neutral corm species, *Polianthes* (Chang *et al*., 1999).

Cytokinin involvement in floral initiation of long day plants, *Sinapsis alba* (Kinet *et al.*, 1993; Havelange *et al.*, 2000) and *Arabidiopsis thaliana* (Corbesier *et al.*, 2003) is thought to involve the physiological interaction of sucrose/cytokinin between the leaf as perceptive site, roots and shoot apical meristem.

Long day (LD) photoperiodic induction of floral transition in *Sinapsis alba*, indicated involvement of a long-distance signal. Early during the LD photo-extension, the movement of an essential signal, identified as sucrose, from the shoot to the root in the phloem was indicated by ring-barking experiments (Havelange *et al.*, 2000). It was hypothesized that arrival of the sugar signal at the root, caused newly synthesized (mainly zeatin-type cytokinins) to be released in the xylem (Lejeune *et al.*, 1994). The importance of cytokinin export was confirmed by experiments that restored inhibited flowering, effected by ring-barking at the 8th hour, with cytokinin application on the apical bud at the 16th hour.

Increased cytokinin supply from the roots apparently results in elevated levels of cytokinin in the mature leaves of induced plants. Subsequently, cytokinin (predominantly as isopentenyladenine riboside) is then exported out of the leaves in the phloem to the apical bud (Lejeune *et al.*, 1994). Cytokinin content in the apical meristem continues to increase and a mitotic wave, with shortening of the major phases of the cell cycle and other events associated with flowering such as starch accumulation, increased cell numbers and decreased vacuole size, is induced (Havelange *et al.*, 1986; Houssa *et al.*, 1990). A similar shoot-to-root signal under photoperiodic control and affecting cytokinin synthesis/release has also been proposed for the short day plant, *Xanthium strumarium* (Kinet *et al.*, 1994).

Furthermore, although cytokinin is instrumental in the floral transition of *Sinapsis alba*, exogenous application alone was insufficient to cause flowering (Bernier *et al*., 1993). Kinet *et al.* (1993) determined that plant organs are not all equally affected by cytokinin and suggested that sensitivity towards cytokinin action may change during the growth cycle.

Exogenous cytokinin applications to *Bougainvillea* (Anthony *et al.*, 1974); *Chrysanthemum* (Pharis, 1972), *Pharbitis nil* (Ogawa and King, 1980), *Lemna paucicostata* (Gupta and Maheshwari, 1969) and *Wolffia microscopica* (Venkataraman *et al.*, 1970) have been reported to result in floral initiation under non-inductive conditions, whilst in other species floral initiation has been inhibited (Bernier *et al*., 1998). These varying reports strongly suggest the effect of exogenous cytokinin application to be dependent on other factors such as, applied concentration, time and site of application and environmental conditions.

Of particular interest for this study, because of similarities in tree phenology to *Protea*, is the effect of benzyladenine (BA) on floral induction in mango (*Mangifera indica* L.) (Chen, 1985). In this study an application of 100 $mg \cdot L^{-1}$ BA to mature shoots enhanced flower bud initiation by 1.5 to two months compared to the untreated control.

Other reports on the role of cytokinin during flowering of woody perennials are often inconsistent and with contradictory findings (Bangerth, 1997). Cytokinin has been implicated in floral initiation of deciduous fruit crops where a putative 'florigenic' component appears to be translocated from the current season spur leaves to promote flowering in bourse buds. Reduction in leaf number, leaf area and low light intensities all negatively impacted on floral induction in bourse buds (Davenport, 2000). Cytokinin has been found in substantial quantities in spur leaves of apple (Greene, 1975). Cytokinin has been associated with the above-mentioned florigenic signal as exogenously applied cytokinin was able to replace the requirement for leaves in the formation of floral buds (Ramirez and Hoad, 1981). It would therefore appear that the role of exogenous cytokinin in flower initiation in deciduous crops is dependent on both the endogenous gibberellin to cytokinin ratio, and the application site (Bubán, 2003).

Similarities exist between the flowering model of *Protea* and that of tropical and subtropical crops such as mango, lychee and citrus. For flowering to occur in these crops, the resting bud first initiates growth with the onset of rapid shoot development (budbreak). Provided the required inductive conditions are present at the time of initiation, reproductive shoot development then follows vegetative shoot initiation (Davenport, 2000). Hence, management of flowering essentially entails management of shoot initiation. It has been suggested that budbreak is regulated by the interactive ratio of accumulated cytokinin (exported from roots) and a decline in auxin production and transport from leaves of increasing age. A further hypothesis suggests that the type of shoot that differentiates soon after budbreak is determined by the interaction of a putative temperature-regulated florigenic promoter, originating in the leaves, and an age-dependent vegetative promoter (Davenport, 2003). Control of flowering of mango, citrus and lychee outside these natural flowering times relies on the management of shoot initiation at a time when the ratio of these two

assumed promoters are favourable for flowering (Davenport, 2003). Although cytokinin has been implicated as a component or regulator of these florigenic promoter(s), with GA a likely vegetative promoter, the mechanism by which it operates remains poorly understood.

Management for 'out of season' flowering in woody perennial tree crops.

In contrast to photoperiodic or cold-requiring herbaceous plants where flower induction is often triggered by an external, single, 'all-or-none' environmental events, floral initiation in woody perennials is more complex and less easily manipulated (Crabbé, 1984).

Flower bud formation in fruit tree species is often influenced by a combination of environmental and intrinsic factors such as endogenous hormones and the phenology of the vegetative growth cycle. Cultural and management techniques may interact with these floral determining factors and can therefore be used to modify the flowering response to deliver 'out of season' flowering (Greene, 1996).

For apples and pears, as examples of deciduous fruit trees adapted to temperate climates, floral buds initiate in mid to late summer and differentiate until trees become dormant in early autumn in preparation for winter. Usually, cultivar-specific chill unit accumulation is a prerequisite to permit renewed growth and development (Dennis, 2003). When cultivars with a low chilling requirement are grown in the tropics, defoliation alone or in combination with branch-bending soon after harvest, induces budbreak (Edwards and Notodimedjo, 1987). As the flowering cycle in spurs begins soon after the break of dormancy, such an advancement of budbreak allows for two crops per year to be harvested.

Although photoperiod plays an insignificant role in apple flowering under field conditions, solar radiation has been shown to significantly increase flowering intensity. Pruning to open trees to sunlight is therefore generally encouraged (Sedgley, 1990). Summer pruning of peaches has the added advantage of earlier flowering (Marini, 1986).

As in *Protea* plants, individual stems of tropical, evergreen tree species such as citrus, mango and lychee undergo periodic vegetative growth flushes, with dormant phases between successive flushes (Davenport, 2003). The number of vegetative flushing cycles can vary from one to several times per year depending upon the cultivar, size of the tree and growth conditions, such as water and nitrogen availability. Inhibition of lychee bud development during the dormancy phase appears to involve the immature leaves as the removal of young leaves shortens the intervals between flushes (Menzel *et al.*, 2000 in Olesen, 2005).

Reproductive flushes of mango and lychee are typically produced once per year, with no dormancy phase in the development of the reproductive flushes as found in most temperate fruit trees (Núñez-Elisea *et al.*, 1993). As previously mentioned, initiation of shoot growth in these tropical crops is the first event in the sequence leading to flowering. Frequent flushing, with shorter rest periods between flushes, typically occurs in young trees or in mature trees where high nitrogen levels and an abundance of water are present. Other factors that may stimulate vegetative shoot initiation include stem pruning, defoliation, foliar nitrogen sprays and ethylene (Davenport, 2003). The frequent flushing and increased growth vigour resulting from these permissive growth conditions then favours vegetative shoot induction as opposed to reproductive flush initiation. Therefore, floral management strategies for 'out of season' flowering of lychee and mango in the tropics focuses on prevention of new shoot initiation in order for resting stems to reach sufficient maturity to induce flowering shoots (Núñez-Elisea and Davenport, 1995; Davenport, 2003).

Davenport (2003) outlined a management program for 'out of season' flowering in mango which required tip pruning of all stems to synchronize vegetative growth on the tree. Apart from encouraging uniform flushing, pruning has the added advantage of removing all growth- and flower-inhibiting factors in stems derived from previous seasons. Careful consideration of pruning depth, fertilizer management during the wet season and accurate

timing of irrigation during the dry season is done to ensure that only one vegetative flush occurs approximately a month after pruning. This eliminates the formation of an early, undesirable second flush. In addition, triazole plant growth retardants, which act by gibberellin biosynthesis inhibition, can be applied in combination with floral-stimulating nitrate sprays. If low night temperatures coincide with the scheduled spray program, earlier stimulation of flowering is very likely (Davenport, 2003).

Citrus in the subtropics reliably flowers as a result of chilling temperatures during the winter months. In contrast, flowering in the tropics is generally induced by water stress (Southwick and Davenport, 1986; Davenport, 1990). Growth flushes containing both vegetative and reproductive shoots are typically initiated with the first major rain directly after the dry season. To achieve 'out of season' flowering in citrus it is therefore critical to prevent the normal flowering cycle. This can be attained by irrigation of the entire root zone throughout the dry period so that trees are never subject to drought stress (Davenport, 2003).

In the absence of low temperatures or water stress, the ability of citrus to flower was directly correlated with the age of the terminal unit in tropical climates (Davenport, 2000). Therefore, as with mango, maintaining low leaf nitrogen levels is critical to depress early additional flushes of vegetative growth, particularly during the rainy season. Shoot initiation (budbreak) can be stimulated by foliar application of low-biuret urea (LBU) a month before the envisaged flowering date, and is most effective used in combination with a soil application of a complete fertilizer mix two month prior to the proposed flowering date (Ali and Lovatt, 1994). Initiation of reproductive shoots in citrus is most likely if LBU is applied on sufficiently mature shoots when floral inductive conditions prevail.

Cool winter temperatures are known to stimulate lychee flowering (Batten and McConchie, 1995). However, when this crop is grown in the lower latitude tropics where chilling temperatures are rarely experienced, the stem age of the terminal flush is of key

importance in the floral initiation process (O'Hare, 2002). Whereas brief dormancy periods are satisfactory for areas where the winter temperatures reach 10°C or below for short periods, flowering can only be achieved in areas without sufficient chilling by imposing longer stem rest duration (Olesen *et al.* 2002; Davenport, 2003). Strategies available to manage flowering time include: the pruning of non-productive vegetative stems immediately after harvest to ensure better, synchronized flush growth combined with water restriction and by maintenance of leaf nitrogen levels in autumn to ≤1.7% to reduce undesirable vegetative growth immediately prior to the winter flowering season (Menzel and Simpson, 1990).

Conclusion.

From the above discussion it appears that 'out of season' flowering in woody perennial tree crops is attained by strategies that rely upon the prevention of the normal flowering cycle, followed by the synchronized stimulation of new bud growth with resultant shoots which are then managed to maximise their intrinsic capacity to flower.

The purpose of this study was to attempt 'out of season' flowering in *Protea* cv. Carnival with BA treatments. The effect of time of BA application in autumn, method of BA application and concentration on bud sprouting and on 'out of season' flower initiation was assessed. The heat unit requirements for the phenological phases, treatment date of BA to green point; green point to completion of flush expansion and the latter to anthesis were established. Shoot characteristics such as number of flushes, level of maturation, stem thickness, leaf area, accumulated stem and leaf dry mass, leaf number and position relative the terminal bud on the efficacy of BA to induce 'out of season' flowering was determined. Lastly, the effect of flowers on the efficacy of BA to induce non-flowering shoots on the same plant to flower 'out of season' was revealed.

Literature cited

Abou-Haidar, S.S., E. Miginiac, and R.M. Sachs. 1985. [^{14}C]-assimilate partitioning in photoperiodically induced seedlings of *Pharbitis nil*. The effect of benzyladenine. Physiol. Plant. 64:265-270.

Ali, A.G. and C.J. Lovatt. 1994. Winter application of low-biuret urea to the foliage of 'Washington' navel orange increased yield. J. Amer. Soc. Hort. Sci. 119:1144-1150.

Anthony, T., Y. Tse, A. Ramina, W.P. Hackett, and R.M. Sachs. 1974. Enhanced inflorescence development in *Bougainvillea* 'San Diego Red' by removal of young leaves and cytokinin treatments. Plant Physiol. 54:404-407.

Bangerth, F. 1997. Can regulatory mechanisms in fruit growth and development be elucidated through the study of endogenous hormone concentrations? Acta Hort. 463:77-87.

Batten, D.J. and C.A. Monchie. 1995. Floral induction in growing buds of lychee (*Litchi chinensis*) and mango (*Mangifera indica*). Aust. J. Plant Physiol. 22:783-791.

Bernier, G. 1988. The control of floral evocation and morphogenesis. Ann. Rev. Plant. Physiol. Plant. Mol. Biol. 39:175-219.

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

Bernier, G., L. Corbesier, C. Périlleux, A. Havelange, and P.Lejeune. 1998. Physiological analysis of the floral transition. In: K.E. Cockshull, D. Gray, G.B. Seymour, and B. Thomas (eds). CAB International, Wallingford, UK. pp. 103-109.

Blazquez, M.A. 2000. Flower development pathways. J. Cell Sci. 113:3547-3548.

Bubán, T. 2003. Hormonal aspects of flower formation and fruit set. In: P. Kozma, J. Nyéki, M. Soltész, and Z. Szabó (eds.) Floral biology, pollination and fertilisation in

temperate zone fruit species and grape. Akadémiai Kiadó, Budapest, Hungary. pp. 3-24.

Chang, S-T., W-S. Chen, C-Y. Hsu, H-C.Yu, B-S. Du, and K-L. Huang. 1999. Changes in cytokinin activities before, during and after floral initiation in *Polianthes tuberosa*. Plant Physiol. Biochem. 37:679-684.

Chen, W-S. 1985. Flower induction in mango (*Mangifera indica* L.) with plant growth substances. Proc. Natl Sci. Counc. B ROC 9:9-12.

Coetzee, J.H. and G.M. Littlejohn. 2001. *Protea*: A Floricultural Crop from the Cape Floristic Kingdom. Hort. Rev. 26:1-48.

Colasanti, J. and V. Sundaresan. 2000. 'Florigen' enters the molecular age: long-distance signals that cause plants to flower. TIBS 25:236-240.

Corbesier, L., E. Prinsen, A. Jacqumard, P. Lejeune, H. Van Onckelen, C. Périlleux, and G. Bernier. 2003. Cytokinin levels in leaves, leaf exudate and shoot apical meristem of *Arabidopsis thaliana* during floral transition. J. Exp. Bot. 54: 2511-2517.

Crabbé, J.J. 1984. Vegetative vigor control over location and fate of flower buds, in fruit trees. Acta Hort. 149:55-63.

Criley, R.A., P.E. Parvin, and S. Lekawatana. 1990. Flower development in *Leucospermum cordifolium* 'Vlam'. Acta Hort. 264:61-70.

Davenport, L. 1990. Citrus flowering. Hort. Rev. 12:349-408.

Davenport, L. 2000. Processes influencing floral initation and bloom: the role of phytohormones in a conceptual flowering model. HortTechnol. 10:733-739.

Davenport, L. 2003. Management of flowering time in three tropical and subtropical fruit tree species. HortScience 38:1331-1335.

Dennis, F Jr. 2003. Flowering, pollination and fruit set and development. In: D.C. Ferree and I. J. Warrington (eds.) Apples: Botany, Production and Uses. CAB International. pp. 153-166.

De Swardt, D.C. 1989. Aspekte van die vegetatiewe en reproduktiewe ontwikkeling by geselekteerde Proteaceace kultivars. M.Sc. thesis, Univ. of Stellenbosch, Stellenbosch, S. Africa.

Dupee, S.A. and P.B. Goodwin. 1990a. Effect of temperature, daylength and growth regulators on flowering in *Protea*, *Telopea* and *Leucospermum*. Acta Hort. 264:79-84.

Dupee, S.A. and P.B. Goodwin. 1990b. Flower initiation in *Protea* and *Telopea*. Acta Hort. 264:71-78.

Dupee, S.A. and P.B. Goodwin. 1992. Flowering and vegetative growth of *Protea neriifolia* and *Protea cynaroides*. Acta Hort. 316:81-89.

Edwards, G.R. and S. Notodimedjo. 1987. Defoliation, bending, and tip pruning of apple under tropical conditions. Acta Hort. 199:125-127.

Fetene, M. and E. Beck. 1993. Reversal of the direction of photosynthate allocation in *Urtica dioica* L. plants by increasing cytokinin import into the shoot. Bot. Acta 106:235-240.

Gerber, A.I. 2000. Inflorescence initiation and development, and the manipulation thereof, in selected cultivars of the genus *Protea*. Ph.D. diss., Univ. of Stellenbosch, Stellenbosch, S. Africa.

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

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001a. Manipulation of flowering time by pruning of *Protea* cv. Sylvia (*P. eximia* × *P. susannae*). HortScience 36:909-912.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001b. Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars. J. Amer. Soc. Hort. Sci. 126:182-187.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001c. The role of leaves and carbohydrates in flowering of *Protea* cv. 'Lady Di'. HortScience 36:905-908.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2002. Defoliation alters spring growth flush characteristics and inhibits flowering in *Protea* cv. Carnival. Scientia Hort. 94: 345-350.

Grayling, A. and D.E. Hanke. 1992. Cytokinins in exudates from leaves and roots of red *Perilla*. Phytochem. 31:1863-1868.

Greene, D.W. 1975. Cytokinin activity in the xylem sap and extracts of MM106 apple rootstocks. Hortscience 10:73-74.

Greene, D.W. 1996. Flower development. In: M. Marl, P. Andrew, G. Lang, and K. Mullinex (eds.) Tree fruit physiology: Growth and development. Good Fruit Grower. Yahima, Washington. pp. 91-98.

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

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. 5:25-28.

Gupta, S. and S.C. Maheshwari. 1969. Induction of flowering by cytokinins in a short-day plant, *Lemna paucicostata*. Plant Cell Physiol. 10:231-233.

Hansen, C.E., C. Kopperud, and O.M. Heide. 1988. Identity of cytokinins in *Begonia* leaves and their variation in relation to photoperiod and temperature. Physiol. Plant. 73: 387-391.

Havelange, A.M. Bodson, and G. Bernier. 1986. Partial floral evocation by exogenous cytokinin in the long-day plant *Sinapsis alba*. Physiol. Plant. 67:695-701.

Havelange, A., P. Lejeune, and G. Bernier. 2000. Sucrose/cytokinin interaction in *Sinapsis alba* at floral induction: a shoot-to-root-to-shoot physiological loop. Physiol. Plant. 109:343-350.

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. 54:440-444.

Hettasch, H.B. 1999. Studies of the vegetative development of *Protea* cv. Sylvia and Cardinal. MSc thesis. Univ. of Stellenbosch, Stellenbosch, S. Africa.

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

Hettasch, H.B. and G. Jacobs. 2006. Effect of daylength on flower initiation of *Leucadendron*. Acta Hort. in press.

Houssa, C., A. Jacqmard, and G. Bernier. 1990. Activation of replicon origins as a possible target for cytokinin in shoot meristems of *Sinapsis*. Planta 181:324-326.

Jacobs, G. and G.E. Honeyborne. 1978. Delaying the flowering time of *Leucospermum* cv. Golden Star by deheading. Agroplantae 10:13-15.

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.

Kaiser Associates Report, 2000. Floriculture Cluster Study. South African Flower and Export Council, South Africa.

Katsumi, M. 1962. Physiological effects of kinetin effect on thickening of etiolated pea stem sections. Physiol. Plant. 15:115-120.

Kinet, J-M., P. Lejeune, and G. Bernier. 1993. Shoot-root interactions during floral transition: a possible role for cytokinins. Environ. Exp. Bot. 33:459-469.

Kinet, J-M., P. Houssa, M-C. Requier, and G. Bernier. 1994. Alteration of cytokinin levels in root and leaf exudates of the short day plant *Xanthium strumarium* in response to a single long night inducing flowering. Plant Physiol. Biochem. 32:379-383.

Lejeune, P., G. Bernier, M-C. Requier, and J-M. Kinet. 1994. Cytokinins in phloem and xylem sap of *Sinapsis alba* during floral induction. Physiol. Plant. 90:522-528.

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.

Leonhardt, K.W. and R.A. Criley. 1999. Proteaceae floral crops: Cultivar development and underexploited uses. In: J. Janick (ed.). Perspectives on new crops and new uses. ASHS Press, Alexandria, USA. pp. 410-430

Levy, Y.Y. and C. Dean. 1998. The transition to flowering. Plant Cell 10:1973-1989.

Macháčková, I., J. Krekule, J. Eder, F. Seidklová, and M. Strnad. 1993. Cytokinins in photoperiodic induction of flowering in *Chenopodium* species. Physiol. Plant. 87:160-166.

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

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

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. 316:91-97.

Marini, R.P. 1986. Defoliation, flower bud cold hardiness, and bloom date of peach as influenced by pruning treatments. J. Amer. Soc. Hort. Sci. 106:593-596.

Menzel, C.M., T. Olesen, C. McConchie, N. Wiltshire, Y. Diczbalis, and C. Wicks. 2000. Lychee, longan and rambutan. Optimising canopy management. Rural Industries Research and Development Corporation, Publication No. 00/29, Canberra, ACT.

Menzel, C.M. and D.R. Simpson. 1990. Nutritional studies on lychee trees in subtropical Australia. Acta Hort. 275:581-585.

Nielsen, T.H. and P. Ulvskov. 1992. Cytokinin and leaf development in sweet pepper (*Capsicum annuum* L.) II. Sink metabolism in relation to cytokinin-promoted leaf expansion. Planta 188:78-84.

Nieuwoudt, G. 2006. The effect of pruning on the growth and development of *Protea* cv. Pink Ice (*P. compacta* R. Brown × *P. susannae* Phill). M.Sc. thesis, Univ. of Stellenbosch, Stellenbosch, S. Africa.

Núñez-Elisea, R. and T.L. Davenport. 1995. Effect of leaf age, duration of cool temperature treatment, and photoperiod on bud dormancy release and floral initiation in mango. Scientia Hort. 62:63-73.

Núñez-Elisea, R., T.L. Davenport, and M.L. Caldeira. 1993. Bud initiation and morphogenesis in 'Tommy Atkins' mango as affected by temperature and triazole growth retardants. Acta Hort. 341:192-198.

Ogawa, Y. and R.W. King. 1979. Indirect action of benzyladenine and other chemicals on flowering of *Pharbitis nil* Chois. Plant Physiol. 63:643-649.

Ogawa, Y. and R.W. King. 1980. Flowering in seedlings of *Pharbitis nil* induced by benzyladenine applied under a non-inductive daylength. Plant Cell Physiol. 21:1109-1116.

O'Hare, T.J. 2002. Interaction of temperature and vegetative flushing maturity influences shoot structure and development of lychee (*Litchi chinensis* Sonn.). Scientia Hort. 95:203-211.

O' Hare, T.J. and C.G.N. Turnbill. 2004. Root growth, cytokinin and shoot dormancy in lychee (*Litchi chinensis* Sonn.). Scientia Hort. 102:257-266.

Olesen, T., C.M. Menzel, N. Wiltshire, and C.A. McConchie. 2002. Flowering and shoot elongation of lychee in eastern Australia. Aust. J. Agric. Res. 53:977-983.

Olesen, T. 2005. The timing of flush development affect the flowering of avocado (*Persea Americana*) and macadamia (*Macadamia integrifolia* × *tetraphylla*). Aust. J. Agr. Res. 56:723-729.

Parvin, P.E., R.A. Criley, and J.H. Coetzee. 2003. Proteas - a dynamic industry. Acta Hort. 602:123-126.

Pharis, R.P. 1972. Flowering of *Chrysanthemum* under non-inductive long days by gibberellins and N^6-Benzyladenine. Planta 105:205-212.

Ramirez, H. and G.V. Hoad. 1981. The effect of growth substances on fruit-bud initiation in apple. Acta Hort. 120:131-136.

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

Rupp, H-M., M. Frank, T. Werner, M. Strnad, and T. Schmülling. 1999. Increased steady state mRNA levels of the STM and KNAT1 homeobox genes in cytokinin overproducing *Arabidiopsis thaliana* indicate a role for cytokinin in the shoot apical meristem. Plant J. 18:557-563.

SAPPEX, 2004. Statistics. SAPPEX Newsletter 119: 30.

Sedgley, M. 1990. Flowering of deciduous perennial fruit crops. Hort. Rev. 12:223-264.

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

Smart, M. 2005. Physiology of floral induction in *Protea* spp. M.Sc. thesis. Univ. of Stellenbosch, Stellenbosch, S. Africa.

Southwick, S.M. and T.L. Davenport. 1986. Characterization of water stress and low temperature effects on flower induction in citrus. Plant Physiol. 81:26-29.

Thomas, B. and D. Vince-Prue. 1997. Photoperiodism in plants. San Diego, California. Academic Press.

Venkataraman, R., P.N. Seth, and S.C. Maheshwari. 1970. Studies on the growth and flowering of a short-day plant, *Wolffia microscopica*. I. General aspects and induction of flowering by cytokinins. Z. Pflanzenphysiol. 62:316-327.

Vogt, M.M. 1977. *Protea cynaroides* (L)L. Intensive cut flower production. Part II. Farming in South Africa No. B10/1977.

2. PAPER I. Exogenous cytokinin induces 'out of season' flowering in *Protea* cvs. Carnival and Pink Ice.

Exogenous cytokinin induces 'out of season' flowering in *Protea* cvs. Carnival and Pink Ice.

Abstract

The cytokinin concentration in the xylem sap of *Protea* cv Carnival shoots was determined at regular intervals from 11 weeks before until 10 weeks after spring budbreak. Cytokinin levels were high during the early phases of spring shoot growth which coincided with the period of inflorescence initiation. 6-Benzyladenine (BA) as ABG-3062 (2% $^{w}/_{w}$ active ingredient) at 50, 250 or 500 mg·L^{-1} was applied to the whole shoots on 22 February, 12 April or 22 May 2001 or only to terminal buds on the last date at 500 mg·L^{-1}. Ninety-five percent of the terminal buds sprouted and initiated an inflorescence when BA application was directed to terminal buds only whereas much lower flowering percentages of 0-35% was achieved when the entire shoot was treated. In addition, after whole shoots were treated with BA in April 2001, between 5-45% floral reversion was observed. High flowering percentages of 87-93% was recorded when BA as MaxCel™ (active ingredients: BA 1.9% $^{w}/_{w}$) was applied at 500mg·L^{-1} to the terminal bud in the dormant state or up to the stage when sprouting buds reached green point. Later applications were less effective at 42-43% inflorescence initiation. BA-containing regulators ABG-3062, Promalin® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 1.8% $^{w}/_{w}$) and Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) applied in May 2002 to terminal buds of *P.* cv. 'Pink Ice' induced budbreak in 95% of shoots. In the case of ABG-3062 or Accel® *ca.* 40% inflorescences initiated. No inflorescences were initiated on flushes

induced by Promalin®. The flowering time of BA-induced inflorescences was significantly advanced compared to flowers that initiated naturally on the spring flush.

Members of the genera *Protea*, *Leucospermum* and *Leucadendron* are predominantly grown in South Africa for export to Europe. Manipulation of flowering time in these cut flowers is important in order to take advantage of periods with high prices (September to January) as well as to smooth supply and reduce periods of over production. *Leucospermum* flowering times can be controlled by day length manipulation (Malan *et al.*, 1994). However, this technology is currently not used in South Africa due to the poor shoot qualities that result under artificial long days applied in winter (Malan and Jacobs, 1994). Instead, manual removal of the *Leucospermum* primary inflorescence that permits a secondary, and later maturing inflorescence to develop, is practiced to smooth out over production during September and October (Jacobs and Honeyborne, 1978). Hettasch and Jacobs (2006) recently reported that flowering in *Leucadendron* is also photoperiod sensitive. However, commercial application of this technology still awaits implementation.

A number of *Protea* species such *P. magnifica*, *P. grandiceps, P. eximia* and some ecotypes of *P. cynaroides* do flower in the desired marketing window (September-January). However, many of the superior hybrid *Protea* cultivars currently available to growers flower from March to June. *Protea eximia* exhibits an ability to initiate flowers throughout the year. Although hybrids of *P. eximia* such as 'Sylvia' (*P. eximia* × *P. susannae*) and 'Cardinal' (*P. eximia* × *P. susannae*) have inherited this characteristic, most inflorescences still predominantly develop on the spring flush with harvest dates then subsequently spread over January to April (Gerber, 2000). 'Sylvia' shoot growth is synchronized by pruning plants in July or August to achieve a flowering peak 14-16 months later (October to December) (Gerber *et al.*, 2001a). Although

effective in 'Sylvia' and also commercial applicable to 'Cardinal', this approach has been unsuccessful on hybrid cultivars that initiate inflorescences almost exclusively on the spring flush such as 'Carnival' (*P. compacta* × *P. neriifolia*) (Jacobs, pers. comm., 2002).

Gerber *et al.* (2001b) showed that flower initiation in 'Carnival' coincided with the early phases of spring flush elongation. In a number of perennial woody plants, spring budbreak is correlated with an increased concentration of cytokinins in the xylem sap just prior to budbreak (Van Staden and Davey, 1979; Tromp and Ovaa, 1990). Furthermore, exogenous application of cytokinins can promote budbreak during late dormancy (Jones, 1973; O'Hare and Turnbill, 2004). Cytokinins have also been implicated in flower initiation in many herbaceous species (Bernier *et al.*, 1998; Corbesier *et al.*, 2003) and a number of perennial horticultural crops (Davenport, 2000).

This paper reports on the increase of endogenous cytokinin prior to budbreak in the xylem sap of 'Carnival' and on the 'out of season' initiation of inflorescences on flushes induced by exogenous cytokinin-containing growth regulator applications in *Protea* cvs. Carnival and Pink Ice.

Materials and Methods

Plant material. Experiments on *Protea* cvs. Carnival (*P. compacta* × *P. neriifolia*) and Pink Ice (*P. compacta* × *P. susannae*) were carried out in commercial plantations of six-year old plants grown from cuttings in the Stellenbosch-Paarl district (33°55′S; 18°50′E), South Africa. The climate is Mediterranean-like with cool, wet winters and dry, hot summers. Annual rainfall is 600-700 mm. Plants were spaced 1 m in the row and 4 m between rows. 'Carnival' plants were pruned during August of the previous year to the trial, to effect biennial cropping as described by Gerber *et al.* (1995). Apart from a harvesting cut (harvesting window ranges from

mid-January to mid-April), 'Pink Ice' plants received no pruning treatment. Plants were not irrigated or fertilized. Pest control focused on the control of thin-line leaf miner (*Phyllocnistis* sp.) and the speckled protea borer (*Orophia* spp.) and were applied by risk analysis which included the emergence and extension of the immature flush and during early inflorescence development.

Cytokinins in xylem sap of 'Carnival' shoots. Xylem sap was collected at three to four week intervals from June 2001 to November 2001. Shoots harvested prior to budbreak consisted of three fully elongated flushes whereas shoots collected post-budbreak included a new developing spring flush. Budbreak refers to the stage where green leaves protrude from the covering bud scale. Budbreak was recorded on 4 September 2001.

Entire shoots were cut 2 cm from their point of inception before 10h00, stripped of leaves and xylem sap extracted according to Belding and Young (1989). Approximately 4 cm of bark was carefully removed from the stem adjacent to the basal cut and rinsed with distilled water to eliminate possible contaminants. This stem section was inserted through a rubber stopper that was fitted into a side-arm vacuum flask. Mild vacuum of 4 kPa was applied to the flask, which allowed for sap to flow in small quantities from the cut end for ≈ 1 min, where-after flow stopped due to constriction at the distal end of the stem section. Continuously removal of 2 cm from the distal end would allow the resumption of flow from the cut end. After the stem was cut down to the stopper, all extractable sap was transferred to cryotubes and xylem exudates were rapidly frozen in liquid nitrogen, freeze-dried and stored at -80°C until analysis. Five three-flush shoots was selected per extraction date and the xylem sap of each shoot was collected and stored separately to serve as replicates. The *t*-zeatin riboside (ZR) concentration of xylem sap was determined by radioimmunoassay using a monoclonal ZR specific antibody as

described by Cook *et al.* (2001). Belding and Young (1989) have suggested cross reactivity with other cytokinins with this sensitive bioassay. Hence, for simplicity, detected *t*-zeatin riboside-like cytokinin activity shall be referred to as cytokinin activity. Results are expressed in ng activity per 100µℓ xylem sap.

Growth regulator treatments on 'Carnival'. 'Carnival' shoots consisting of three flushes from point of inception were used. BA solutions were prepared by diluting ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$), supplied by Abbott Laboratories, North Chicago USA, with water. No additional wetting or penetrating agent was needed. Shoots were treated with BA at a concentration of 50, 250 or 500 $mg \cdot L^{-1}$ on 22 February (late -summer), 12 April (mid-autumn) or 22 May 2001 (late-autumn). The solutions were applied with a paint brush to both the leaves and stem of the entire shoot. A treatment with 500 $mg \cdot L^{-1}$ BA was applied to the terminal bud only of the 2nd summer flush on 22 May 2001. Distilled water was used as the control treatment. Twenty shoots were used per treatment in a completely randomized design. The occurrence of an autumn flush as well as the ability of the autumn flush to terminate in an inflorescence was recorded for all shoots. On 5 December 2001, when the first flowers were at the commercially 'soft tip' harvestable stage (florets still enclosed in involucral bracts, but with the distal end of the inflorescence indenting to touching), all shoots were harvested. The presence or absence of an inflorescence, inflorescence diameter (measured at the widest basal portion) and dry mass (60°C; 72h) were determined. The number of shoots that showed signs of floral reversion was also recorded. Floral reversion was considered to have occurred when a shoot developed from a bud that was previously determined to be floral. In this study floral reversion is revealed by presence of many involucral bracts at the upper intercalation of the reverted vegetative terminal flush.

Verification of optimum BA concentration for inflorescence initiation in 'Carnival' on application to terminal buds. A trial to verify the optimum BA concentration for inducing budbreak and inflorescence initiation in *P*. cv. Carnival when applied to the terminal buds alone was conducted on 6 May 2005. BA as contained in MaxCel™ (active ingredients: BA 1.9% $^{w}/_{w}$; Valent BioSciences Corporation, Libertyville, USA) was used. Only the terminal bud of three-flush shoots was treated at concentration levels of 50, 100, 250 or 500 mg·L^{-1} BA prepared by diluting MaxCel™ with distilled water. Distilled water was used as the control treatment. Ten shoots were used per treatment repeated five times in a randomized complete block design.

BA application to different growth stages of the apical bud in 'Carnival'. On 30 April 2004, mature three-flush shoots where the terminal bud exhibited different growth stages were selected (Figure 1). The different growth stages of the terminal bud that constituted the different treatments included the following categories: dormant (bud enclosed by bud scales, with no visible macroscopic bud activity, Figure 1A); swollen (bud noticeably swollen with bud scales beginning to fold back to reveal green leaves, Figure 1B); green tip (developing flush elongated up to 1.5 cm, representing an intact bulbous 'torpedo' shape, Figure 1C); elongation stage I (developing flush elongated up to 2.5 cm, with proximal leaves beginning to unfold, Figure 1D); elongation stage II (developing flush elongated up to 4 cm, leaves proximal and distal folded away slightly; the stem slightly exposed at the distal end, Figure 1E). On the day of selection, all shoots received a single treatment of BA at 500 mg·L^{-1} (MaxCel™) applied with a paint brush as described earlier. Five shoot were used per treatment repeated three times in a randomized complete block design.

Growth regulator treatments on 'Pink Ice'. 'Pink Ice' shoots consisting of six flushes from the point of inception were selected. On 4 April 2002, terminal buds received a single treatment

of one of the following growth regulator solutions: BA (ABG-3062, active ingredient: BA 2% $^{w}/_{w}$) at a concentration of 250 or 500 mg·L^{-1}; Promalin® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 1.8% $^{w}/_{w}$; Abbott Laboratories, North Chicago, USA) at a concentration of 500 mg·L^{-1}, Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$; Valent Biosciences Corporation, Illinois, USA) at a concentration of 500 mg·L^{-1}, 2% $^{w}/_{w}$ active ingredient. After maturation of the flush as was induced by the April growth regulator application, all solutions were reapplied on 12 May 2002 to the dormant terminal bud of the induced flushes. Distilled water was used as a control in both application dates.

On 18 December 2001 when the first flowers were at the commercial 'soft tip' harvestable stage, all shoots were cropped. Presence or absence of an inflorescence; inflorescence diameter (measured at the widest basal portion) and the dry mass (60°C; 72 h) was determined. Thirteen shoot were used per treatment in a complete randomized design.

Axillary bud development that was stimulated following cytokinin-containing growth regulator applications to both the entire shoot and to terminal buds of 'Carnival' and 'Pink Ice' were removed by hand at regular intervals.

Statistical analysis. Categorical data of flowering incidences in 'Carnival' and 'Pink Ice' as induced by growth regulator treatments, including the assessment of the time of application in 'Carnival' (Tables 1,2 & 5) was subjected to the M-L Chi-Square test using Statistica version 7.1 (Stasoft, 2005). In the M-L Chi-Square tests, flowering incidences of controls was compared to that of growth regulator treatments, whilst comparisons within growth regulator treatments was also performed. Descriptive statistics for inflorescence characteristics (Table 3) were also obtained using Statistica version 7.1 (Stasoft, 2005). Standard analysis of variance was performed using the General Linear Method procedure generated by SAS® program (Statistical

Analysis Systems Institute, 2000, Cary, USA). Logit transformation was performed on the data describing the percentages budbreak and inflorescence initiation (Table 4, 5). Mean separation was accomplished by least significant difference (LSD), where applicable. The SAS® program was also used to fit correlations (Pearson correlation coefficient) and orthogonal contrasts were appropriate (Figure 4).

Results

Cytokinins in xylem sap of 'Carnival' shoots. Levels of cytokinin activity in xylem sap increased from mid June 2001, approximately 80 days before budbreak to peak at a time coincidental with spring budbreak in early September, before significantly declining by mid November (Figure 2).

Growth regulator treatments on 'Carnival'. When the entire shoot length was treated with BA in February or May, apical buds failed to sprout (Table 1). Terminal buds sprouted when the entire shoot was treated in April or when BA was applied in May to the terminal bud alone. In addition to the release of the terminal buds, axillary buds also sprouted in the April treatment, but not in the May treatment along the entire shoot (data not presented). Terminal flushes formed after BA applications in either April or May, but demonstrated variable inflorescence initiation ability (Table 1). Application of BA (500 $mg \cdot L^{-1}$) to terminal buds alone in May resulted in significantly greater floral initiation compared to any other treatments. Budbreak and inflorescence initiation in control shoots that received no BA application was insignificantly low for all treatment dates (Table 1).

Significant floral reversion (40-45%) occurred in shoots treated in April in their entirety with BA solutions of 250 and 500 $mg \cdot L^{-1}$ (Table 2). This floral reversion was indicated by the

development of many involucral-like bracts at the base of the induced terminal spring flush (Figure 3).

Inflorescence development was significantly advanced in BA-induced inflorescences compared to the normal inflorescence initiation phase on the spring flush (Table 3), although the final harvest dates was not recorded. Flowers borne on BA-induced flushes resulting from the April treatment reached commercially harvestable stage first (5 December 2001). The basal diameters of the inflorescences that resulted from the April treatment as compared that of inflorescences of the May treatment 5 weeks later was comparable. Inflorescence dry mass accumulation in inflorescences initiated on shoots treated with BA in May was approximately 50% less than to that of inflorescences initiated with the April treatment (Table 3).

Verification of optimum BA concentration for inflorescence initiation in 'Carnival' with application to terminal buds. Budbreak incidence following any BA application was significantly higher than in untreated control shoots (Figure 4). Budbreak and inflorescence initiation increased linearly with an increase in BA concentration (Figure 4). Budbreak incidence at 250 and 500 $mg \cdot L^{-1}$ BA did not differ significantly at the 5% level(F-value:0.95; Pr>F: 0.3440), but did differ significantly (F-value:28.86; Pr>F: <.0001) from budbreak incidence observed with 50 and 100 $mg \cdot L^{-1}$ BA applications.

BA at 250 and 500 $mg \cdot L^{-1}$ was found to be the most effective concentration to initiate inflorescences on three-flush shoots on 'Carnival' in early May. No inflorescences were induced in autumn on shoots that did not receive BA applications (Figure 4).

BA application to different growth stages of the apical bud. BA treatments were equally effective when applied to the terminal bud when it was at the dormant, swollen or green point growth stages (Table 4). When BA treatments were applied to elongation stages I and II,

inflorescence initiation was significantly lower than the other development stages. Shoot deformities were reported when BA applications were made to developing flushes past an elongation stage of 5 cm (Figure 5).

Growth regulator treatments on 'Pink Ice'. Application of BA as ABG-3062, Promalin® and Accel® solutions to terminal buds alone resulted in significantly more budbreak compared to the untreated control (Table 5). Subsequent inflorescence initiation occurred on ≈ 40% of the flushes induced by ABG-3062 or ≈25% of shoots induced by Accel®, whilst no inflorescence initiation occurred in the Promalin® treated shoots (Table 5).

Inflorescence development on both ABG-3062 and Accel® induced autumn flushes was more advanced than that of inflorescences that initiated on the spring flush of the control.

Discussion

The sharp increase in cytokinin concentration of 'Carnival' xylem sap resembles similar patterns found in other woody perennials (Belding and Young, 1989; Hewett and Wareing, 1973). However, cytokinin peaking values in 'Carnival' were lower than that reported for apple (Cutting *et al.*, 1991; Cook *et al.*, 2001), but is in agreement with results obtained with *Protea aurea* (Collier *et al*., 2000).

Increased cytokinin concentration appears to be responsible for bud sprouting as exogenous cytokinins application is known to release resting buds for new shoot growth (Steffens and Stutte, 1989; Turnbill *et al.*, 1997; O'Hare and Turnbill, 2004).

Shoot growth of 'Carnival' and 'Pink Ice' occurs in distinct flushes with flowers initiating almost exclusively on a spring flush that develops terminally on over-wintering shoots (Gerber, 2000). Flower initiation occurs during early elongation of the spring flush following budbreak (Gerber *et al.,* 2001b), a time that coincides with the peak values of cytokinin determined

(Figure 1). It is possible that inadequate levels of cytokinin in competent shoots may account for the failure of 'Carnival' and 'Pink Ice' to initiate flowers on flushes at other times of the year. This view is supported by the successful inflorescence initiation that can be achieved with exogenous cytokinin application (Tables 1; 5).

Application of BA solutions (50, 250 and 500 $mg \cdot L^{-1}$) to the entire shoot of 'Carnival' in April; or 500 $mg \cdot L^{-1}$ BA to just the 'Carnival' terminal buds in May; and BA applications (250-500 $mg \cdot L^{-1}$) to 'Pink Ice' terminal buds in April followed by a May application led to a significantly higher terminal budbreak than that observed in the control. Furthermore, inflorescences initiated in 'Carnival' in both the 'entire shoot BA treatment' (32%) in April and in 'the May terminal bud BA treatment' (95%), whilst BA treatments (250 and 500 $mg \cdot L^{-1}$) on 'Pink Ice' resulted in a 54% and 42% increase in initiation respectively. The BA-induced increase in inflorescence initiation is noteworthy as shoots following the natural *Protea* phenology rarely terminate in an inflorescence on an autumn flush (Jacobs, pers. comm., 2001).

Cytokinin is considered one of several components in a multi-factorial model for flowering control of angiosperms where different floral initiation factors may become limiting in different plants or under different growth conditions (Bernier *et al.*, 1993). However, the role of cytokinin in the flowering of woody perennials is not as clear as that of herbaceous annuals and appears to be inconsistent (Bangerth, 1997).

Protea phenology has similarities to that of some tropical and subtropical tree crops, such as mango and lychee, where vegetative shoots exhibit periodic extension (flushing) terminated by production of an inflorescence. Reproductive flushes in these crops are generally initiated after extended periods of stem rest in the low-latitude tropics or, immediately following periods of cool night temperatures in the higher latitude tropics or subtropics (Davenport, 2000).

Promotion of flower bud formation and the advancement of flowering time by eight weeks through cytokinin (BA) application have been demonstrated in mango (Chen, 1985). However, application of thidiazuron (TDZ), another growth regulator with high cytokinin-like activity, only released bud dormancy, but did not cause floral induction (Núñez-Elisea and Davenport, 1995). It is possible that in crops exhibiting ephemeral flush growth with periodic shoot dormancy, floral induction may be present before shoot initiation, with floral initiation only occurs after bud sprouting. The cytokinin peak in 'Carnival' that coincides with the onset of inflorescence initiation may therefore be directly involved in the initiation process of competent shoots as part of the floral signal.

BA at a concentration of 250 or 500 $mg \cdot L^{-1}$, when applied to just the terminal bud of three-flush shoots of 'Carnival', was found to be optimal for induction of budbreak and inflorescence initiation (Figure 4). BA applications to the entire shoot in February, soon after extension of the 2^{nd} summer flush, or with late autumn in May, did not initiate any terminal budbreak (Table 1). BA application either in February, when dry mass accumulation in the terminal flush was still minimal, or at the onset of bud rest with the relatively low temperatures in late-May, may have inhibited terminal budbreak.

For 'Carnival', in April, terminal budbreak was consistently induced by BA application, irrespective of the concentration (Table 1). However, floral reversion (Figure 3) was observed where the whole shoot was treated with BA, but not in shoots where only the terminal bud was treated in May (Table 1; Figure 4). Floral reversion is not frequently reported as plants usually initiate flowers under conditions that will result in normal flower development. Incomplete flowering may however be encountered when plants were grown at the limits of their range or grown 'out of season' and exposed to different environmental conditions (Battey and Lyndon,

1990). Though reversion in woody plants is considered to occur rarely, the phenomenon has been observed in the formation of the massive inflorescence of *Protea cynaroides*, under conditions where leaves were under heavy predation pressure (G. Jacobs, pers. com, 2001). Witness to the reversion process in this study on 'Carnival' is the additional bract-like appendages (Figure 3) that developed just above the upper intercalation on reversed shoots. From field observations of *P. cynaroides* and evidence presented here on 'out of season' induced inflorescence initiation, it would appear as if the apical meristem in *Protea* may not become fully committed to flowering until florets begin to form.

Floral reversion may be related to the large number of axillary buds that sprouted with the BA application (data not presented). Diversion of resources away from the apical meristem to sink activities, created by exogenous cytokinin application all along the shoot, may have reduced potential resources available for floral induction and initiation. The importance of both spatial and temporal regulation of cytokinin levels to facilitate flowering has been clearly demonstrated in transgenic *Arabidiopsis* mutants. Over-expression of the hsp70ipt gene that led to cytokinin enrichment of *Arabidiopsis* plants as a whole led neither to earlier, nor enhanced flowering (Medford *et al.*, 1989). Similarly, in *Protea*, the application to cytokinin to the whole shoot was shown to be less successful than the localized stimulation of the terminal bud. It is therefore feasible that BA application should be focused to the terminal bud to secure higher flowering percentages rather than a spray application to the whole plant or shoot. (Table 1).

The actual growth stage of the terminal end of the upper flush also appears to be an important criterion in determining the efficacy of BA to induce inflorescence initiation (Table 4). Based on studies of the apical meristem during the transition from vegetative to reproductive growth, Gerber *et al.* (2001b) established that inflorescence initiation takes place in

the early stages of the elongation of the flush that will subtend the inflorescence. Results from this study (Table 4) restrict the window in which the decision regarding the reproductive or vegetative nature of the succeeding flush is made, to the stage where elongation of the new flush has advanced to approximately 1.5 cm (green point stage). Inflorescence initiation was significantly less successful when BA was applied to the succeeding, more advanced vegetative growth stages (elongation stage I & II) of the new developing flush (Table 4). Application of BA to the new developing flush past an elongation stage of 5 cm resulted in arrested bud growth, shoot deformities ascribed to the release of lateral buds from apical dominance and extreme woodiness of the stem; but not inflorescence initiation (Figure 5).

Similar to our observations in 'Carnival', the differentiation of the shoots into inflorescences in lychee took place soon after the resumption of bud sprouting, when the buds were no more than a few millimetres in length, but not at later stages of shoot elongation (Batten and McConchie, 1995). Likewise, in avocado and macadamia (another member the Proteaceace), the time of induction is believed to occur during early flush development (Olesen, 2005). Furthermore, the buds in lychee seemed unresponsive to florally inductive environmental conditions during the greater part of the quiescent period (Olesen *et al.*, 2002). This raises the question as to whether the dormant bud in *Protea* is able to perceive inductive conditions, even though floral initiation only takes place after bud sprouting.

Whereas cytokinin appears to be promotive for inflorescence initiation, growth regulator combinations containing a high gibberellin to cytokinin content were completely unsuccessful in initiating inflorescences on induced flushes. As a naturally occurring phytohormone, gibberellins have been known to have a strong influence on flowering. Gibberellins promote flowering in many photoperiodic long day- and cold-requiring rosette plants grown under non-

inductive conditions (Bernier, 1988), and have been known to stimulate precocious cone production in gymnosperms (Pharis *et al.*, 1987). However, gibberellins have been identified as potent floral inhibitors exported from the seeds of developing apple fruit, preventing flowering of bourse buds (Bangerth, 1997). Cytokinin derived from leaves and gibberellins exported from the developing fruit may interact to determine the fate of bourse buds. Spurs with more than six leaves are known to overcome the inhibitory effect of fruit on floral development in bourse buds. In addition, the inhibitory influence of gibberellins on floral initiation in apple can be overcome by BA application (McLaughlin and Greene, 1984). The possible role of $GA_{4/7}$ as a vegetative promoter and cytokinin as floral stimulus in 'Pink Ice' are supported by the findings that Promalin® (BA:$GA_{4/7}$; 50:50) failed to initiate flowers on the autumn flush whereas Accel® treatments (BA:GA ; 80:20) effectively caused inflorescence initiation.

Inflorescence initiation on flushes of 'Carnival' and 'Pink Ice' induced by treatment with BA or Accel® in April or May advanced flowering from the 'normal' time of March-May the following year to such an extent that flowers were ready for the Christmas market in the same year as the treatment application (Hoffman, personal observation). It is possible that application of cytokinin to control flowering time in 'Carnival' and 'Pink Ice' may also be effective in related cultivars since flower initiation during the early elongation phase of flush development appears to be a common strategy in proteas (Gerber *et al.*, 2001b). The effective use of cytokinin to initiate inflorescences in spring-flowering *Protea* cultivars, 'Carnival' and 'Pink Ice', without the exposure of the shoot to inductive winter conditions, is a first report for Proteaceace and may have significant commercial potential for *Protea* as a cut flower crop.

Literature cited

Bangerth, F. 1997. Can regulatory mechanisms in fruit growth and development be elucidated through the study of endogenous hormone concentrations? Acta Hort. 463:77-87.

Batten, D.J. and C.A. McConhie. 1995. Floral induction in growing buds of lychee (*Litchi chinensis*) and mango (*Mangifera indica*). Aust. J. Plant Physiol. 22:783-791.

Battey, N.H. and R. F. Lyndon. 1990. Reversion of flowering. Bot. Rev. 56:162-189.

Belding, R.D. and E. Young. 1989. Shoot and root temperature effect on xylary cytokinin levels during budbreak in young apple trees. HortScience 24:115-117.

Bernier, G. 1988. The control of floral evocation and morphogenesis. Ann. Rev. Plant. Physiol. Plant Mol. Biol. 39:175-219.

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

Bernier, G., L. Corbesier, C. Périlleux, A. Havelange, and P. Lejeune. 1998. Physiological analysis of the floral transition. In: K.E Cockshull, D. Gray, G.B.D. Seymour, and B. Thomas (eds.). Genetic and Environmental Manipulation of Horticultural Crops. CAB International, Wallingford, UK. pp. 103-109.

Chen, W-S. 1985. Flower induction in mango (*Mangifera indica* L.) with plant growth substances. Proc. Natl. Sci. Counc. B. Roc. 9:9-12.

Collier, M.D., S. Huitson, and D.E. Hanke. 2000. Altering N-nutrition changes the cytokinin content of leaves of woody plants. Phyton 40:13-16.

Cook, N.C., D.U. Bellstedt, and G. Jacobs. 2001. Endogenous cytokinin distribution patterns at budburst in 'Granny Smith' and 'Braeburn' apple shoots in relation to bud growth. Scientia Hort. 87:53-63.

Corbesier, L., E. Prinsen, A. Jacqumard, P. Lejeune, H. Van Onckelen, C. Périlleux, and G. Bernier. 2003. Cytokinin levels in leaves, leaf exudate and shoot apical meristem of *Arabidopsis thaliana* during floral transition. J. Exp. Bot. 54:2511-2517.

Cutting, J.G.M., D.K. Strydom, G. Jacobs, D.U. Bellstedt, K.J. Van der Merwe, and E.W. Weiler. 1991. Changes in xylem constituents in response to rest-breaking agents applied to apple before budbreak. J. Amer. Soc. Hort. Sci. 116:680-683.

Davenport, L. 2000. Processes influencing floral initiation and bloom: the role of phytohormones in a conceptual flowering model. HortTechnol. 10:733-739.

Gerber, A.I. 2000. Inflorescence initiation and development, and the manipulation thereof, in selected cultivars of the genus *Protea*. Ph.D. diss. Uni. of Stellenbosch, Stellenbosch, S. Africa.

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

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001a. Manipulation of flowering time by pruning of *Protea* cv. Sylvia (*P. eximia* × *P. susannae*). HortScience 36:909-912.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001b. Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars. J. Amer. Soc. Hort. Sci. 126:182-187.

Hettasch, H. and G. Jacobs. 2006. Effect of daylength on flower initiation of *Leucadendron*. Acta Hort. In press.

Hewett, E.W. and P.F. Wareing. 1973. Cytokinins in *Populus* × *robusta*: changes during chilling and bud burst. Physiol. Plant 28:393-399.

Jacobs, G. and G.E. Honeyborne. 1978. Delaying the flowering time of *Leucospermum* cv. Golden Star by deheading. Agroplantae 10:13-15.

Jones, O.P. 1973. Effects of cytokinins in xylem sap from apple trees on apple shoot growth. J. Hort. Sci. 48:181-188.

Malan, D.G. and G. Jacobs. 1994. Influence of day length, irrigation and pruning on shoot growth of *Leucospermum* 'Red Sunset'. J. S. Afr. Soc. Hort. Sci. 4:24-25.

Malan, D.G., J.G.M. Cutting, and G. Jacobs. 1994. The role of the developing inflorescence in the loss of responsiveness to flower inducing short days in *Leucospermum* 'Red Sunset'. J. S. Afr. Soc. Hort. Sci. 4:32-36.

McLaughlin, J.M. and D.W.Greene. 1984. Effect of BA, GA_{4+7} and Daminozide on fruit set, fruit quality, vegetative growth, flower initiation and flower quality in 'Golden Delicious' apple. J. Amer. Soc. Hort. Sci. 109:34-39.

Medford, J.L., R. Horgan, Z. El-Sawi, and H. J. Klee. 1989. Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. Plant Cell 1:403.413.

Núñez-Elisea, R. and T.L. Davenport. 1995. Effect of leaf age, duration of cool temperature treatment, and photoperiod on bud dormancy release and floral initiation in mango. Scientia Hort. 62:63-73.

O' Hare, T.J. and C.G.N. Turnbill. 2004. Root growth, cytokinin and shoot dormancy in lychee (*Litchi chinensis* Sonn.). Scientia Hort. 102:257-266.

Olesen, T. 2005. The timing of flush development affect the flowering of avocado (*Persea americana*) and macadamia (*Macadamia integrifolia* × *tetraphylla*). Aust. J. Agr. Res. 56:723-729.

Olesen, T., C.M Menzel, N. Wiltshire, and C.A. McConchie. 2002. Flowering and shoot elongation of lychee in eastern Australia. Aust. J. Agric. Res. 53:977-983.

Pharis, R.P., J.E. Webber, and S.D. Ross. 1987. The promotion of flowering in forest trees by gibberellin $GA_{4/7}$ and cultural treatments. A review of the possible mechanisms. Ecol. Man. 19:65-84.

Statistical Analysis Systems Institute Inc. 2000. SAS®/STAT User's Guide, Version 9.1, SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

Statsoft Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com

Steffens, G.L. and G.W. Stutte. 1989. Thidiazuron substitution for chilling requirement in three apple cultivars. J. Plant Growth Regul. 8:301-307.

Tromp, J. and J.C. Ovaa. 1990. Seasonal changes in the cytokinin composition of xylem sap of apple. J. Plant Physiol. 136:606-610.

Turnbill, C.G.N., M.A.A. Raymond, I.C. Dodd, and S.E. Morris. 1997. Rapid increases in cytokinin concentration in lateral buds of chickpea (*Cicer arietinum* L.) during release of apical dominance. Planta 202:271-276.

Van Staden, J. and J.E. Davey. 1979. The synthesis, transport and metabolism of endogenous cytokinin. Plant Cell Environ. 2:93-106.

Table 1. Effect of benzyladenine (BA) as ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on percentage budbreak and flowering outcome of three-flush shoots of *Protea* cv. Carnival. BA solutions were applied to the entire shoot in February, April and May 2001 or to the terminal bud alone of the 2nd summer flush in May 2001. Data collection took place on 5 December 2001 when the first BA-induced flowers were at the commercially harvestable 'soft tip' stage.

APPLICATION DATE	22 February 2001		12 April 2001	
APPLICATION SITE	Complete shoot		Complete shoot	
BA ($mg \cdot L^{-1}$)	Budbreak on 2nd summer flush	Flowering	Budbreak on 2nd summer flush	Flowering
0 (control)	5^{a}	0^{a}	10^{a}	5^{a}
50	0^{a}	0^{a}	75^{b}	35^{b}
250	15^{a}	0^{a}	95^{b}	25^{b}
500	10^{a}	5^{a}	100^{b}	35^{b}
APPLICATION DATE	22 May 2001		22 May 2001	
APPLICATION SITE	Complete shoot		Terminal bud	
BA ($mg \cdot L^{-1}$)	Budbreak on 2nd summer flush	Flowering	Budbreak on 2nd summer flush	Flowering
0 (control)	0^{a}	0^{a}	15^{a}	10^{a}
50	0^{a}	0^{a}	0^{a}	0^{a}
250	5^{a}	0^{a}	0^{a}	0^{a}
500	0^{a}	0^{a}	95^{b}	95^{b}

Percentages (n=20) within columns and treatment date followed by superscripts of the same letter are not significantly different at the 5% level, M-L Chi-Square test.

Table 2. Effect of benzyladenine (BA) as ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) concentration as applied to entire shoots (12 April 2001) on inflorescence initiation and floral reversion of *Protea* cv. Carnival. Data is expressed as percentage values of inflorescences subtended by an induced autumn flush; percentage autumn flush shoots showing evidence of flowering buds but which reverted to vegetative flush extension; and percentage inflorescences initiated exclusively on spring flush shoots, that is, shoots which under no circumstances exhibited any floral initiation on the autumn flush.

BA ($mg \cdot L^{-1}$)	Inflorescence initiation on autumn flush	Autumn flush floral reversion	Inflorescence initiation on spring flush
Control (0)	5^{a}	0^{a}	95^{a}
50	35^{b}	5^{a}	60^{b}
250	25^{b}	45^{b}	30^{c}
500	35^{b}	40^{b}	25^{c}

Percentages (n=20) within columns followed by superscripts of the same letter are not significantly different at the 5% level, M-L Chi-Square test.

Table 3. Inflorescence diameter and dry mass of *Protea* cv. Carnival determined when the first inflorescences were at the commercial harvestable stage of 'soft tip' (5 December 2001). BA solutions as ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) at 50, 250 and 500 mg·L^{-1} were applied to the complete shoot on 12 April 2001 or to the apical bud alone (500 mg·L^{-1}) on 22 May 2001. Inflorescence characteristics of the spring flush are the control treatment (no BA application) in which inflorescence initiation followed the natural phenology.

	Number of shoots	Inflorescence diameter (mm)[z]	Inflorescence dry mass (g)
Inflorescence on spring flush (untreated control)	76	19.4 ± 0.76[z]	1.7 ± 0.15[z]
Inflorescence on BA-induced autumn flush			
April application[y]	54	39.9 ± 1.35	23.5 ± 4.41
May application	19	37.6 ± 1.14	11.3 ± 1.18

[y]Data of BA concentrations (50, 250 and 500 mg·L^{-1}) were pooled.

[z] Measured at widest basal portion

Table 4. Effect of benzyladenine (BA) at 500 mg·L^{-1} as MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) on flowering incidence when applied (30 April 2004) to different terminal bud growth stages of *Protea* cv. Carnival.

Growth stage of terminal bud	% Flowering
Dormant	93.3[a]
Swollen	86.7[a]
Green tip	91.7[a]
Elongation stage I	43.3[b]
Elongation stage II	42.2[b]

Values followed by different superscripts differ significantly (p=0.05; LSD test).

Table 5. Effect of benzyladenine (BA) as ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$), Promalin® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 1.8% $^{w}/_{w}$) and Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) solutions on budbreak on the 2nd summer flushes and flowering incidence on the induced autumn flushes of *Protea* cv. Pink Ice. Growth regulators were applied on 4 April 2002 and re-applied on 12 May 2002. Diameter (mm) and dry weight determination (g) of inflorescences born either on an autumn or spring flush were determined on 18 December 2002.

Growth Regulator	Concentration ($mg \cdot L^{-1}$)	Budbreak	Flowering	Flowering flush	Inflorescence diameter (mm) [z]	Inflorescence dry weight (g)
Control	0	1[a]	1[a]	Spring	31.0[b]	10.7[b]
ABG-3062	250	13[b]	7[b]	Autumn	40.8[a]	19.7[a]
ABG-3062	500	12[b]	5[b]	Autumn	44.9[a]	22.6[a]
Promalin®	500	12[b]	0[a]	Spring	34.2[b]	9.4[b]
Accel®	500	13[b]	3[b]	Autumn	39.1[a]	15.9[b]

Data presented are real observations out of 13 shoots for the incidence of budbreak and flowering. Values (n=13) within columns followed by superscripts of the same letter are not significantly different at the 5% level, M-L Chi-Square test for bud break and flowering. Values for diameter and dry weight followed by superscripts of the same letter are not significantly different (p=0.05; LSD test).

[z] Measured at widest basal portion

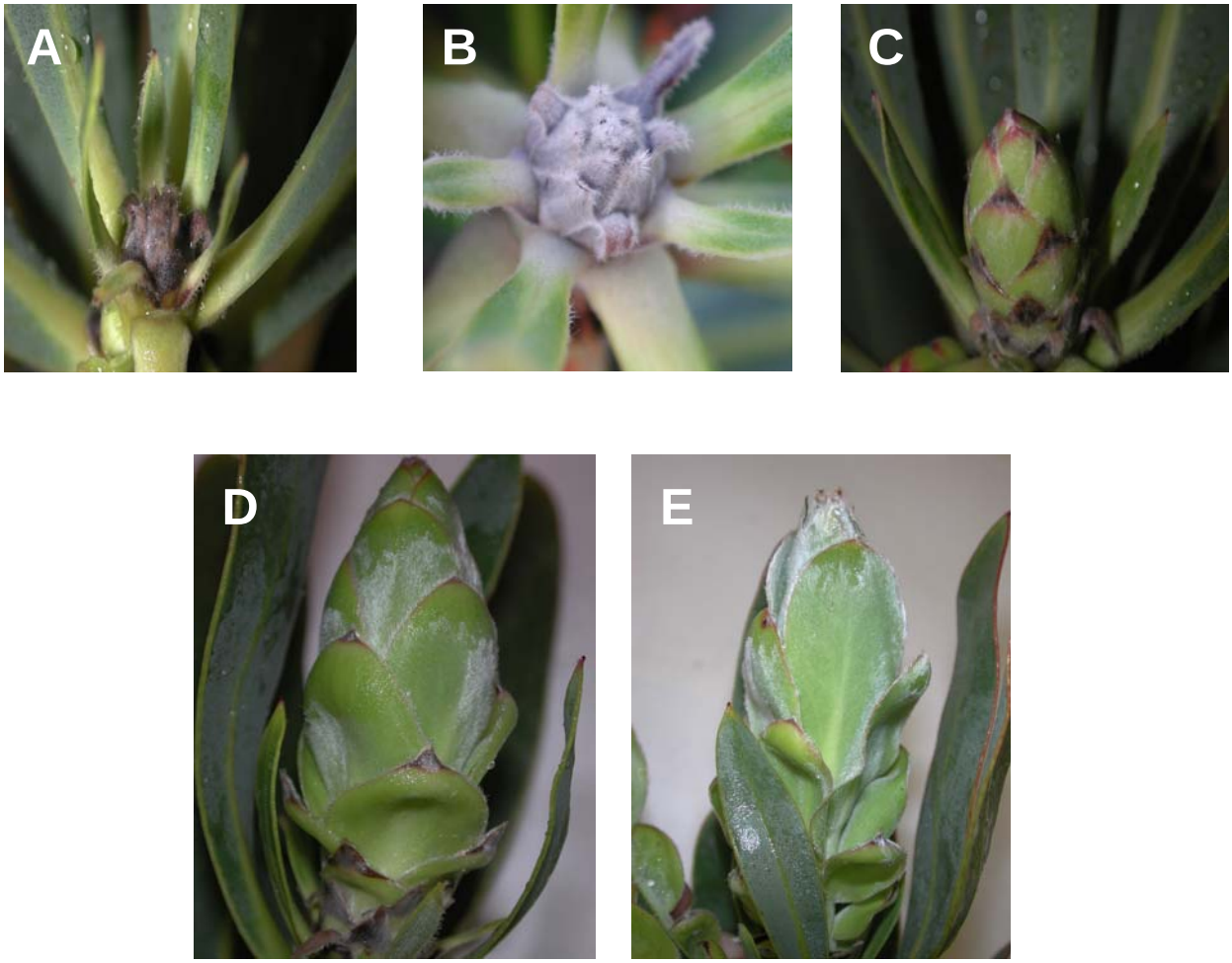


Figure 1. Morphological characteristics of the different stages of the terminal bud development in *Protea* cv. Carnival. A. Dormant B. Swollen C. Green point D. Elongation I E. Elongation II.

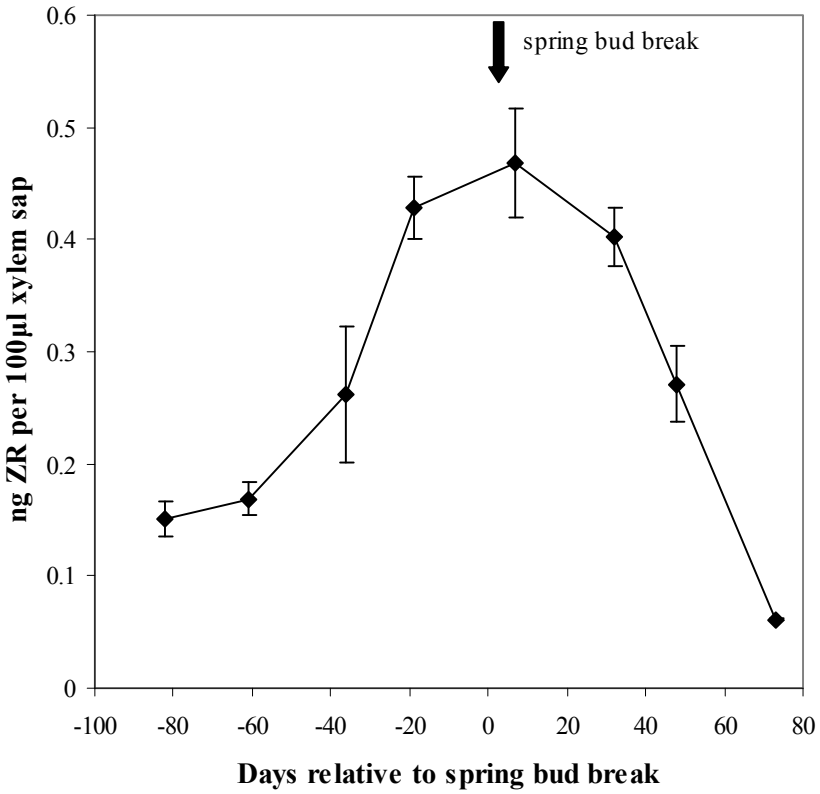


Figure 2. *t*-Zeatin riboside (ZR) concentration (ng·100µL^{-1}) in xylem sap of *Protea* cv. Carnival before, during and after spring budbreak (4 September 2001). Time is expressed as days relative to spring budbreak. Data presented are means of five replicates +/- SE.

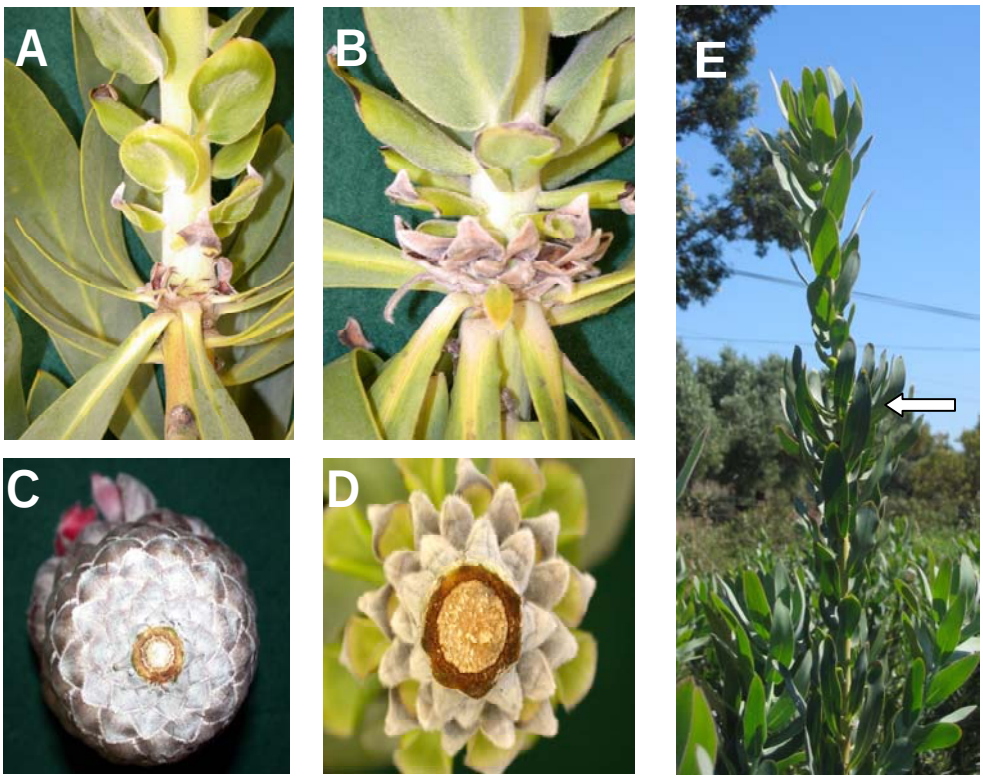
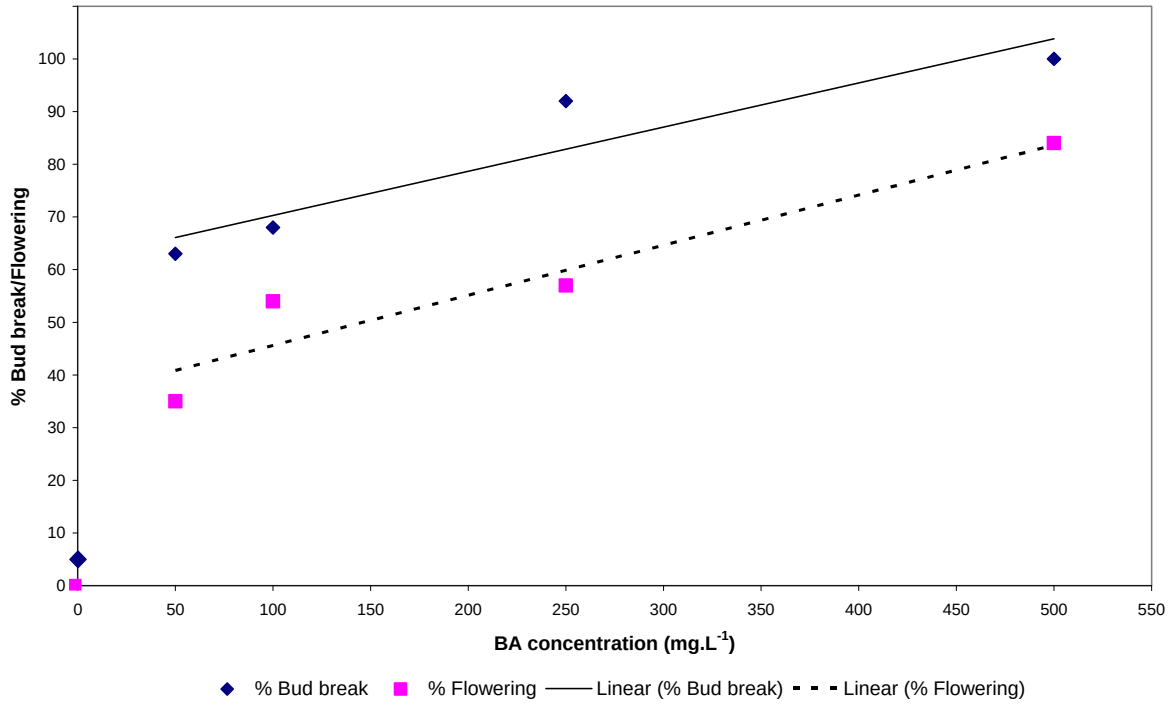


Figure. 3. Floral reversion as observed in *Protea* cv. Carnival when entire shoots were treated with ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) solutions (250 and 500 mg·L^{-1}) on 12 April 2001. A. Intercalation between autumn and spring flushes with no floral reversion observed. Bracts visible are bud scales which protect the terminal bud prior to flush extension. B. Intercalation between autumn and spring flushes where floral reversion was recorded. Bracts visible resemble first involucral bracts of the inflorescence. C. Abaxial view showing involucral bracts of the inflorescence. D. Abaxial view at autumn and spring intercalation on floral reversion. E. Floral reversion as evident from the 'cup-shape' (indicated by the arrow) formation of vegetative leaves typically surrounding an inflorescence.



ANOVA

Source	*F-value*	*Pr>F*	*Significance*
Budbreak			
Control vs BA	187.57	<.0001	***
Concentration linear	12.02	0.0047	***
Flowering			
Control vs BA	362.24	<.0001	***
Concentration linear	9.64	0.0091	***

Figure 4. Effect of BA as MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) applied on 6 May 2005 to the terminal bud alone of *Protea* cv. Carnival shoots on percentage bud break and percentage inflorescence initiation.

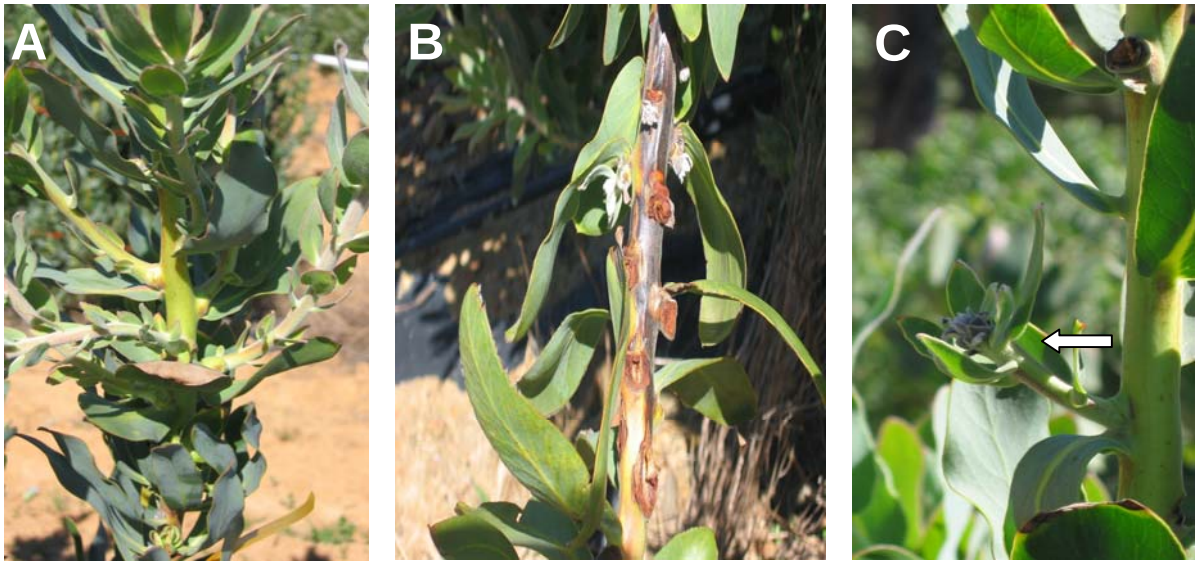


Figure 5. Shoot deformities found on BA-induced flushes of *Protea* cv. Carnival when BA applications as MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) were made to developing flushes past an elongation stage of 5 cm. A. Excessive release of axillary buds from apical dominance. B. Extreme woodiness of the main stem. C. Premature flowering of side-branches.

3. PAPER II. Inflorescence initiation through benzyladenine-containing growth regulator induction and the manipulation thereof to control flowering time in *Protea* cv. Carnival.

Inflorescence initiation through benzyladenine-containing growth regulator induction and the manipulation thereof to control flowering time in *Protea* cv. Carnival.

Abstract

Dormant, terminal buds on *Protea* cv. Carnival shoots were treated in autumn with 500 mg·L^{-1} benzyladenine (BA), prepared by diluting with distilled water either ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) or Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$). To compare the efficacies of the two BA sources to cause 'out of season' flower initiation in autumn, three-flush shoots of eight-year old plants were treated. Two and three flush shoots of 12-year old plants were also compared in their ability to initiate inflorescences 'out of season' following treatment in autumn with a concentration of 500 mg·L^{-1} BA (ABG-3062). All shoots received a single application of growth regulator and treatment dates were spaced two weeks apart, starting 14 March to 22 May 2003. With the exception of the last two treatment dates (May) on two flush shoots, buds on most shoots sprouted in response to the applied BA.

Irrespective of the source of BA, the percentage inflorescence initiation following treatment of three-flush shoots increased progressively with later application dates and percentages as high as 90% was achieved. Low flowering percentages recorded for two-flush shoots were due to a combination of low percentages bud break and inflorescence initiation. Compared to flowers that initiated naturally on the spring flush, flowering times of the early treatments were advanced by more than three months and occurred pre-Christmas, without compromising product quality.

Manipulation of *Protea* flowering time to take advantage of market demand requires an understanding of the mechanisms fundamental to inflorescence initiation and development.

The phenology of *Protea* cv. Carnival (*P. neriifolia* × *P. compacta*), when pruned in a biennial cropping system (Gerber *et al.*, 1995), follows a vegetative growth cycle exhibiting strong seasonality (Greenfield *et al.*, 1994). Pruning of flowering and vegetative shoots back to 15 cm bearers releases lateral buds from apical dominance, which develop vegetatively by elongation of successive growth flushes. Vegetative growth on plants of prime producing capacity is typified by three-flush shoots. These shoots comprise of a spring flush, starting in August/September, and two summer flushes, commencing December and February-April respectively (Greenfield *et al.*, 1994). In addition to the above-mentioned three-flush shoots, depending on the plant vigour, a limited number of shoots may initiate an additional autumn flush. This flush, if present, appears on strong shoots during middle to late April (Hoffman, pers. obs.). In older, less vigorous plants, a full growth season ending in dormancy might often only produce two flushes. During late August to early September, a highly synchronized spring budbreak in both two- and three-flush over-wintering shoots marks the onset on the new vigorous spring growth season. It is then this resultant spring flush that typically terminates in an inflorescence.

The precise factor(s) that control flower initiation in 'Carnival' remains unclear (Gerber, 2000). However, a minimum requirement of at least two vegetative flushes (Greenfield *et al.*, 1994) and the presence of mature leaves on an over-wintering flush (Gerber *et al.*, 2002) appear to be essential before a shoot will initiate an inflorescence.

'Carnival' may show some degree of plasticity in its flowering initiation pattern when the natural synchrony of vegetative flushing is rescheduled through selective pruning treatments. Pruning of 'Carnival' back to bearers in late May (Greenfield *et al.*, 1994), advanced the spring flush by a month compared to other pruning schedules. Subsequently,

inflorescence initiation took place mainly on the 1st summer flush. No pruning treatment could effect inflorescence initiation on the 2nd summer or autumn flushes (Greenfield *et al.*, 1994). Limited leaf area and carbohydrate availability were proposed as a reason for the absence of inflorescence initiation on the autumn flush.

Indeed, autumn flushes rarely initiate inflorescences (G. Jacobs, pers. comm., 2001), even when subtended by a spring and two summer-flushes and where sufficient shoot quality, in terms of leaf number, stem diameter and stem length, apparently exists.

Contrary to the natural flushing cycle of 'Carnival', where most three-flush shoots by late summer and autumn were entering or had already achieved a state of dormancy, budbreak and new flush growth were induced in shoots treated with BA in April and May (Paper I: table 1, pg. 54; table 4, pg.57; figure 4, pg.62). The subsequent cytokinin-induced autumn flush-growth then terminates inconsistently in an inflorescence.

BA-induced inflorescences on the autumn flush may be harvested earlier (November-December) compared to the later peak production time (February-March) of normal spring-initiated inflorescences (Paper I, table 3, pg. 56). The ability of BA to induce a vegetative flush which terminates in an inflorescence outside of the natural flushing cycle, opens up possibilities for greater control of *Protea* flowering time in order to meet market demands.

This study provides information on the composition of a suitable cytokinin-containing growth regulator, the relevance of application times and the shoot characteristics considered favourable to inflorescence initiation in *Protea* cv. Carnival. Inflorescence quality and harvest distribution under cytokinin induction in autumn is compared with spring-initiated inflorescences following the natural flushing cycle. The possible commercial application of cytokinin growth regulators to advance flowering time of 'Carnival' in South Africa to meet the peak European winter demand, is explored.

Materials and Methods

Plant material. Experiments on *Protea* cv. Carnival were carried out in commercial plantations located in the Stellenbosch district (33°55'S; 18°50'E), South Africa. The climate is Mediterranean-like with cool, wet winters and hot dry summers. The annual rainfall is 600-700 mm, concentrated during the winter months. All plants had been pruned back to bearers in August 2002 to effect biennial cropping as described by Gerber *et al.* (1995). Plants were spaced 1m in the row and 4m between rows. Plants were not irrigated or fertilized. Pest control focused on the control of thin-line leaf miner (*Phyllocnistis* sp.) and the speckled protea borer (*Orophia* spp.) and were applied by risk analysis which included the emergence and extension of the immature flush and during early inflorescence development**.**

Growth regulator application. To compare the effacicies of BA from two different sources, three-flush shoots of eight-year old 'Carnival' plants were used. Growth regulator treatments consisted of either a single application of aqueous 6-benzyladenine (BA) as ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$; Abbott Laboratories, North Chicago, USA) at a concentration of 500 mg·L^{-1}, or Accel® at a concentration of 500 mg·L^{-1} (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$; Valent Biosciences, Illinois, USA). No surfactants were needed to facilitate applications. Growth regulator solutions were applied to the dormant terminal bud using a paint brush on 14 March, 26 March, 9 April, 23 April, 7 May, and 21 May 2003. Distilled water was used as a control treatment. At each treatment date stem diameters (mm) were measured between the upper position at the intercalation of the1st and 2nd summer flush. Ten shoots were used per treatment. Treatments were replicated five times in a randomized complete block design.

In a second trial where the efficacy of BA was evaluated on shoots of different quality, two-and three-flush shoots of 12-year old 'Carnival' plants were used. The 12-year old plants

differed from the eight-year old plants with respect to tree complexity and reduced growth vigour. Growth regulator application date and methods were similar to the trial performed above on the eight-year old plants, except that ABG-3062 was the only growth regulator that was used. Distilled water was again used as the control treatment. At each treatment date, stem diameters (mm) at the upper position of the intercalation of the spring and summer flushes and 1st and 2nd summer flushes were measured for two- and three-flush shoots respectively. Ten shoots were used per treatment. Treatments were replicated five times in a randomized complete block design.

Field observations. Developmental stages of the terminal bud, or developing flush, for all control- and treatment shoots were observed on a two-weekly basis from date of growth regulator application until initiated inflorescences reached the commercially viable 'soft tip' stage, signalling harvest time. Budbreak and inflorescence initiation for each treatment as well as the harvest date of control and growth regulator-induced inflorescences were recorded.

Temperature was recorded as air temperature on an hourly basis for the whole duration of the experiment and was from the Somerset West-Stellenbosch area, as a courtesy from the Deciduous Fruit Producers Trust (DFPT), South Africa.

Induced flush characteristics and inflorescence quality. Shoot characteristics and inflorescence quality of ten representative growth regulator-induced inflorescence-bearing and control shoots were assessed at harvest. Shoot characteristics assessed were dry mass (g) of the separated stem and leaves. Inflorescence quality parameters included basal diameter (mm) and inflorescence length (mm) (measured from the inflorescence base to involucral bract tips), number of florets and involucral bracts, and inflorescence dry weight (g) (60°C; 72h).

Colour measurements on inflorescences were performed immediate post-harvest using a colour-guide 45°/0° colorimeter (Cat no. 6805; BYK-Gardner, USA) in the upper 8cm region of the involucral bract tips using five representative shoots per treatment. Chromaticity coordinate, a* (red-green range) was determined with use of the CIELab colorimetric space (MINOLTA, 1998.)

Statistical analysis. Standard analysis of variance was performed on the data using the General Linear Model Procedure generated by the SAS® program (SAS Institute, 2000). LSMeans and LSD values were calculated at a 5% significance level. The SAS® program was also used to fit regressions (Pearson correlation coefficient) and orthogonal contrasts. Logit transformation of data was performed on all values expressed as percentages. Descriptive statistics were obtained through the use of Statistica version 7.1 (Statsoft, 2005).

Results

Effect of growth regulators on budbreak. Accel® and ABG-3062 application in autumn effected a significant higher percentage budbreak (≥92%) on three-flush 'Carnival' shoots compared to autumn budbreak percentages (24%) of untreated controls (Figure 1A). However, there was no significant difference in percentages budbreak between shoots treated with growth regulators in autumn and spring budbreak percentages of untreated control (Figure 1A). Furthermore, no significant difference in percentage budbreak was found between growth regulators. No interaction between time of growth regulator application and percentage budbreak (Figure 1A) or significant correlation between average temperature from application to budbreak and budbreak incidence was found over application time (Figure 1B).

Budbreak in ABG-3062-treated two- and three flush shoots of 12-year old 'Carnival' plants was significantly higher than that of untreated two- and three flush control shoots initiated in autumn (6% and 24% respectively) (Figure 2A). A quadratic relationship

between the time of ABG-3062 application and budbreak incidence of both two- and three-flush shoots was found to describe decreasing budbreak (Figure 2A).

Although the average temperature recorded between growth regulator application and budbreak for two- or three-flush shoots of 12-year old plants decreased over time, there was no significant correlation (5% level) between temperature and percentage budbreak over the treatment period (Figure 2B).

Effect of growth regulators on inflorescence initiation. Percentage inflorescence initiation increased linearly with time in three-flush shoots on eight-year old plants treated with either Accel® or ABG-3062 application (Figure 3A). Inflorescence initiation on growth regulator-induced shoots was significantly greater than that recorded for the control (2%). No significant difference in inflorescence initiation was found between the two formulations of growth regulator applications on the induced autumn flushes (Figure 3A). The temperature decrease observed from green point to cessation of the induced flush elongation, the period during which inflorescence initiation is known to occur (Gerber *et al.*, 2001a), was negatively correlated with the percentages of flowering observed over time (Figure 3B).

ABG-3062 application to three-flush shoots of 12-year old plants resulted in increased inflorescence initiation, described by a quadratic relationship over time (Figure 4A). Inflorescence initiation varied from 16% (14 March) to 92% (21 May). In comparison, low (2%) inflorescence initiation occurred on the 24% three-flush shoots of the control which naturally initiated an autumn flush (Figure 4A).

Inflorescence initiation in two-flush shoots subjected to ABG-3062 treatment was significantly lower than that of three-flush shoots (Figure 4A). ABG-3062-induced inflorescence initiation on two-flush shoots was described by a cubic relationship over time (Figure 4A). Two-flush shoots did not initiate inflorescences on the induced autumn flush for the first three treatment dates. In the late April application date (23 April), inflorescence

initiation increased to an average of 30% in the two-flush shoots, before declining in the May treatment dates (Figure 4A). When data is expressed as percentage flowering of shoots that successfully initiated an autumn flush with ABG-3062 application (Figure 2A), instead of percentage flowering of the total treatment population (n=50), the percentage flowering incidence increases from 8 to 10.5% and 22% to 64.7% for 7 May and 21 May application dates respectively. No inflorescences initiated spontaneously in autumn on two-flush shoots, compared to an 80% flowering incidence resulting from normal inflorescence initiation subsequent to spring flush elongation (Figure 4A). Although the temperature declined for the period 'green point to cessation of induced flush elongation' for the respective application dates (Figure 4A), there was no significant correlation with flowering incidence and temperature for that period at the 5% level for either two- or three-flush shoots (Figure 4B).

The trend in percentage flowering for both Accel® and ABG-3062 treatments with time was significantly correlated with the intercalation diameter of the three-flush shoots of eight-year old plants at the time of treatment (Figure 5A). Stem diameter of two-flush shoots, prior to growth regulator application was significantly lower than that of three-flush shoots for all treatment dates (Figure 5B). This smaller stem diameter of two-flush shoots is reflected in a lower percentage flowering compared to that of three-flush shoots (Figure 5B). Two-flush shoots were only able to initiate inflorescences in up to 30% of shoots on the last three treatment dates (Figure 5B). Stem diameter of both two- and three flush shoots on 12-year old plants prior to growth regulator treatment was not correlated with flowering success (Figure 5B).

Harvest distribution. Harvest time for inflorescences initiated on three-flush shoots of eight-year old plants with Accel® and ABG-3062 application on the first (14 March) and last treatment date (21 May) respectively reached the commercially harvestable stage approximately 99 and 30 days earlier than the normal harvest time recorded for comparable

untreated three-flush shoots in February 2004 (Table 1). Harvest times subsequent to successful inflorescence initiation with ABG-3062 application (23 April, 7 May and 21 May) on two-flush shoots of 12-year old plants peaked approximately 79 to 31 days earlier than in two-flush untreated control shoots (Table 2). BA-initiated inflorescences on three-flush shoots of 12-year old plants harvested approximately 95 to 31 days earlier than three-flush control shoots (Table 2).

The harvest distribution indicated that harvest of flowers initiated by earlier treatment dates to be more condensed than that of treatment dates. Harvest of all inflorescence-bearing three-flush shoots treated with Accel® and ABG-3062, with the exception of the ABG-3062 treatment dates of 28 March and 9 April, were focused to the extent that 80% of the harvest was completed within 14 days or 27-30 days for the first two and last four treatment dates respectively (Table 1). Harvest distribution of both two-flush untreated control and BA initiated shoots was focused so that 80% of the harvest was completed within 27-28 days. The total harvest of three-flush shoots treated with ABG-3062 in March and early April, was completed within 13-16 days (Table 2). For later treatment dates, 80% of the harvest of three-flush shoots was completed within 27-28 days which was similar to the 30 days required to harvest 80% of three-flush control shoots (Table 2).

No significantly difference (5% level) was detected between Accel and ABG-3062 treatments with respect to the number of days that were required from the date of application to harvest for inflorescences that was initiated on three-flush shoots of eight-year old plants (Figure 6A). An average of 240 days from application to harvest was calculated for Accel and ABG-3062 (Figure 6A), with no linear or quadratic relationship emerging between treatment dates. In two-flush shoots of 12-year old plants, ≈255 days was required from the date of application to harvest compared to the ≈248 days that was required for inflorescences

developing on three-flush shoots on the same age plants (Figure 6B), with no linear nor quadratic trend observed between treatment dates.

Characteristics of the induced flush and inflorescence quality. No significant difference in subtending flush characteristics or inflorescence quality was found between Accel® and ABG-3062 treatments for any of the measured parameters and therefore a mean value of these treatments are presented in Table 3. At harvest, the growth regulator-induced flush had a significantly greater mean stem diameter than that measured for control shoots (Table 3). The significantly lower total dry mass of the induced flushes could be attributed to the lower leaf dry mass, as no differences were found in stem dry mass between the treatment and the control flush. The terminal flush characteristics of stem and total dry mass did not differ between application dates, but shoot diameter and leaf dry mass increased in a quadratic or linear relationship respectively over time.

Except for inflorescence length, significant differences were recorded between all the inflorescence parameters measured on growth regulator-initiated inflorescences on the autumn flush versus spring-initiated control inflorescences (Table 3). Inflorescences initiated on control shoots in spring were smaller (lower dry weight and smaller diameter) and less dense (lower number of involucral bract and florets) than inflorescences induced by cytokinin application onto an autumn flush. The inflorescences on control shoots were less intensely coloured (less red) than inflorescences induced by the two earliest treatment dates (March), but not to later treatment dates.

There was no significant difference in inflorescence length ($p=0.2179$) or number of involucral bracts ($p=0.2147$) between inflorescences initiated on two- and three-flush shoots of 12-year old plants (Table 4). Inflorescences that initiated on the ABG-3062-induced autumn flush of two-flush shoots had a significantly smaller basal diameter ($p<0.0001$), a significantly lower number of florets ($p=0.0078$) and a significantly reduced total dry weight

($p<0.0001$) compared to inflorescences induced by ABG-3062 on three-flush shoots (Table 4). No relationship could be established between the most of the characteristics of the inflorescences that induced on two-flush shoots and the treatment dates, with the exception of the involucral bracts that decreased in a quadratic relationship with later treatment dates. For inflorescence that was initiated on three-flush shoots, the inflorescence length, number of involucral bracts and dry weight varied linearly with application dates, whereas inflorescence diameter and number of florets changed in a quadratic relationship with application dates (Table 4).

Delayed spring harvest time of vegetative growth regulator-induced autumn shoots. Green point on non-flowering, growth regulator-induced autumn flushes of eight-year old plants was observed in the spring to occur 18 to 31 days later than green point in untreated, control shoots (Table 5). Subsequently, a later harvest date of up to 10 days may then occur for spring-initiated inflorescences on growth regulator treated shoots when compared to the untreated control.

Onset of the spring flush (green point) of shoots of 12-year old plants that did not flower after treatment with BA in autumn was, in most cases, later than the onset of the spring flush for control shoots (Tables 5, 6). However, in spite of the delay to reach the green point stage, the harvest time for inflorescences that developed on the spring flush of the growth regulator-treated two-flush shoots was in close proximity or earlier than that of the two-flush control shoot (Table 6). In three-flush shoots, the harvest time of the spring-flush induced inflorescence of the first and last treatment date, was some 13 to 17 days later than of the control, whilst other treatment harvest dates were similar to that of the control (Table 6).

Discussion

Effect of growth regulators on budbreak. The first stage of inflorescence development, the differentiation of the involucral bracts, is believed to occur soon after budbreak (Gerber

et al., 2001a) when buds are only a few millimetres in length (Paper I, table 4, pg. 57). The timing of this phase of the vegetative cycle within inductive conditions is of critical importance as shoots that have not been induced either before or during early elongation will remain vegetative.

The terminal bud rest observed in 'Carnival' shoots between successive flushes and over an extended period starting in late summer through winter to early-spring, can be defined as paradormancy (Lang *et al.*, 1987). Results from this study show that terminal bud rest in most three-flush shoots may be overcome by growth regulators such as BA. In contrast, only 24% of three-flush untreated control shoots acquired the intra-plant state in the autumn necessary to produce a flush unassisted compared to the almost 100% incidence with the natural spring budbreak (Figures 1, 2).

A decrease in temperature was found to be an important factor in determining the time required from application to budbreak for 'Carnival' (Paper III, figure 1, pg.122). However, temperature was not a determining factor in the incidence of budbreak of three-flush shoots (Figure 1B). The regulation of dormancy during autumn in 'Carnival' appears to be controlled by factors outside the bud, but within the plant.

An interaction between time of application and possible shoot assimilate availability emerge as a variable in determining the reactivity of a shoot to respond to BA application for induced budbreak (Figure 2A). Two-flush shoots treated in May demonstrated an increasing unresponsiveness to BA application. Not only was the additional flush on a three-flush shoot found to be beneficial in reducing the number of days from application to green point in three-flush shoot (Paper III, figure 2, pg. 123), but it also appeared to promote budbreak in late autumn compared to that of two-flush shoots (Figure 2A).

The cause of the unresponsiveness of two-flush shoots to flush with BA in late autumn is uncertain. Temperature may be implicated, but is only correlated with percentage

budbreak incidence at the 10% significance level (Figure 2B). Alternatively, the inability of two-flush shoots to react to BA application may reflect a low inherent vigour, with a possibility that these shoots may only respond to application of higher BA concentration levels. Vegetative development in *Banksia hookeriana* was found to be strongly affected by temperature (Rieger and Sedgley, 1996). Limited vegetative flushing with no growth extension occurred under a controlled environmental regime of 8 or 16 hour day length and low temperatures of 15/10°C (day/night).

Effect of growth regulators on inflorescence initiation. The number of sequential steps required for woody plant floral initiation is apparently greater than that necessary for many herbaceous annuals, where one promotive factor, such as day-length, may predictably and repeatedly induce flowering (Jackson and Sweet, 1972). The floral initiation process of most woody perennials appears to be preferably dictated by intrinsic factors with environmental stimuli considered to be more effective enhancers than inducers of flowering (Bangerth, 1997).

The increase in percentage of three-flush shoots initiating inflorescences with later growth regulator treatment dates (Figures 3A, 4A) could be related to one, or both, of the following factors. Firstly, the later the date of application, the longer the interval from completion of the 2nd summer flush extension until application, thereby implicating enhanced maturity. The vegetative flushing phenology of *Protea* has similarities to tropical and subtropical crops such as mango, lychee, citrus and avocado. In these crops, management of flowering entails management of shoot initiation. Vegetative growth is initiated with budbreak and flowering occurs if induction is coincidental with this early stage of shoot initiation (Davenport, 2000). Vegetative flush production is stimulated following a series of frequent flushing as would typically arise in young, vigorous plants or plants grown under a regime of high nitrogen levels with an abundance of water. Reproductive flushes are

favoured when shoot initiation takes place on resting stems of sufficient maturity (Davenport, 2003).

Secondly, apart from flush maturity, it has been shown in lychee, mango and Mediterranean woody perennials such as olive and macadamia (an Australian evergreen of the Proteaceace family) that if high temperatures prevail prior to inflorescence initiation, flowering incidence is reduced. In a recent review on the macadamia physiology, Huett (2004) found low temperature, particularly in late summer to early autumn, to be important to promote flower bud initiation. Moncur *et al.* (1985), stated that floral initiation in macadamia occurred during May (day length 10h 40-50 min), with minimum temperatures between 11°C and 15°C, followed by a dormant bud phase lasting 50-96 days. Flower bud dormancy release occurred earliest at the cooler sites, after a rise in temperature and good rain. In olive, the floral stimulus is also believed to be induced by exposure of the shoot to cold conditions (Hackett and Hartmann, 1967) while for lychee, a combination of low temperatures (15°C/17°C or 18°C/13 °C, day/night) and advanced vegetative maturity were prerequisites for floral initiation to occur (O'Hare, 2002). Critical temperatures in mango for floral induction in potted trials were below 20°C (day) and 15°C (night), with the reliability of induction increasing when night temperatures were held at 10°C (Núñez-Elisea and Davenport, 1991). Furthermore, growth of induced buds during exposure to cool temperature, appeared necessary for floral initiation. Buds that resumed growth under warm temperatures (28°C/22°C, day/night) after receiving a cool temperature inductive treatment, produced vegetative flushes (Núñez-Elisea and Davenport, 1995).

Gerber *et al.* (2001a) has shown that inflorescence initiation in *Protea* occurs soon after bud sprouting and that involucral leaves differentiate during elongation of the flush that subtends the inflorescence. It is therefore argued that temperatures that prevail from the time of growth regulator application up to budbreak or when the terminal bud has extended up to

5cm (Paper I, table 4, pg.57), may affect floral initiation in 'Carnival'. The significant negative correlation between percentage inflorescence initiation and mean daily temperatures for the particular application date in three-flush shoots of productive eight-year old plants support the argument that high temperatures may be the reason for the poor flowering response on early growth regulator application dates.

In this study 'Carnival' is observed to initiate inflorescences from March (under cytokinin regulation) to September (natural initiation cycle). The photoperiod of this initiation window falls approximately within the dates of equinox where day length is ≤12 hours. Floral initiation for 'Carnival' outside this photoperiod window in similar three-flush shoots in its natural vegetative cycle is the exception and even under BA induction it is poor and infrequent (unpublished data). Though flowering does not appear to be under photoperiodic control in most woody perennials (Chuine *et al.*, 1999), photoperiod has been known to play a role in the floral induction of other Proteaceace members. *Serruria florida* requires the natural short days of winter for flowering (Malan and Brits, 1990), whilst *Leucospermum patersonii* has been classified as an obligatory long-short day plant under moderate temperature regimes (22°C/17°C, day/night) (Wallerstein, 1989). In the latter study it was found that the short-day requirement could be overcome by low temperatures (17°C/12 °C, day/night) under non-inductive long-day conditions.

Budbreak percentages for two-flush shoots were comparable to that of three-flush shoots for the first four application dates. However, no flowering was recorded for two-flush shoots at the first three application dates. Since both two-and three-flush shoots were exposed to the same environmental conditions and exhibited comparable shoot maturity (Paper IV), a further explanation, in addition to temperature, photoperiod or flush maturity should be considered.

Leucospermum 'Red Sunset' is known to be photoperiod sensitive (Malan and Jacobs, 1990), but light intensity (solar radiation) was also found to be important as heavy shading, applied during summer, reduced the number of stems forming an inflorescence (Jacobs, 1983). Jacobs (1983) also observed long stems to be less detrimentally affected by non-inductive low light treatments than shorter shoots. This is of particular significance as the importance of shoot quality for successful inflorescence initiation was also observed here for 'Carnival' when two-flush and three-flush shoots of 15-year old plant were compared.

Current photosynthates and the presence of leaves have been identified as key requirements for floral initiation in *Protea* (Gerber *et al.*, 2001b; Gerber *et al.*, 2002). Therefore, the better flowering response achieved by three-flush shoots compared to two-flush shoots could be related to the presence of more leaves and thus a better photosynthate supply. The poor flowering of two-flush shoots when BA was applied on the two last treatment dates was largely due to poor budbreak (Figure 2A). However, even when the percentage inflorescence initiation is expressed as a percentage of shoots with BA-induced budbreak, floral initiation was still significantly lower than that of three-flush shoots. Thicker shoots with three growth flushes had a higher propensity to initiate inflorescences with the cytokinin treatment than thinner three-flush or two-flush shoots (Figure 5). This appears to be particularly true for the earlier treatment dates, when shoots were exposed to potentially suboptimal inductive conditions of higher temperatures and longer days. The importance of intercalation diameter and other shoot characteristics such as leaf number and the position of the leaves to the terminal bud in determining the responsiveness of the shoot to BA application will receive more attention in Papers IV and V.

Harvest distribution. The harvest distribution of BA-induced inflorescences for three- (Table 1) and two-flush shoots (Table 2) was concentrated so that 80% of the harvest of all treatment dates was completed within a month. A short flowering period for spring-initiated

inflorescences is not unusual for the western-Cape production area. A focused flowering time resulting from a known and controllable trigger such as a growth regulator application, ensures predictability of harvest time, a desirable trait in the design of management strategies to align a product to market demand.

Inflorescences from earlier treatment dates were the most advanced compared to the control. However, the number of days that were required from growth regulator application to harvest was not significantly different between the treatment dates (Figure 6). Shoots that receive an early growth regulator application had a relatively short response time to bud break, but required a longer inflorescence development period than later treatment dates. In contrast inflorescences that resulted from later treatment dates needed a longer time to bud break and a shorter inflorescence development period (Paper III).

Characteristics of the growth regulator-induced flush and inflorescence quality. Growth-regulator-induced flush and inflorescence appearance is similar or even enhanced when compared to inflorescences borne on the spring flush of untreated shoots (Hoffman, pers. obs.). The thicker terminal stems that resulted from the cytokinin treatment did not lead to heavier flushes as the leaves on the induced flushes made a smaller contribution than was the case in control shoots (Table 3).

Inflorescences that resulted from BA treatments had a more complex appearance with an increased number of appendages and a more intense colour than control inflorescences, especially when treated early (Tables 3, 4). Improvement of flower quality of *Leucospermum* by a single application of a low concentration BA solution prior to floret initiation was also achieved by increasing shoot diameter and the number of florets initiated (Napier *et al.*, 1986).

An early BA application on three-flush shoots of 'Carnival' induced a harvestable product as early as November compared to the normal spring flowering time of late February

(Table 1). This harvest date is desirable as it falls within the margins for Christmas (25 December). Flowers destined for this holiday period, could then take advantage of travelling by sea container, as opposed to the more expensive air freight. This may present significant savings to the South African grower. However, in earlier application dates, where bud extension and inflorescence initiation proceeds under proposed suboptimal inductive conditions, a reduced flowering incidence is obtained compared to later treatment dates (Figures 3, 4). The later, but more successful treatment dates from the end of April through to the end of May, position the resulting harvest dates to fall well within production deadlines for Valentine's day, even for the slower developing two-flush shoots (Tables 1, 2). Application of BA and BA-related growth regulators may indeed be valuable tools to advance flowering time in 'Carnival'.

Delayed spring harvest time of vegetative growth regulator-induced autumn shoots. If BA application does not induce flowering in the autumn flush, there is a significant delay in the spring budbreak of those shoots whose total length was extended by an additional vegetative autumn flush compared to the untreated control (Tables 5, 6). This lag period recorded for spring budbreak of up to a month, is reduced over the inflorescence development period, so that harvest times in those shoots were delayed, but only up to one or two weeks compared to the control shoots. However, in 'Carnival', this treatment-induced delay could still make a significant impact on prices if normal flowering time is postponed to beyond Valentines' day, a time when demand for flowers is at an all-time low.

Alternatively, the delay in flowering time imposed by BA on shoots not responding to the treatment can possibly be exploited to manipulate the flowering time of those cultivars whose flowering period immediately precedes that of an optimum marketing window. Such cultivars may include 'Brenda' or 'Lady Di' where harvests peak from April to July. Delayed budbreak, together with retarded inflorescence development during winter, may possibly

shift the harvest period into the optimum European marketing window which starts in September.

To conclude: The efficacy of BA to induce 'out of season' flowering is affected by the percentage buds induced to sprout, and may also be affected by any one or a combination of the following: flush maturity, number of leaves, shoot diameter and temperatures during the period from treatment to budbreak.

Inflorescence quality is not compromised by the exogenous cytokinin application and harvest time is focused. Inflorescence initiation induced by BA application may provide opportunities for the grower to control *Protea* flowering time to meet market demands.

Literature cited

Bangerth, F.K. 1997. Can regulatory mechanism in fruit growth and development be elucidated through the study of endogenous hormone concentrations? Acta Hort 463:77-87.

Chuine, I., P. Cour, and D.D. Rousseau. 1999. Selecting models to predict the timing of flowering of temperature trees: Implications for tree phenology modeling. Plant Cell Environ. 22:1-3.

Davenport, T.L. 2000. Processes influencing floral initiation and bloom: the role of phytohormones in a conceptual flowering model. HortTechnol. 10:733-739.

Davenport, T.L. 2003. Management of flowering in three tropical and subtropical fruit tree species. HortsSience 38:1331-1335.

Gerber, A.I. 2000. Inflorescence initiation and development, and the manipulation thereof, in selected cultivars of the genus *Protea*. Ph.D. diss. Univ. of Stellenbosch, Stellenbosch, S. Africa.

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.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001a. Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars. J. Amer. Soc. Hort. Sci. 126:182-187.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001b. The role of leaves and carbohydrates in flowering of *Protea* cv. 'Lady Di'. HortScience 36: 905-908.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2002. Defoliation alters spring growth flush characteristics and inhibits flowering in *Protea* cv. Carnival. Scientia Hort. 94:345-350.

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. 4:42-46.

Hackett, W.P. and H.T. Hartmann. 1967. The influence of temperature on floral initiation in the olive. Physiol. Plant 20:430-436.

Huett, D.O. 2004. Macadamia physiology review: A canopy light response study and literature review. Austr. J. Agri. Res. 55:609-624.

Jackson, D.I. and G.B. Sweet. 1972. Flower initiation in temperate woody plants. Hort. Abstr. 42:9-24.

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

Lang, G.A., J.D. Early, G.C. Martin, and R.L. Darnell. 1987. Endo-, Para-, and Ecodormancy: Physiological terminology and classification for dormancy research. HortScience 22:371-377.

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. and G. Jacobs. 1990. Effect of photoperiod and shoot decapitation on flowering of *Leucospermum* cv. Red Sunset. J. Amer. Soc. Hort. Sci. 115:131-135.

MINOLTA. 1998. Precise colour communication. Minolta Corp., Ramsey, New Jersey. pp1-59.

Moncur, M.W., R.A. Stephenson, and T. Trochoulias. 1985. Floral development of *Macadamia integrifolia* Maiden & Betche under Australian conditions. Scientia Hort. 27:87-96.

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

Núñez-Elisea, R. and T.L. Davenport. 1991. Effect of duration of low temperature treatment on flowering of containerized 'Tommy Atkins' mango. HortScience 26:751.

Núñez-Elisea, R. and T.L. Davenport. 1995. Effect of leaf age, duration of cool temperature treatment, and photoperiod and bud dormancy release and floral initiation in mango. Scientia Hort. 62:63-73.

O'Hare, T.J. 2002. Interaction of temperature and vegetative flush maturity influences shoot structure and development of lychee (*Litchi chinensis* Sonn.) Scientia Hort. 95:203-211.

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

Statistical Analysis Systems Institute Inc. 2000. SAS®/STAT User's Guide, Version 9.1, SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

Statsoft Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com

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

Table 1. Harvest distribution of *Protea* cv. Carnival inflorescences on eight-year old plants as initiated by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 solutions (500 mg·L^{-}1; active ingredient: BA 2% $^{w}/_{w}$) over treatment dates. Distilled water was used as a control. Values are expressed as percentages of the total harvest. Shaded blocks represent the three peak harvest dates for each treatment. The advancement of flowering time represents the difference in days between the dates when the cumulative number of stems harvested was ≥60% for control and treated shoots.

		HARVEST DATES 2003-2004												
TREATMENT	**Advancement of harvest (days)**	**03-Nov**	**10-Nov**	**17-Nov**	**24-Nov**	**01-Dec**	**12-Dec**	**28-Dec**	**12-Jan**	**25-Jan**	**11-Feb**	**23-Feb**	**01-Mar**	**08-Mar**	**17-Mar**
Control										2.2	**50**	**41.3**	**4.3**	0	2
Accel®															
14-Mar	99	**16.7**	**50**	**16.7**	4.2	4.2	8.3								
28-Mar	96	10.8	**45.9**	**29.7**	**13.5**										
09-Apr	72		3.2	3.2	3.2	**25.8**	**51.6**	**12.9**							
23-Apr	59				**10.5**	2.6	**55.3**	**28.9**	2.6						
07-May	44							**36.84**	**52.63**	**10.53**					
21-May	30							2.3	**61.4**	**27.3**	**9.1**				
ABG-3062															
14-Mar	99	**8.3**	**83.3**	**8.3**											
28-Mar	85	8.6	14.3	**22.9**	**31.4**	**20**	2.9								
09-Apr	59			4.76	4.76	**14.29**	**42.86**	**19.05**	9.52	4.76					
23-Apr	66				4.8	**21.4**	**40.5**	**26.2**	7.1						
07-May	44						3.2	**25.8**	**54.8**	**16.1**					
21-May	30					2.2	0	4.3	**32.8**	**42.6**	**11.5**	6.5			

Table 2. Harvest distribution of *Protea* cv. Carnival inflorescences on 12-year old plants as initiated on two-and three flush shoots respectively with ABG-3062 solutions at 500 mg·L^{-1} (active ingredient: BA 2% $^{w}/_{w}$) over six treatment dates in 2003. Distilled water was used as a control. Values are expressed as percentages of the total harvest. Shaded blocks represent the three peak harvest dates for each treatment. The advancement of flowering time represents the difference in days between the dates when the cumulative number of stems harvested was ≥60% for control and treated shoots.

		HARVEST 2003-2004															
TREATMENT	**Advancement of harvest (days)**	**10-Nov**	**24-Nov**	**10-Dec**	**23-Dec**	**06-Jan**	**20-Jan**	**03-Feb**	**17-Feb**	**26-Feb**	**04-Mar**	**11-Mar**	**17-Mar**	**24-Mar**	**30-Mar**	**08-Apr**	**20-Apr**
Two-flush shoot																	
Control									**2.6**	**7.7**	**17.9**	**25.6**	**20.5**	**7.7**	**2.6**	**10.3**	**5.1**
***ABG-3062* 14 Mar**	*																
***ABG-3062* 28 Mar**	*																
***ABG-3062* 9 Apr**	*																
***ABG-3062* 23 Apr**	79			**21.1**	**21.1**	**47.4**	**5.3**	**5.3**									
***ABG-3062* 7 May**	51					**50**	**25**	**25**									
***ABG-3062* 21 May**	31					**12.5**	**12.5**	**75**									
Three-flush shoot																	
Control							**6.3**	**12.5**	**20.8**	**33.8**	**12.5**	**6.3**	**4.2**	**0**	**2.1**	**0**	**2.1**
***ABG-3062* 14 Mar**	95	**25**	**25**														
***ABG-3062* 28 Mar**	80		**95**	**5**													
***ABG-3062* 9 Apr**	66			**56.3**	**43.8**												
***ABG-3062* 23 Apr**	66		**2.3**	**25.6**	**60.5**	**11.6**											
***ABG-3062* 7 May**	45			**10.3**	**13.8**	**27.6**	**37.9**	**3.4**	**6.9**								
***ABG-3062* 21 May**	31				**6.8**	**34.1**	**36.4**	**22.7**									

Table 3. Shoot characteristics of the subtending flush to the inflorescence and inflorescence quality at the commercial 'soft tip' harvest stage of *Protea* cv. Carnival for inflorescence initiated on an induced autumn flush of eight-year old plants with Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 applications (500 $mg \cdot L^{-1}$; active ingredient: BA 2% $^{w}/_{w}$) over six treatment dates in 2003. Controls comprise of spring-initiated inflorescences. Ten single shoot replicates were used per treatment. As there was no statistical difference in any parameters measured between Accel® and ABG-3062, values for these groups were pooled. Inflorescence quality parameters measured were length (mm) (measured from base of inflorescence to involucral bract tips), basal diameter (mm), the number of florets and involucral bracts, dry weight (g) and colour (expressed as the a* chromaticity coordinate in the CIELab colorimetric space). [z] Data not obtained.

	Subtending flush to inflorescence				Inflorescence characteristics					
Treatment Date	Intercalation Diameter (mm)	Stem Dry Mass (g)	Leaf dry mass (g)	Total dry mass (g)	Length (mm)	Diameter (mm)	No of florets	No involucral bracts	Dry weight (g)	Colour (a*)
Control (spring)	10.59^{c}	12.51^{a}	24.29^{a}	36.81^{a}	134.8^{b}	39.2^{c}	225^{d}	101^{c}	20.4^{c}	12.4^{b}
14 Mar	11.40^{b}	13.49^{a}	16.25^{d}	29.74^{c}	123.2^{c}	46.6^{b}	260^{c}	125^{b}	24.1^{b}	14.3^{a}
26 Mar	11.16^{b}	13.21^{a}	17.76^{c}	30.97^{b}	124.5^{c}	45.1^{b}	266^{c}	121^{b}	24.2^{b}	14.2^{a}
9 Apr	11.82^{b}	12.56^{a}	18.12^{c}	30.68^{b}	138.9^{ab}	44.0^{b}	276^{ab}	127^{a}	25.9^{ab}	12.3^{b}
21 Apr	11.69^{b}	13.61^{a}	18.99^{bc}	32.59^{b}	134.3^{b}	44.8^{b}	274^{ab}	122^{b}	26.1^{a}	12.8^{b}
7 May	13.69^{a}	11.88^{b}	18.20^{c}	30.08^{b}	142.9^{a}	49.0^{a}	282^{a}	127^{a}	25.8^{ab}	-^{z}
22 May	13.77^{a}	12.71^{a}	19.99^{b}	32.70^{b}	137.4^{ab}	46.1^{b}	267^{bc}	109^{c}	23.3^{bc}	-^{z}
CONTRAST	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Control vs ABG-3062 & Accel	<0.0001	0.4354	<0.0001	<0.0001	0.6098	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ABG-3062 & Accel Time Lin.	<0.0001	0.0909	0.0003	0.1005	<0.0001	0.2371	0.0042	0.0298	0.8959	-^{z}
ABG-3062 & Accel Time Qua.	0.0015	0.7693	0.5923	0.8323	0.0013	0.1001	0.0003	0.0212	0.0006	-^{z}

Means (n=20) within columns followed by superscripts with the same letter are not significantly different at LSD (p=0.05) values (5%).

Table 4. Inflorescence quality at the commercial 'soft tip' harvest stage in *Protea* cv. Carnival for inflorescences initiated on an autumn flush induced on two- and three flush shoots respectively by ABG-3062 application (500 mg·L^{-1}) over six treatment dates in 2003. Inflorescence quality parameters measured were inflorescence length (mm) (measured from the base of inflorescence to involucral bract tips), basal diameter (mm), number of florets and involucral bracts and inflorescence dry weight (g).

	Number of shoots		Length (mm)		Diameter (mm)		Number of involucral bracts		Number of florets		Dry weight (g)	
Treatment Date	Two-flush	Three-flush	Two-flush	Three-flush	Two-flush	Three-flush	Two-flush	Three-flush	Two-flush	Three-flush	Two-flush	Three-flush
14 Mar	0	8	-[z]	118.6[c]	-[z]	53.9[a]	-[z]	165.2[a]	-[z]	253.2[c]	-[z]	22.1[b]
26 Mar	0	10	-[z]	120.5[c]	-[z]	49.2[ab]	-[z]	126.1[b]	-[z]	261.1[c]	-[z]	25.2[a]
9 Apr	0	10	-[z]	134.0[ab]	-[z]	40.2[c]	-[z]	130.6[b]	-[z]	274.9[b]	-[z]	23.2[a]
21 Apr	10	10	134.4[a]	131.2[b]	41.1[a]	45.8[b]	123.2[a]	126.6[b]	272.4[a]	281.5[ab]	22.9[a]	26.2[a]
7 May	4	10	130.9[a]	139.7[a]	42.0[a]	48.9[b]	113.6[a]	123.4[b]	279.0[a]	290.8[a]	21.3[a]	26.3[a]
22 May	10	10	133.3[a]	136.7[ab]	43.0[a]	46.5[b]	111.2[c]	109.4[c]	266.6[a]	277.4[b]	21.3[a]	25.7[a]
CONTRASTS			Pr >F	Pr >F	Pr >F	Pr >F	Pr >F	Pr >F	Pr >F	Pr >F	Pr >F	Pr >F
ABG-3062 Time Linear				<.0001		0.0247		<.0001		<.0001		0.0333
ABG-3062 Time Quadratic			0.9641	0.0762	0.0586	0.0003	0.0339	0.0921	0.3502	0.0010	0.1362	0.4139

Values within columns followed by superscripts with the same letter are not significantly different at LSD (p=0.05) values (5%).

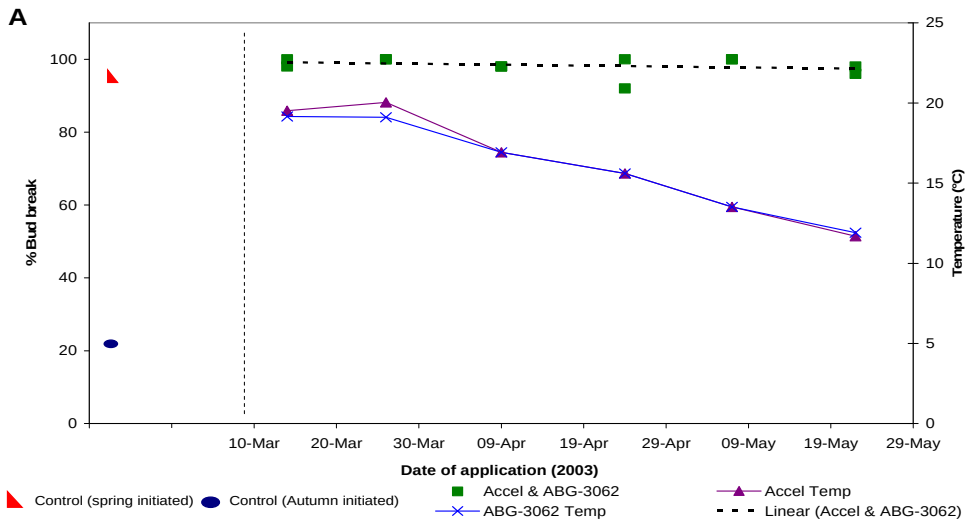
[z] Inflorescence initiation did not occur

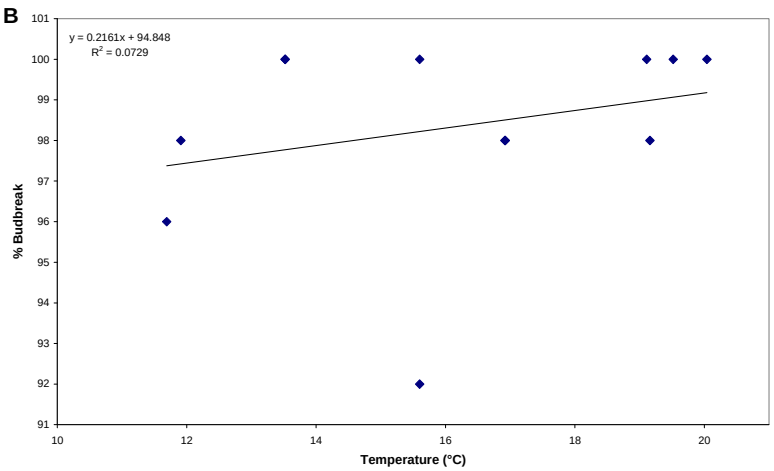
Table 5. Average dates (± SE) in 2003-2004 for spring-initiated green point (GP) and harvest of spring-initiated inflorescences, together with the delay (in days) of green point and the subsequent harvest as recorded for shoots that did not initiate inflorescences on either the Accel® (500 mg·L^{-1}: active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (500 mg·L^{-1}; active ingredient: BA 2% $^{w}/_{w}$) -induced autumn flush on six treatment dates in 2003.

	Number of shoots		Accel®		ABG-3062		Accel®		ABG-3062	
Application date	Accel®	ABG-3062	Date of GP	Delay of GP (days)	Date of GP	Delay of GP (days)	Date of GP	Delay of GP (days)	Date of GP	Delay of GP (days)
14 Mar	19	33	29 Sept ± 1.5	24	24 Sept ± 1.4	19	21 Feb ± 1.3	4	18 Feb ± 2.4	1
26 Mar	10	13	2 Oct ± 1.6	27	1 Oct ± 1.6	26	24 Feb ± 1.4	7	18 Feb ± 0.9	1
9 Apr	17	27	28 Sept ± 1.3	23	26 Sept ± 1.8	21	21 Feb ± 2.2	4	19 Feb ± 1.1	2
24 Apr	8	5	23 Sept ± 3.9	18	6 Oct ± 2.6	31	21 Feb ± 1.8	4	14 Feb ± 2.3	-3
7 May	8	15	3 Oct ± 4.2	28	5 Oct ± 1.2	30	23 Feb ± 1.9	6	20 Feb ± 2.9	3
21 May	1	1	30 Sept ± 0.0	25	30 Sept ± 0.0	25	23 Feb ± 0.0	6	27 Feb ± 3.6	10
Control	47		5 Sept ± 1.2				17 Feb ± 1.1			

Table 6. Average dates (± SE) in 2003-2004 for spring-initiated green point and harvest of spring-initiated inflorescences, together with the delay (in days) of green point and the subsequent harvest as recorded for shoots which did not initiate inflorescences on the ABG-3062 autumn-flush (500 mg·L^{-1}: active ingredient: BA 2% $^{w}/_{w}$) on two- and three-flush shoots on six treatment dates in 2003.

	Number of shoots		Two-flush shoot		Three-flush shoot		Two-flush shoot		Three-flush shoot	
Application Date	Two-flush shoot	Three-flush shoot	Date of GP	Delay of GP (days)	Date of GP	Delay of GP (days)	Date of GP	Delay of GP (days)	Date of GP	Delay of GP (days)
14 Mar	37	40	3 Oct ± 1.1	11	28 Sept ± 2.1	11	12 Mar ± 4.1	-4	8 Mar ± 6.3	13
26 Mar	46	25	2 Oct ± 1.9	10	8 Oct ± 5.3	21	8 Mar ± 1.6	-8	27 Feb ± 4.3	4
9 Apr	43	31	20 Sept ± 2.1	-2	23 Sept ± 1.3	6	7 Mar ± 2.6	-9	27 Feb ± 2.4	4
24 Apr	23	4	29 Sept ± 1.17	7	24 Sept ± 3.0	7	12 Mar ± 3.9	-4	24 Feb ± 4.1	1
7 May	40	22	4 Oct ± 0.5	12	26 Sept ± 1.2	9	5 Mar ± 3.5	-11	26 Feb ± 2.5	3
21 May	36	4	8 Oct ± 1.6	16	17 Sept ± 0.3	0	26 Mar ± 2.0	10	12 Mar ± 0.0	17
Control	50	47	22 Sept ± 1.9		17 Sept ± 3.0		16 Mar ± 1.6		23 Feb ± 4.6	





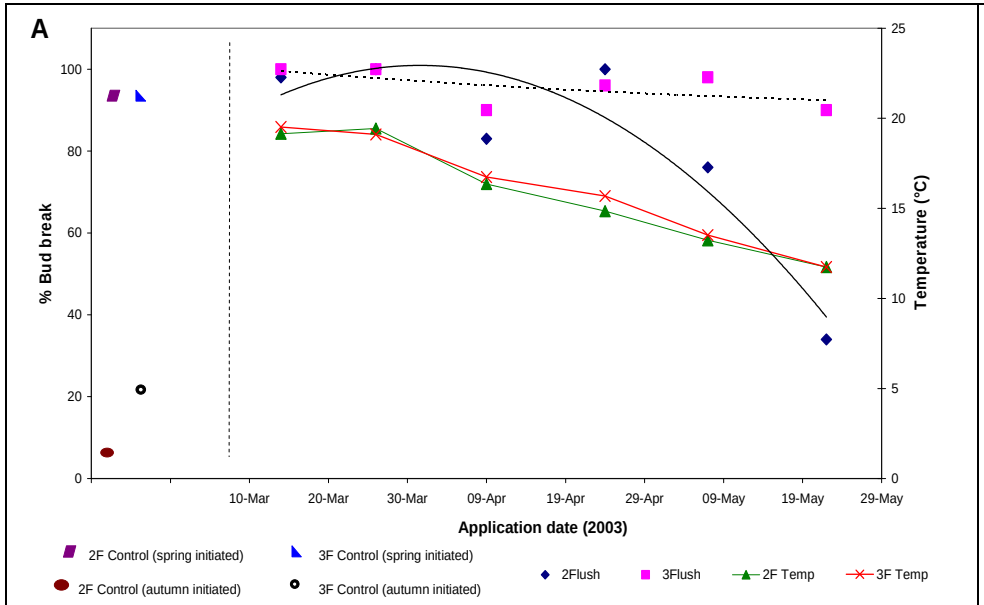
ANOVA

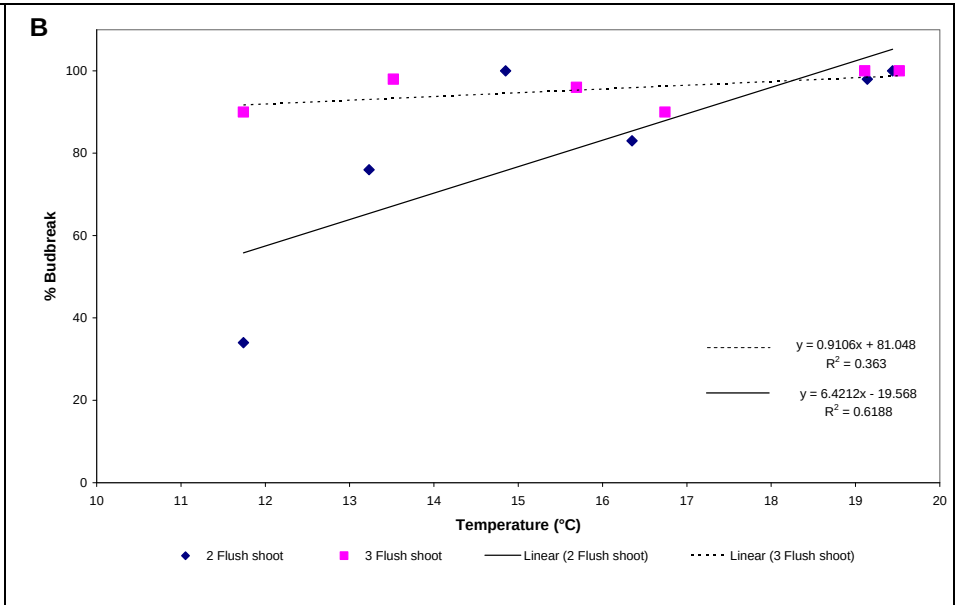
CONTRASTS	*F value*	*Pr>F*	*Significance*
Control (autumn) vs. Treatment	316.94	<.0001	***
Control (spring) vs. Treatment	1.61	0.2112	ns
Accel ® vs ABG-3062	1.11	0.298	ns
Time Lin Treatment	1.02	0.319	ns
Time Qua Treatment	0.14	0.711	ns
Time Lin* Accel® vs ABG-3062	1.15	0.289	ns
Time Qua* Accel® vs ABG-3062	1.84	0.182	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
Temperature (Accel® & ABG-3062) vs % Budbreak	0.27	0.396	ns

Figure 1. A. Percentage budbreak in *Protea* cv. Carnival with Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) application to three-flush shoots of eight-year old plants, including the average temperatures from application to budbreak on six application dates in autumn 2003. B. Relationship between % budbreak and the average temperature recorded during each respective period.





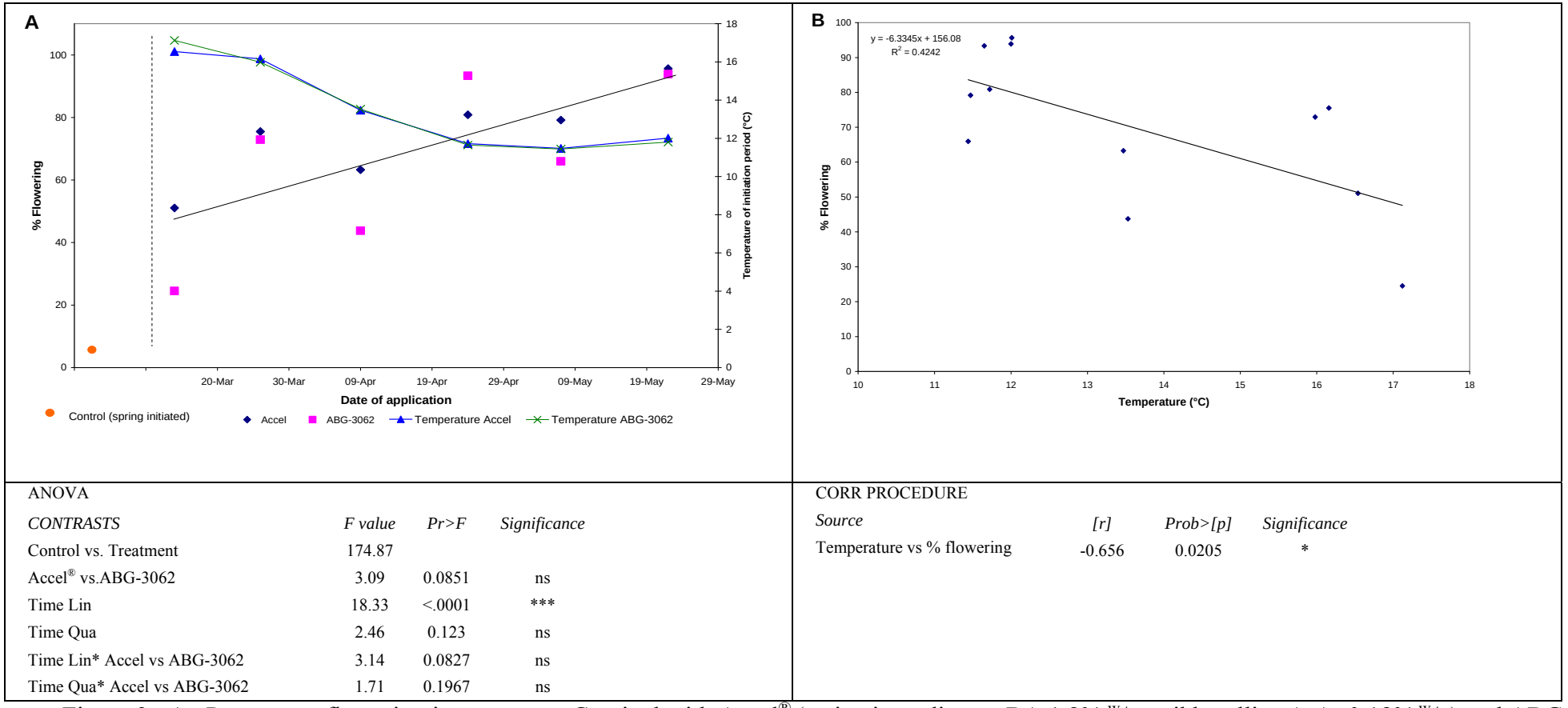
ANOVA

CONTRASTS	*F value*	*Pr>F*	*Significance*
2Flush control (autumn) vs. 2Flush treatment	150.29	<.0001	***
3Flush control (autumn) vs. 3Flush treatment	91.77	<.0001	***
2Flush vs 3Flush Treatment	17.27	0.0001	***
Time Lin	18.91	<.0001	***
Time Qua	10.46	0.0092	**
Time Lin* 2Flush vs 3Flush	41.10	<.0001	***
Time Qua* 2Flush vs 3Flush	7.42	0.0092	**

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
2F Temperature vs % Budbreak	0.787	0.0634	ns
3F Temperature vs % Budbreak	0.603	0.2056	ns

Figure 2. A. Percentage budbreak in *Protea* cv. Carnival with ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) application to two-and three-flush shoots of 12-year old plants on six application dates in autumn 2003, including the average temperatures from application to budbreak. B. Relationship between % budbreak and the average temperature recorded during each respective period.



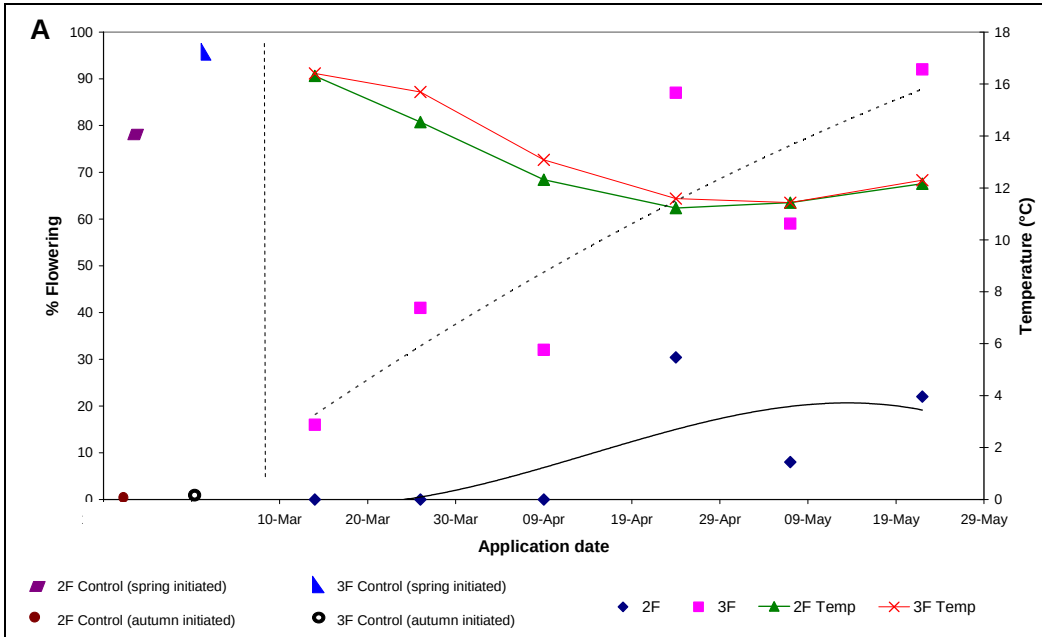
ANOVA

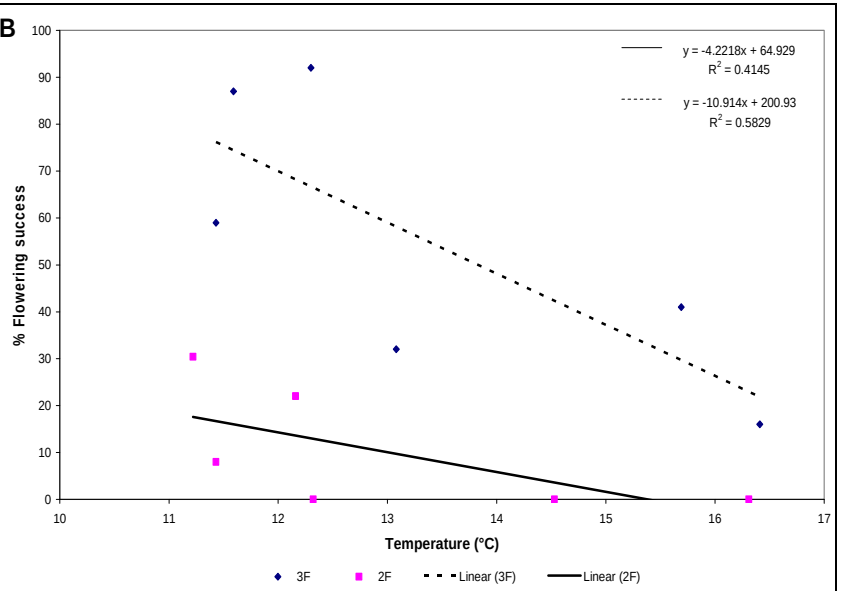
CONTRASTS	*F value*	*Pr>F*	*Significance*
Control vs. Treatment	174.87		
Accel® vs.ABG-3062	3.09	0.0851	ns
Time Lin	18.33	<.0001	***
Time Qua	2.46	0.123	ns
Time Lin* Accel vs ABG-3062	3.14	0.0827	ns
Time Qua* Accel vs ABG-3062	1.71	0.1967	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
Temperature vs % flowering	-0.656	0.0205	*

Figure 3. A. Percentage flowering in *Protea* cv. Carnival with Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) application to three-flush shoots of eight-year old plants on six application dates (2003), including the average temperatures from growth regulator application to cessation of induced flush elongation. B. Relationship between percentage flowering and the average temperature recorded during each respective application period .





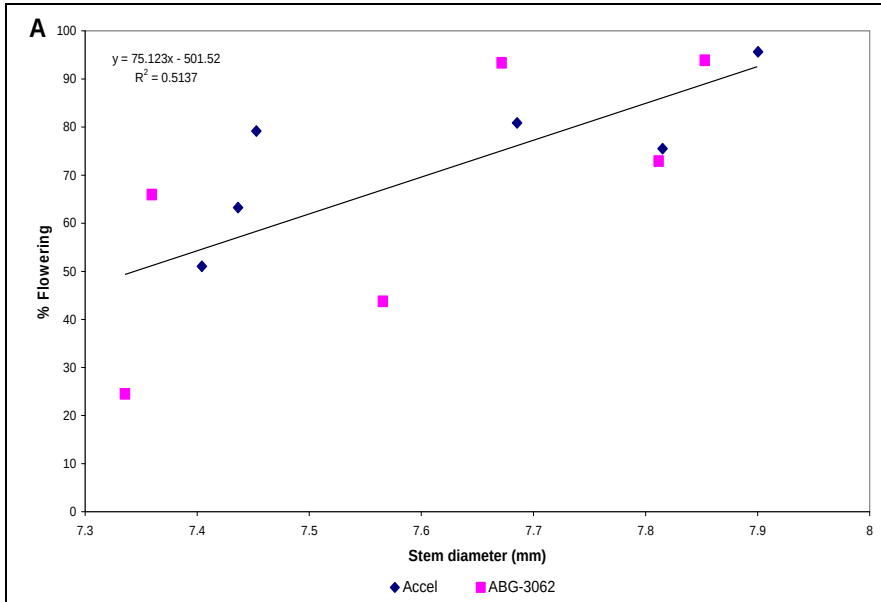
ANOVA

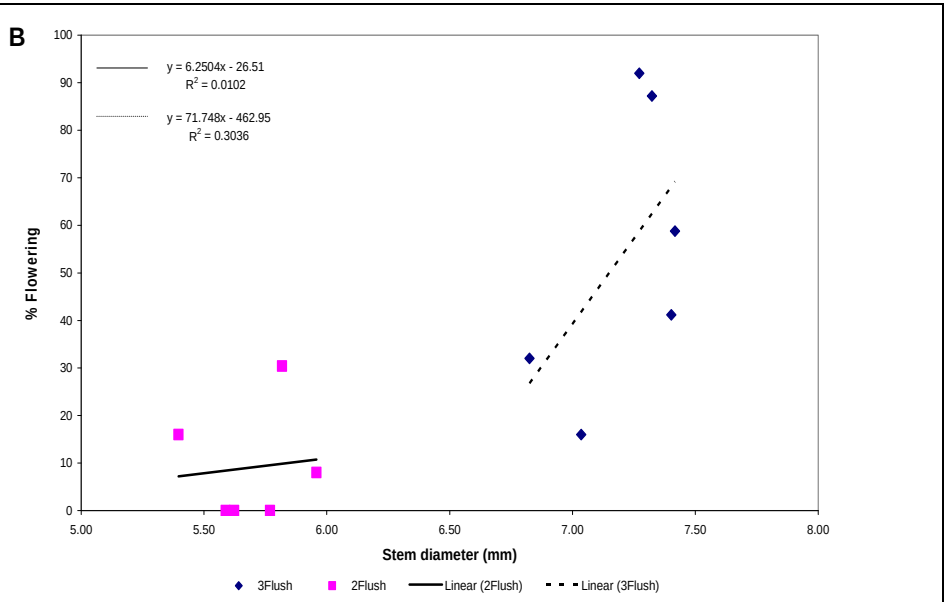
CONTRASTS	F value	Pr>F	Significance
2F vs 3F	139.9	<.0001	***
Time Lin	52.8	<.0001	***
Time Qua	4.69	0.0358	*
Time Lin* 2F vs 3F	0.55	0.4632	ns
Time Qua* 2F vs 3F	1.71	0.4472	ns
Time Cub* 2F vs 3F	5.08	0.0292	*

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	Significance
3F Temperature vs 3F % flowering	-0.7635	0.0773	ns
2F Temperature vs 2F % flowering	-0.6439	0.1677	ns

Figure 4. A. Percentage flowering in *Protea* cv. Carnival with ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) to two (2F)- and three-flush (3F) shoots of 12-year old plants on six application dates (2003), including the average temperatures from growth regulator application to cessation of induced flush elongation. B. Relationship between % flowering and the average temperature recorded during each respective application period.





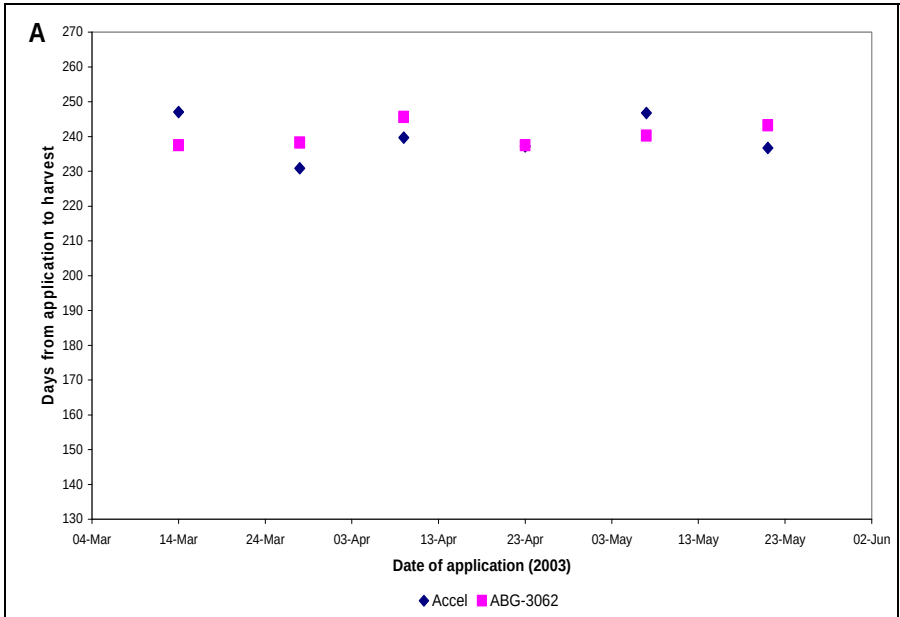
CORR PROCEDURE

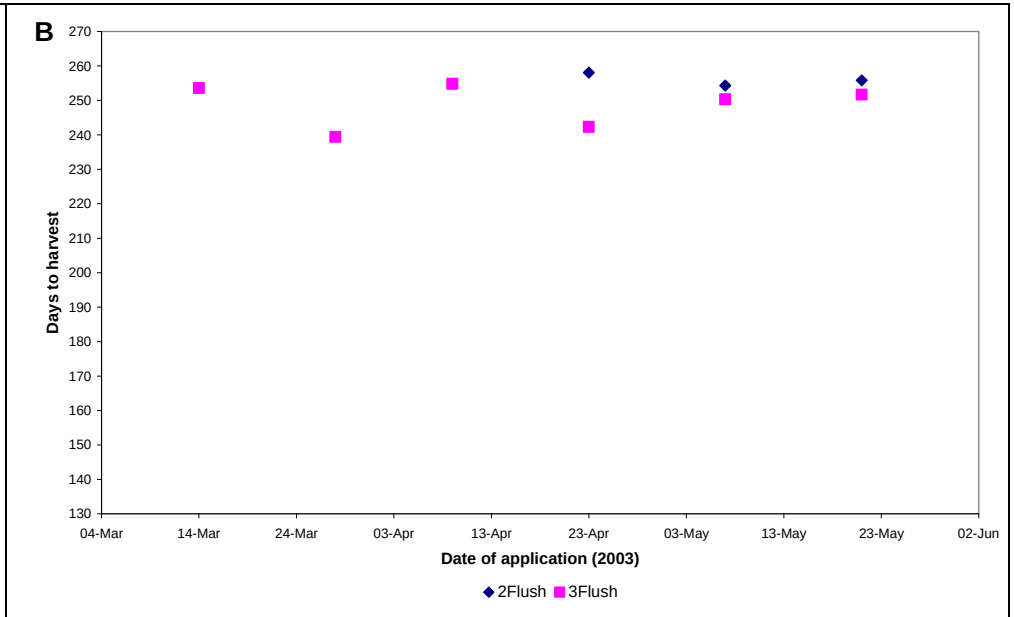
Source	*Prob>[r]*	*p*	*Significance*
Stem diameter vs % flowering	0.717	0.0087	***

CORR PROCEDURE

Source	*Prob>[r]*	*p*	*Significance*
2F Stem diameter vs % flowering	0.07174	0.8926	ns
3F Stem diameter vs % flowering	0.5455	0.2629	ns

Figure 5. A. Relationship between % flowering as induced by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) at 500 mg·L^{-1} and shoot diameter (mm) of three-flush shoots of eight-year old plants of *Protea* cv. Carnival. B. The relationship between % flowering as induced by ABG-3062 in *Protea* cv. Carnival on two- and three-flush shoots of 12-year old plants and stem diameter. Growth regulators were applied on six treatment dates from March to May 2003. Stem diameter was measured at the intercalation between the terminal and sub-terminal flush.





ANOVA

CONTRASTS	F value	Pr>F	Significance
Accel® vs ABG-3062	0.09	0.7690	ns
Time Lin	0.55	0.4613	ns
Time Qua	0.70	0.4063	ns

ANOVA

CONTRASTS	F value	Pr>F	Significance
2Flush Lin	0.98	0.4694	ns
2Flush Qua	1.50	0.3080	ns
3Flush Time Lin	0.14	0.7159	ns
3Flush Time Qua	1.39	0.2671	ns

Figure 6. A. The number of days required from Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) application to harvest for inflorescences initiated on three-flush shoots of eight-year old plants of *Protea* cv. Carnival B. The number of days required from ABG-3062 application to harvest for inflorescences initiated on two- and three-flush shoots of 12-year old plants of *Protea* cv. Carnival. Growth regulators were applied on six treatment dates from March to May 2003.

4. PAPER III. Phenology of, and heat unit requirements for vegetative growth and inflorescence development induced by benzyladenine-containing growth regulators in *Protea* cv. Carnival.

Phenology of, and heat unit requirements for vegetative growth and inflorescence development induced by benzyladenine-containing growth regulators in *Protea* cv. Carnival.

Abstract

Dormant, terminal buds on mature, three-flush shoots of eight-year old plants of *Protea* cv. Carnival were treated with 500 mg·L^{-1} benzyladenine (BA), prepared by diluting either ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) or Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$). In a separate trial, dormant terminal buds of two- and three-flush shoots of 12-year old plants were also treated with BA (ABG-3062) at a concentration of 500mg·L^{-1}. All shoots received a single application of growth regulator. Treatment dates were spaced two weeks apart, starting 14 March to 22 May 2003. Three phenological stages and the days required to complete each stage was recorded for all treatments. Monitored stages included: date of BA application to green point; green point to completion of induced shoot expansion and from the latter until anthesis. Base temperatures of 8°C, 6°C and 1°C were determined for the respective stages. For two-and three-flush shoots, between 13-65 days were required from date of growth regulator application to green point. The number of days required to green point was negatively correlated with the mean temperature that prevailed for that period. Three-flush shoots required between 5400-6000 and two-flush shoots an additional 1000 growing degree hours (GDH) to complete this phenological phase. Between 39-58 days were required for two-and three-flush shoots, to complete the phenological phase 'green point to completion of shoot expansion'. The relationship between temperature and the required number of days to complete this phase was not clear. Two-flush shoots needed 8500 and three-flush shoots 600 more

GDH to complete the cessation of induced flush elongation. The last phenological phase needed between 121-187 days to completion and the duration was negatively correlated with the prevailing temperatures. Inflorescences on three-flush shoots required between 53000 and 56000 and those on two-flush shoots, 59000 GDH to anthesis.

Benzyladenine (BA) has been shown to induce budbreak and inflorescence initiation in *Protea* cv. Carnival outside the natural flushing- and spring inflorescence initiation cycle (Paper I: table 1, pg. 54; Paper II: tables 1, 2, pg. 87-88). Inflorescences initiated by cytokinin growth regulator treatments were harvested significantly earlier than of the untreated control. This advanced inflorescence initiation offers the potential for manipulation of the harvest time of 'Carnival', and possibly other *Protea* to meet periods of peak demand. Vegetative and reproductive development of shoots induced by BA application in late summer and autumn takes place under different environmental conditions of light (irradiance and photoperiod) and temperature than the normal spring-initiated inflorescences. An understanding of how environmental conditions regulate *Protea* growth and development is required to optimize crop production. This information is particularly important for production outside the natural distribution of *Protea* or when management tools such as pruning or growth regulator application are employed.

In *Protea*, leaf primordia of the succeeding flush differentiate during the current flush extension so that the terminal bud of each flush is preformed, containing the shoot and full leaf complement for the next flush. Involucral bract differentiation takes place during elongation of the flush subtending the inflorescence. Floral bract and floret formation commences up on completion of subtending flush elongation (Gerber *et al.*, 2001a). Progression from a vegetative to a reproductive shoot involves the distinct phenological stages of budbreak, cessation of shoot elongation, macroscopic inflorescence initiation,

inflorescence growth and development, finally leading to anthesis. The onset and timing of the inflorescence initiation and thus the subsequent harvest date, is therefore directly linked to the cessation of preceeding flush elongation.

In order to better predict the time of harvest, quantification of the individual vegetative and reproductive phases through a variable representing the physiological age of each phase is important. Thermal (heat) unit calculation using environmental variables of temperature and occasionally light is commonly employed to describe crop development (Pasian and Lieth, 1994). Developmental rates increase in an approximately linear fashion as a function of air temperature where heat units are a measure of the length of development time at various temperatures.

Heat units can be calculated as growing degree-hours (GDH) or growing degree-days (GDD). One degree-hour is observed when the air temperature is one degree above a lower threshold temperature for one hour. GDD can be estimated by approximating diurnal temperature trends, but are more accurately calculated as total degree-hours for a day, total GDH/24 (Snyder *et al.*, 1999). Development rate is assumed to be insignificant when the air temperature is below a particular threshold value (known as the base temperature) that signals the onset of the growing season. When the temperature determined is more than one degree above the threshold temperature for one hour, the number of degree-hours then equals the observed air temperature minus the threshold temperature. Therefore, a bigger difference between air and base temperature implies more degree-hours and a faster rate of development. In determining the onset of a developmental stage, such as flowering or budbreak, the concept of temperature sum is used. The 'temperature sum' is the accumulated temperature above the base temperature from a certain starting date, calculated by the progressive addition of growing degree hours (GDH) or growing degree days (GDD). Quantification of a phenological stage is based on the assumption that the product of time

until a phenological event, and the average temperature during this time period is a constant. (Diekmann, 1996).

No information on the pattern of cytokinin-induced vegetative growth, or inflorescence initiation and development as observed on the autumn flush, is available for *Protea*. In this paper, the phenology of the vegetative flush induced by cytokinin-containing regulators applied over a period of 12 weeks (late summer-autumn), on the terminal flush of two- and three flush shoots of *Protea* cv. Carnival is reported. The heat unit requirements and base temperatures for phenological stages 'date of BA application to green point'; 'green point to completion of shoot expansion' and 'from completion of shoot expansion to anthesis' were determined.

Materials and Methods

Plant material. Experiments on *Protea* cv. Carnival were carried out in commercial plantations grown from cuttings in the Stellenbosch district (33°55´S; 18°50´E), South Africa. The climate is Mediterranean-like with cool, wet winters and hot dry summers. The annual rainfall is 600-700 mm, concentrated during the winter months. All plants were pruned back to bearers in August 2002 to effect biennial cropping as described by Gerber *et al.* (1995). Plants were spaced 1 m in the row and 4 m between rows. Plants were not irrigated or fertilized. Pest control focused on the control of thin-line leaf miner (*Phyllocnistis* sp.) and the speckled protea borer (*Orophia* spp.) and were applied by risk analysis which included the emergence and extension of the immature flush and during early inflorescence development**.**

Growth regulator application. Three-flush shoots of eight-year old 'Carnival' plants were used. Growth regulator treatments consisted of either a single application of benzyladenine (BA) as ABG-3062 (Abbott Laboratories, North Chicago, USA) at a concentration of 500 $mg \cdot L^{-1}$ (active ingredient: BA 2% $^{w}/_{w}$) or Accel® at a concentration of

500 mg·L^{-1} (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) as obtained from Valent Biosciences, Illinois, USA. Growth regulator solutions were applied on 14 March, 26 March, 9 April, 23 April, 7 May, and 21 May 2003. Growth regulator solutions were applied to the dormant terminal bud using a paint brush. Distilled water was used as the control treatment. At each treatment date stem diameters (mm) on the 1st and 2nd summer flush intercalation was determined. Ten shoots were used per treatment. Treatments were replicated five times in a randomised complete block design.

A second trial two-and three-flush shoots on 12-year old 'Carnival' plants were used. The 12-year old plants differed from the eight-year old plants with respect to tree complexity and reduced growth vigour. Growth regulator application date and method were as described for eight-year old plants, with the exception that only BA was used as a growth regulator. Distilled water was again used as the control treatment. At each treatment date stem diameter (mm) at the intercalation of the spring and summer flushes and that for the 1st and 2nd summer flushes was determined for two- and three-flush shoots respectively. Ten shoots were used per treatment. Treatments were replicated five times in a randomised complete block design.

Phenological stages. Developmental stages of all treatment shoots were observed from date of growth regulator application until inflorescences reached the commercially mature 'soft tip' stage. The following phenological stages were documented: green point (developing flush elongated up to 1.5 cm, representing an intact slender torpedo shape, with leaves intact), cessation of elongation of the induced flush (all leaves unfolded with the terminal bud macroscopically visible), and inflorescence development.

Thermal Unit Calculations. Heat units were calculated as growing degree hours (GDH) for each hour, where

GDH = (Measured temperature (T_m°C)) – (Base temperature (T_b°C))

with GDH = 0 when $T_b > T_m$, as negative values do not support the idea that development rate are related to heat units.

Heat unit requirement for the phenological stages of budbreak, full shoot elongation and harvest stage were calculated as the number of GDH accumulated from cytokinin application to green point; from green point to cessation of shoot growth and from the latter stage to the commercial harvestable 'soft tip' stage respectively. Accumulated Growing Degree Days (GDD) was obtained as follows:

GDD = (total GDH)/24

Base temperatures fitted with $T_b = i \ldots n$ with values for budbreak: on eight-year old plants (i=6, n=12) and 12-year old plants (i=6, n=11); for full shoot elongation: on eight-year old plants (i=1, n=9) and 12-year old plants (i=5, n=10), and the harvest stage, for both eight- and 12-year old plant (i=0 and n=5).

The best base temperature for each phenological stage observed in two-and three flush shoots with BA application and on three-flush shoots with Accel® application was identified as the temperature where the GDH sum displayed the minimum coefficient of variation (Rattigan and Hill, 1986).

Base temperature (T_b) was calculated by a second method (Arnold, 1959), by finding the x-intercept of the regression line between rates of development, p (% day^{-1}) and mean air temperature (°C). The number of days (D) necessary to reach a specific phenological stage was expressed by a reciprocal transformation, 100/D. This transformation, assuming that development rate is linearly related to temperature, then express percentage daily average rate of development (DARD) towards the achieving of the specific phenological phase. Therefore, if it takes 50 days to reach budbreak at a specific temperature, the DARD=100/50= 2% per day = 2 DARD (Werner *et al.*, 1988).

The x-intercept method was used as a validation tool for base temperatures calculated from GDH approach. This method, however, may represent an extrapolation far from the mean and the closest observed data point. Such an extrapolation may sometimes result in negative x-values. In those cases, the x-intercept estimation was omitted as the result does not support biological principles.

Temperature was recorded as air temperature on an hourly basis for the whole duration of the trials and was from for the Somerset-West-Stellenbosch area, as a courtesy from the Deciduous Fruit Producers Trust (DFPT) of South Africa.

Statistical analysis. Standard analysis of variance was performed, using the General Linear Method generated by the SAS® program (SAS Institute, 2000). Regression (Pearson correlation coefficient) and orthogonal contrasts were also fitted with aid of the SAS program (SAS Institute, 2000).

Results

Green point. The number of days required for a shoot to reach green point after applications of Accel® and ABG-3062, increased progressively with time in a quadratic relationship for three-flush shoots of eight-year old 'Carnival' plants (Figure 1A). No significant difference was obtained between the growth regulators Accel® and ABG-3062 for the number of days required to reach green point. The lag phase between the application date and green point was significantly negatively correlated with temperature (Figure 1B).

The relationship between date of application and the number of days required to reach green point stage in two-and three flush shoots of 12-year old 'Carnival' plants when treated with ABG-3062 (Figure 2A) were similar to that recorded for the growth regulator application on eight-year old plants as described above (Figure 1A). However, across treatment dates, with exception of the last treatment date, significantly more days were required to reach green point in two-flush shoots than in three-flush shoots (Figure 2A). An

interaction of a quadratic nature between date of application and days to green point is apparent (Figure 2A). Both two- and three flush shoots displayed a significant negative correlation with temperature as the number of days required to green point increased as temperatures declined in early to late autumn (Figure 2B).

Base temperatures of 9°C for green point induction by both Accel® and ABG-3062 respectively on three-flush shoots of eight-year old plants proved to yield the lowest %CV for the range base temperatures tested (Table 1). Similarly, analysis of data to determine the base temperature for green point in two- and three flush shoots of 12-year old plants treated with BA displayed base temperatures of 8 °C and 9 °C respectively (Table 2). When the x-intercept method of Arnold (1959) was applied to calculate the daily development rate to reach green point, a linear relationship was demonstrated between mean daily temperature and rate of development for all treatments (Figure 3). Solving the equations in Figure 3A- and B by substituting zero for y (rate of development equals nil), x (the base temperature) generated a value of 6.73°C for Accel® and ABG-3062 collectively on three-flush shoots of eight-year old plants (Figure 3A). A comparative base temperature requirement of 6.58°C and 8.17°C was calculated for green point induction by ABG-3062 application on two-and three flush shoots of 12-year old plants respectively (Figure 3B).

A representative base temperature of 8°C was selected for green point induction in 'Carnival' (Table 7). Two-flush shoots on the 12-year old plants at 6927 required significantly (at 5% confidence level) more GDH than the three-flush shoots of the same age plants at 5952 GDH units (Table 7). In eight-year old plants, no significant difference was obtained between the GDH requirements of Accel and ABG-3062 treated plants (Table 7).

Cessation of flush elongation. All shoots that obtained green point under growth regulator induction or under natural conditions as in the control shoots, reached full flush elongation between 39 to 54 days after green point was recorded (Tables 3, 4). In all

treatments, the number of days required to complete elongation of the induced flush increased in a quadratic relationship with time (Figures 4A, 5A). Flush elongation resulting from the Accel® treatments advanced more rapidly than for the BA application (Figure 4A). However, two- and three-flush shoots showed a similar response to BA application for all treatment dates (Figure 5A). In all treatments, no significant interaction between time and growth regulator type or flush number was achieved at the 5% confidence level (Figures 4A, 5A). A negative correlation existed between days required to cessation of flush elongation and the average temperature monitored over the elongation period in two-and three flush shoots of 12-year old plants when treated with BA (Figure 5B), but was non-significant for three-flush shoots of eight-year old plants when treated with Accel® and BA (Figure 4B).

The non-significant correlation between the number of days required from green point to full flush elongation for three-flush shoots of eight-year old plants with Accel® or BA application (Figure 4B) and the average temperature over this elongation period, were the basis of the failure of the %CV test to predict a base temperature for these treatments (Table 3).

A base temperature 6°C was calculated for the two-and three-flush shoots of 12-year old plants of 'Carnival' that did show a significant relationship between the number of days required from green point to cessation of flush elongation and average temperature for that period (Table 4).

Three-flush shoots of 12-year old plants required more GDH units at 9075 than two-flush shoot at 8455 GDH to achieve full flush elongation when both were treated with ABG-3062 (Table 7).

Harvest. The number of days from cessation of the elongation of the induced flush to the harvestable stage decreased in a quadratic relationship with each progressive application date for both the Accel® and ABG-3062 treatments on three-flush shoots of eight-year old

'Carnival' plants (Figure 6A). The number of days required from cessation of flush elongation to harvest were significantly negatively correlated with the average temperature observed over that period at the 5% confidence level (Figure 6B).

In the ABG-3062 treatment on two-flush shoots of 12-year-old plants, the number of days required from cessation of induced flush elongation declined in a quadratic relationship over time, whereas a linear relationship was observed in three-flush shoots of the same age when treated with BA (Figure 7A). A significant negative correlation of days to harvest with temperature was recorded for all treatments (Figure 6B, 7B) in both plant age groups with the exception of the two-flush shoots where the correlation ($r=-0.995$; $p=0.06$) proved to be non-significant at the 5% level (Figure 7B).

A projected base temperature for inflorescence development of 0°C and 1°C was calculated for three-flush shoots of eight-year old plants treated with Accel® and ABG-3062 respectively (Table 5). Two-flush and three-flush shoots of 12-year old plants treated with ABG-3062 display a similar respective 2°C and 0°C base temperature prerequisite for development (Table 6). By extrapolation, the x-intercept method (Arnold, 1959) estimated a base temperature of 0.4°C (Figure 8A) for three-flush shoots of eight-year old plants treated with Accel® and ABG-3062, with a base temperature value of 1.7 °C for two-flush shoots of 12-year plants treated with BA (Figure 8B). The x-intercept method yielded a negative x-value on zero substitution and was therefore rejected for the prediction of the base temperature for inflorescence development for treatments of three-flush shoots of 12-year old plants.

At a bases temperature of 1°C, more significantly more (5%) accumulated GDH at 59413 was required for two-flush shoots of 12-year old plants treated with BA compared to the requirement of Accel® and BA treatments on three-flush shoots of both plant age groups (Table 7).

Discussion

The first step in the sequence of events leading to flowering in *Protea* is sprouting of a terminal bud that gives rise to a growth flush that subtends the inflorescence. The increase in the number of days required between the date of treatment with Accel® or ABG-3062 on eight-year old plants and budbreak can at least partly be ascribed to the decrease in mean temperatures for the intervals (Figure 1). Comparing two-flush with three-flush shoots of 12-year old plants, a similar relationship between temperature and days required to budbreak were evident (Figure 2). Two-flush shoots however took longer to sprout (Figure 2A). This could possibly also be a reflection of the lower inherent vigour of two-flush shoots caused by correlative effects as both two- and three-flush shoots occurred together on the 12-year old plants.

All procedures employed to estimate the base temperature for bud sprouting under growth-regulator induction in 'Carnival' yielded base temperatures of between 6-9°C (Tables 1, 2; Figure 3). These values are higher than the more common threshold value of 5°C that is normally used to define the beginning of the thermal growing season (Diekmann, 1996). However, cytokinin application on 'Carnival' was made to shoots in autumn and it is conceivable that a higher base temperature may be required to activate growth during periods of reduced vigour. A similar phenomenon of different phenological phases differing in their base temperatures were reported for roses. (Pasian and Lieth, 1994).

The GDH units calculated for the different phenological intervals varied considerably and require verification by data of an additional growth season. Dissimilarity in the accumulation of GDH units for the different application dates may also indicate that temperature might not the only factor that could affect the number of days from treatment to budbreak. This is evident in the two-flush shoots requiring significantly more GDH units to induce budbreak than three-flush shoots (Table 7). In roses, the poor correlation between

days after pruning and budbreak just below the pruning cut was similarly ascribed to endogenous factors such as inter-shoot competition (Pasian and Lieth, 1994).

Accel® and ABG-3062 did not differ significantly at the 5% confidence level (F-value: 0.11; Pr>F: 0.7392) in the number of days required from growth regulator application to budbreak (Figure 1A). However, Accel® treated shoots could complete flush elongation in a shorter time than BA treated shoots (Figure 4A). This may be due to the presence of gibberellins (GA) in Accel®. GA is well-known for promoting vegetativeness and acts as an agent for internodal elongation. Wallerstein and Nissim (1992) found exogenously applied BA and GA_3 both to accelerate the elongation of new shoots, but the effect of BA disappeared with time, whereas the GA_3 effect became stronger. A reduced flush elongation time may allow an earlier inflorescence initiation and therefore an advanced flowering time.

The quadratic relationship between date of ABG-3062 or Accel® application and the number of days from green point up to completion of flush expansion show that only for the last three treatment dates was there an increase in the number of days (Table 3) required to complete flush expansion (Figure 4A). The mean temperature for the respective periods however changed little. In addition, the non-significant correlation between days to full flush elongation and the temperature over that period (Figure 4B), also suggest that factors other than temperature, such as day length and irradiance level, may affect the time required to complete flush expansion.

In contrast, comparing the number of days between green point and complete flush expansion between two-and three-flush shoots of 12-year old plants, the number of days required for flush elongation increased with a decrease in temperature over the first four treatment dates, with the result that in this case, the negative correlation between days and temperature were significant (Figure 5B). The reason for this apparent contradiction is not

clear, but it appears that the more vigorous shoots on younger plants were less affected by temperature as compared to shoots on older plants. Although two-flush shoots took longer to sprout than three-flush shoots, the time required to complete flush expansion did not differ significantly (Table 4; Figure 5). Apparently flush expansion is affected less by correlative effects than budbreak. Due to the poor relationship between days from green point to flush elongation and the average temperature recorded for that period, only the method of the lowest %CV for two-and three-flush shoots of 12-year old plants yielded a meaningful base temperature of 6°C (Table 4). This value is in agreement with other woody, perennial crops where base temperatures of 5.5°C and 5.2°C were reported for shoot development in roses (Pasian and Lieth, 1994) and strawberry guava (Normand and Habib, 2001) respectively.

At 6°C as base temperature, three-flush shoots at 9075 required slightly more GDH units to complete flush expansion than two-flush shoots at 8455 (Table 4). The reason for this observed trend is unclear, but could possibly be related to the fact that a flush following on a three-flush shoot has more leaves to unfold than that following on a two-flush shoot (Gerber *et al.*, 2001a). If it is assumed that an equivalent leaf unfolding rate exist between the two shoot types, it might offer an explanation for the longer period required to flush expansion in three-flush shoots compared to two-flush shoots.

The decrease in the number of days from the completion of flush expansion up to harvest for Accel® and ABG-3062 treated three-flush shoots of eight-year old plants can in part be ascribed to increasing temperatures of each progressive treatment date (Figure 6A). This is supported by the negative correlation coefficient shown to be significant at the 5% confidence level (Figure 6B). Similar results were achieved for three-flush but not for two-flush shoots on the older 12-year old plants. Other reports on inflorescence development of Proteaceace support findings of this study. Gerber *et al.* (2001b) reported a slower development rate for inflorescences subject to cooler weather in winter for 'Sylvia', a cultivar

with an open flowering initiation window, compared to inflorescences initiated on any other flush. Similarly, in a comparison of flowering time for *Protea* cv. Salmon Pink between four different sites, a warm winter was found to advance and enhance flowering compared to cooler areas (Dupee and Goodwin, 1990). Inflorescence development in *Leuospermum. patersonii* and *Banksia ashbyi* was also found be accelerated by moderate temperatures, especially during the primordial stage (Wallerstein and Nissim, 1992). Since budbreak on two-flush shoots of 12-year old 'Carnival' plants was low on the last three treatment dates (Paper II, figure 2, pg. 94), inflorescence initiation was also corresponding low for that period. Although the correlation between temperature and days to harvest for the last three treatment dates give an r-value of 0.995, it was still not significant due to the small data set (Figure 7B).

The ability of 'Carnival' inflorescences to develop at temperatures of between 0° and 2°C is suggested by the thermal unit- (Tables 5, 6) and projected x-intercept methods by extrapolation (Figure 8). Since plants were not grown outside the temperature range measured, a low base temperature of 1°C should be considered as a potential temperature at which inflorescence development is possible. Such a low base temperature should be viewed in the light of many *Protea* species being predominantly winter flowering. This adaptation for inflorescence development under the low temperatures of winter and early spring which is typical of a Mediterranean climate may also be present in *Protea* hybrids despite a different flowering window.

The base temperature estimated for inflorescence development of 'Carnival' at 1°C is lower than the recorded 6°C for *Leucospermum* (Jacobs and Honeyborne, 1979; Criley *et al.*, 1990). However, when the inflorescence development rate is expressed as an average daily heat unit accumulation, the rate for 'Carnival' of 16.3 and 14.2 for two-flush and three-flush shoots respectively is comparable to that of 12.8 calculated for *Leucospermum* cv. Vlam

(Criley *et al.*, 1990). The GDH units calculated for completion of flower development was less variable than that calculated for the phenological phase of green point and cessation of flush elongation. This could imply that temperature is a dominant factor that determines the rate of flower development.

In addition to temperature, the size of the photosynthetic source subtending the inflorescence may also play a determining role in the number of days required for inflorescences to mature. Two-flush shoots accumulated more GDH units to harvest at base temperature of 1°C (Table 7) and reached the harvestable stage slightly later than the three-flush shoots under BA applications and similar growing conditions (Figure 7). Gerber *et al*. (2001b) has shown that flowers of 'Syliva' borne on long shoots require fewer days to reach anthesis as compared to short shoots. This observed trend is most likely due to an increase in the size of the photosynthetic source that accounts for the increase in growth rate. As control of temperature in the orchard is complicated, management practices can focus instead on the production of vegetative shoots of superior quality prior to growth regulator application to in order to further promote the advancement the already 'out of season' flowering time in 'Carnival'.

The presence of GA in Accel® did not offer any further advantages to accelerated flowering times compared to BA, as GDH unit accumulation at a base temperature of 1°C (Table 7) and days required for completion of inflorescence development to harvest were not significantly different between growth regulators (Figure 6A).

In conclusion: The different phenological phases viz. green point, flush expansion and inflorescence development in *Protea* cv. Carnival was calculated to have different base temperatures of 8°C, 6°C and 1°C respectively for the growth season of 2003. Phenological phase development and progression of 'Carnival' as induced by cytokinin-based growth regulators over time showed a definite relationship with temperature, but was also affected

by intra-plant factors, possibly plant age, vigour and shoot characteristics such as shoot length and number of flushes.

Literature Cited

Arnold, C.Y. 1959. The determination and significance of the base temperature in a linear heat unit system. Proc. Amer.Soc. Hort. Sci. 74:430-445.

Criley, R.A., P.E. Parvin, and S. Lekawatana. 1990. Flower development in *Leucospermum cordifolium* 'Vlam'. Acta Hort. 264:61-70.

Diekmann, M. 1996. Relationship between flowering phenology of perennial herbs and meterological data in deciduous forest of Sweden. Can. J. Bot. 74:528-537.

Dupee, S.A. and P.B. Goodwin. 1990. Flower initiation in *Protea* and *Telopea*. Acta Hort. 264:71-78.

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.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001a. Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars. J. Amer. Soc. Hort. Sci. 126:182-187.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001b. Manipulation of flowering time by pruning of *Protea* cv. Sylvia (*P. eximia* × *P. susannae*). HortScience 36:909-912.

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

Normand, F. and R. Habib. 2001. Phenology of strawberry guava (*Psidium cattleianum*) in Réunion Island. J. Hort. Sci. Biotechnol. 76:541-545.

Pasian, C.C. and J.H. Lieth. 1994. Prediction of rose shoot development based on air temperature and thermal units. Scientia Hort. 59:131-145.

Rattigan, K. and S.J. Hill. 1986. Relationship between temperature and flowering in almond. Austr. J. Exp. Agric. 26:399-404.

Snyder, R.L., D. Spano, C. Cesaraccio, and P. Duce. 1999. Determining degree-day thresholds from field observations. Int. J. Biometerol. 42:177-182.

Statistical Analysis Systems Institute Inc. 2000. SAS®/STAT User's Guide, Version 9.1, SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

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.

Werner, D.J., B.D. Mowrey and E. Young. 1988. Chilling requirement and post-rest heat accumulation as related to difference in time of bloom between peach and western sand cherry. J. Amer. Soc. Hort. Sci. 113: 775-778.

Table 1. The number of days between date of treatment with Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) or ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) and green point on three-flush shoots of eight-year old *Protea* cv. Carnival plants. The accumulated growing degree hours (GDH) at various base temperatures (°C) is indicated for the period application to green point. Values indicated in **bold** specify the base temperature with lowest %CV. Mean, standard deviation (σ) and coefficient of variation (CV) are reported.

		Accel									BA						
		Base temperature (T_b)									Base temperature (T_b)						
Time interval (2003)	Days to green point	6°C	7°C	8°C	**9°C**	10°C	11°C	12°C	Time interval (2003)	Days to green point	6°C	7°C	8°C	**9°C**	10°C	11°C	12°C
14 Mar-6 Apr	23	7464	6912	6360	**5808**	5256	4711	4170	14 Mar-1Apr	19	5684	5252	4820	**4388**	3957	3531	3110
26 Mar-8 Apr	13	4381	4068	3756	**3444**	3132	2820	2508	26 Mar-12 Apr	17	5351	4943	4535	**4127**	3723	3325	2931
9 Apr-6 May	28	7078	6430	5783	**5144**	4524	3943	3394	9 Apr-6 May	27	7078	6430	5783	**5144**	4524	3943	3394
24 Apr-22 May	29	6685	5996	5311	**4633**	3978	3366	2797	24 Apr-22 May	29	6685	5996	5311	**4633**	3978	3366	2797
7 May-14 June	38	6935	6100	5300	**4540**	3830	3171	2559	7 May-15 June	39	7137	6275	5451	**4669**	3941	3267	2643
21 May-14 Jul	55	7863	6807	5837	**4959**	4169	3450	2811	21 May-17 Jul	57	8578	7452	6416	**5150**	4626	3855	3168
	Mean	6734	6052	5391	**4755**	4148	3577	3040		Mean	6752	6058	5386	**4685**	4125	3548	3009
	σ	1225	1038	892	**785**	712	666	636		σ	1157	897	674	**407**	362	287	271
	%CV	18.20	17.16	16.54	**16.51**	17.16	18.62	20.93		%CV	17.14	14.81	12.52	**8.69**	8.78	8.09	9.03

Table 2. The number of days required to induce green point on two- and three-flush shoots of 12-year old *Protea* cv. Carnival plants by ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) application on six dates (March-May 2003). The accumulated growing degree hours (GDH) at various base temperatures (°C) is indicated for the period 'application to green point stage'. Values indicated in **bold** specify the base temperature with lowest %CV. The mean, standard deviation (σ) and coefficient of variation (CV) are reported.

		Two-flush shoots								Three-flush shoots					
		Base Temperature (T_b)								Base Temperature (T_b)					
Time interval (2003)	Days to green point	6°C	7°C	**8°C**	9°C	10°C	11°C	Time interval (2003)	Days to green point	6°C	7°C	8°C	**9°C**	10°C	11°C
14 Mar-10 Apr	27	8516	7868	**7220**	6572	5927	5289	14 Mar-6 Apr	23	7464	6912	6360	**5808**	5256	4711
26 Mar-19 Apr	24	7741	7165	**6589**	6013	5441	4876	26 Mar-13 Apr	18	5665	5233	4801	**4369**	3941	3519
9 Apr-16 May	37	9189	8301	**7417**	6543	5694	4897	9 Apr-9 May	31	7730	7010	6291	**5580**	4891	4250
24 Apr-4 Jun	41	8942	7951	**6973**	6018	5114	4281	24 Apr-24 May	32	7215	6478	5745	**5019**	4316	3658
7 May-23 Jun	47	8285	7267	**6299**	5387	4544	3767	7 May-15 Jun	39	7137	6275	5451	**4669**	3941	3267
21 May-25 Jul	65	9509	8236	**7064**	5998	5032	4159	21 May-25 Jul	65	9509	8236	7064	**5998**	5032	4159
	Mean	8697	7798	**6927**	6089	5292	4545		Mean	7453	6691	5952	**5240**	4563	3927
	σ	644	481	**414**	436	499	568		σ	1237	988	792	**655**	573	538
	%CV	7.41	6.16	**5.97**	7.16	9.44	12.49		%CV	16.60	14.77	13.31	**12.50**	12.57	13.69

Table 3. The number of days required for cessation of flush elongation following green point induction with Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) or ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on three-flush shoots of eight-year old *Protea* cv. Carnival plants. Accumulated growing degree hours (GDH) at various base temperatures (°C) is given for the phenological phase 'green point to cessation of flush elongation'. Mean, standard deviation (σ) and coefficient of variation (CV) are reported.

		Accel									BA						
		Base temperature (T_b)									Base temperature (T_b)						
Time interval (2003)	Days to cessation of flush elongation	1°C	2°C	5°C	6°C	7°C	8°C	9°C	Time interval (2003)	Days to cessation of flush elongation	1°C	2°C	5°C	6°C	7°C	8°C	9°C
7 Apr-22 May	45	17499	16371	12987	11863	10742	9626	8522	2 Apr-12 May	40	15863	14879	11927	10943	9959	8979	8007
9 Apr-27 May	48	17822	16647	13448	11947	10778	9617	8473	13 Apr-30 May	47	17613	16437	12909	11737	10568	9412	8278
7 May-20 Jun	44	13470	12390	9204	8193	7206	6263	5373	7 May-17 Jun	41	12930	11898	8852	7881	6934	6027	5168
23 May-10 Jul	48	12896	11722	8318	7264	6269	5356	4531	23 May-10 Jul	48	12928	11730	8253	7338	6332	5410	4575
15 June-3 Aug	49	12854	11657	8242	7203	6229	5339	4527	16 Jun-3 Aug	48	12566	11393	8050	7036	6085	5217	4423
15 Jul-7 Sept	54	15847	14408	10208	8899	7652	6493	5416	18 Jul-7 Sept	51	14772	13404	9421	8184	7007	5915	4899
	Mean	15065	13866	10346	9228	8146	7116	6140		Mean	14445	13290	9902	8853	7814	6826	5891
	σ	2293	2278	2215	2167	2097	1996	1867		σ	2014	2020	2031	1984	1939	1865	1765
	%CV	15.22	16.43	21.41	23.48	25.75	28.06	30.40		%CV	13.94	15.21	20.51	22.41	24.81	27.32	29.95

Table 4. The number of days required for cessation of flush elongation following green point induction by ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on two- and three-flush shoots of 12-year old *Protea* cv. Carnival plants. Accumulated growing degree hours (GDH) at various base temperatures (°C) is given for the phenological phase 'green point to cessation of flush elongation'. Values indicated in **bold** specify the base temperature with lowest %CV. Mean, standard deviation (σ) and coefficient of variation (CV) are reported.

		Two-flush shoots								Three-flush shoots					
		Base temperature (T_b)								Base temperature (T_b)					
Time interval (2003)	Days to cessation of flush elongation	5°C	**6°C**	7°C	8°C	9°C	10°C	Time interval (2003)	Days to cessation of flush elongation	5°C	**6°C**	7°C	8°C	9°C	10°C
11 Apr-20 May	39	10855	**9900**	8947	7999	7062	6153	7 Apr-16 May	40	10958	**9998**	9039	8082	7136	6215
19 Apr-10 Jun	50	12146	**10908**	9689	8498	7347	6256	14 Apr-3 Jun	52	13085	**11866**	10650	9447	8272	7150
17 May-5 Jul	49	9006	**7940**	6927	5988	5130	4358	10 May-29 Jun	52	9951	**8813**	7721	6690	5740	4868
5 Jun-25 Jul	51	5770	**6933**	5987	5132	4367	3674	25 May-17 Jul	55	7746	**7730**	6676	5712	4843	4066
24 Jun-14 Aug	51	8148	**7314**	6318	5399	4551	3770	16 Jun-12 Aug	58	9041	**7994**	6914	5921	5011	4177
26 Jul-14 Sept	50	8654	**7735**	6658	5653	3546	2856	26 Jul-16 Sept	53	9204	**8050**	6949	5920	4958	4056
	Mean	9096	**8455**	7421	6445	5334	4511		Mean	9998	**9075**	7991	6962	5993	5089
	σ	2216	**1582**	1521	1434	1538	1397		σ	1848	**1596**	1563	1499	1409	1305
	%CV	24.37	**18.71**	20.50	22.25	28.83	30.96		%CV	18.48	**17.59**	19.56	21.53	23.51	25.64

Table 5. The number of days required to reach the harvest stage following flush elongation and green point as induced by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on three-flush shoots of eight-year old *Protea* cv. Carnival plants. Accumulated growing degree hours (GDH) at various base temperatures (°C) is given for the phenological stage 'cessation of flush elongation to harvest'. Values indicated in **bold** specify the base temperature with lowest %CV. Mean, standard deviation (σ) and coefficient of variation (CV) are reported.

		Accel®								Benzyladenine					
		Base temperature (T_b)								Base temperature (T_b)					
Time interval (2003-2004)	Days to harvest	**0°C**	1°C	2°C	3°C	4°C	5°C	Time interval (2003-2004)	Days to harvest	0°C	**1°C**	2°C	3°C	4°C	5°C
23 May-14 Nov	175	**56926**	52744	48572	44426	40341	36344	13 May-6 Nov	177	57645	**53392**	49147	44930	40773	36704
28 May-11 Nov	167	**53968**	49978	45996	42041	38146	34341	31 May-18 Nov	171	56039	**51955**	47879	43860	39843	35942
21 Jun-4 Dec	166	**58244**	54279	50322	46391	42508	38688	18 Jun-10 Dec	175	62217	**58036**	53863	49716	45617	41585
11 Jul-15 Dec	157	**58281**	54513	50746	46993	43281	39619	11 Jul-14 Dec	156	57827	**54083**	50340	46616	42923	39285
4 Aug-7 Jan	156	**62494**	58750	55006	51266	47543	43843	4 Aug-1 Jan	150	59001	**55401**	51801	48204	44626	41070
8 Sept-7 Jan	121	**52637**	49734	46831	43928	41025	38122	8 Sept-17 Jan	131	53321	**53296**	50249	47202	44155	41108
	Mean	**57092**	53333	49579	45841	42141	38493		Mean	57675	**54360**	50547	46749	42989	39282
	σ	**3509**	3352	3249	3199	3195	3223		σ	2968	**2122**	2087	2143	2270	2435
	%CV	**6.15**	6.29	6.55	6.98	7.58	8.37		%CV	5.15	**3.90**	4.13	4.59	5.28	6.20

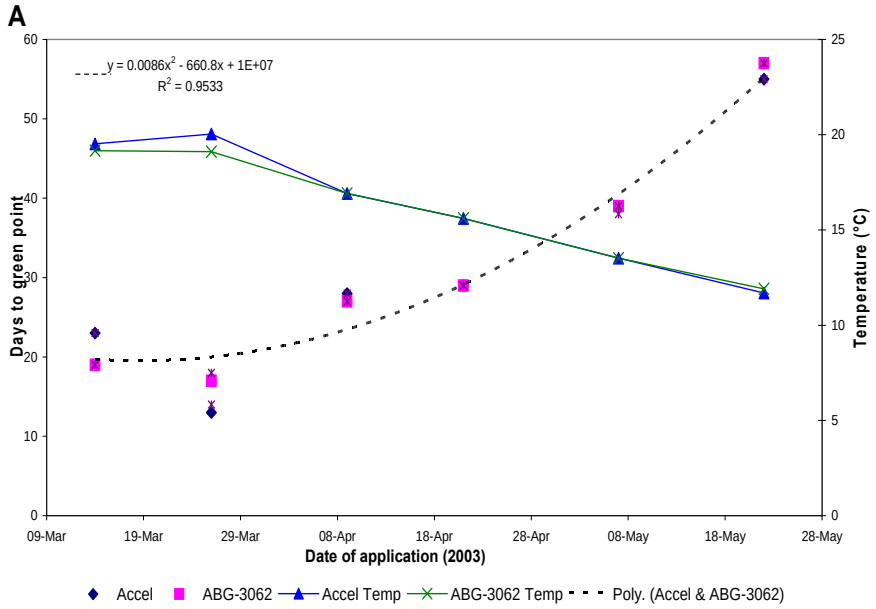
Table 6. Accumulated growing degree hours (GDH) at various base temperatures (°C) to reach the harvest stage following cessation flush elongation and green point induction by ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on two- and three-flush shoots of 12-year old *Protea* cv. Carnival plants. Values indicated in **bold** specify the base temperature with lowest %CV. Mean, standard deviation (σ) and coefficient of variation (CV) are reported.

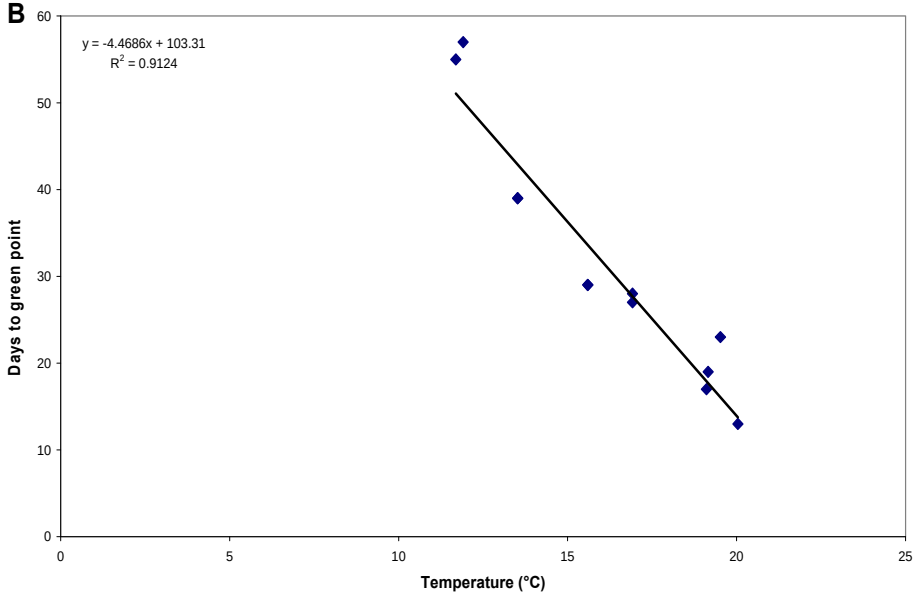
		Two-flush shoots								Three-flush shoots					
		Base temperature (T_b)								Base temperature (T_b)					
Time interval (2003-2004)	Days to harvest	0°C	1°C	**2°C**	3°C	4°C	5°C	Time interval (2003-2004)	Days to harvest	**0°C**	1°C	2°C	3°C	4°C	5°C
	–	–	□	□	□	□	□	17 May-20 Nov	187	**62344**	57851	53366	48908	44511	40202
	□	□	□	□	□	□	□	4 Jun-19 Nov	168	**55598**	51562	47534	43533	39594	35741
	□	□	□	□	□	□	□	30 Jun-19 Dec	172	**63006**	58874	54751	50654	46605	42615
26 Jul-5 Jan	163	64389	60453	**56517**	52587	48678	44801	18 Jul-20 Dec	155	**58726**	54983	51240	47503	43796	40132
15 Aug-13 Jan	151	62999	59351	**55703**	52058	48420	44795	13 Aug-11 Jan	151	**62744**	59072	55400	51731	48069	44421
15 Sept-30 Jan	137	63195	59883	**56571**	53259	49947	46636	17 Sept-26 Jan	131	**60711**	57520	54329	51138	47947	44756
	Mean	63528	59896	**56264**	52635	49015	45411		Mean	**60522**	56644	52770	48911	45087	41311
	σ	753	551	**487**	602	818	1061		σ	**2896**	2887	2946	3061	3211	3373
	%CV	1.18	0.92	**0.87**	1.14	1.67	2.34		%CV	**4.78**	5.10	5.58	6.26	7.12	8.16

Table 7. Accumulated degree growing hours (GDH) in three-flush shoots of eight-year old *Protea* cv. Carnival or in two-and three-flush shoots of 12-year old 'Carnival' plants for the period 'growth regulator application to green point', 'green point to cessation of flush elongation' and 'cessation of flush elongation to harvest' as induced by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) at base temperatures of 8°C, 6°C and 1°C respectively.

	Growth regulator application-Green point		Green point-Cessation of flush elongation		Cessation of flush elongation-Harvest	
	Number of shoots	Base temperature 8°C	Number of shoots	Base temperature 6°C	Number of shoots	Base temperature 1°C
Eight-year old plants						
Accel®	293	5391[a]	292	–	223	53333[a]
ABG-3062	297	5386[a]	295	–	197	54361[a]
Twelve-year old plants						
Two-flush shoots	245	6927[a]	243	8455[b]	30	59413[a]
Three-flush shoots	287	5952[b]	287	9075[a]	164	56644[b]

Mean separation in columns within plant age followed by superscripts with the same letter are not significantly different at LSD $_{(p=0.05)}$





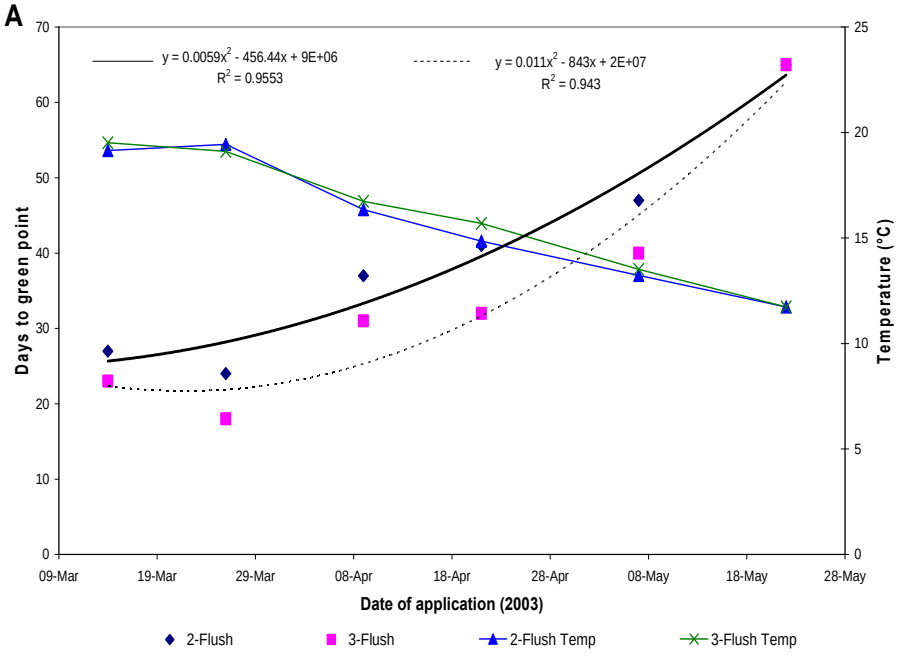
ANOVA

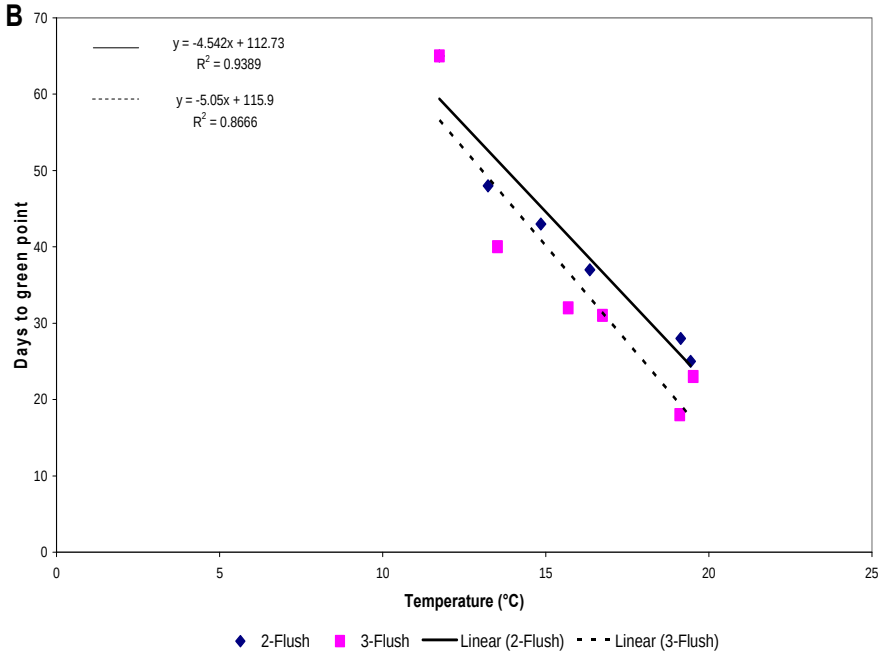
CONTRASTS	*F value*	*Pr>F*	*Significance*
Accel® vs ABG-3062	0.11	0.7392	ns
Time linear	482.15	<0.0001	***
Time quadratic	73.04	<0.0001	***
Time linear*Accel® vs ABG-3062	1.3	0.2611	ns
Time quadratic*Accel® vs ABG-3062	0.3	0.5897	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
Temperature vs Days	-0.95186	<0.0001	***

Figure 1.A. The number of days required to green point as induced on three-flush shoots of eight-year old plants of *Protea* cv. Carnival by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) applications on six application dates (2003). The average temperature (°C) recorded from growth regulator application to green point for each respective treatment date is indicated on the secondary y-axis. B. Relationship between number of days required from growth regulator application to reach green point and the average temperature (°C) recorded for that period.





ANOVA

CONTRASTS	*F value*	*Pr>F*	*Significance*
2-Flush vs 3-Flush	45.6	<.0001	***
Time linear	834.68	<.0001	***
Time quadratic	102.67	<.0001	***
Time linear*2-Flush vs 3-Flush	0.46	0.5015	ns
Time quadratic*2-Flush vs 3-Flush	9.9	0.003	**

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
2Flush Temperature vs Days	-0.96898	0.0014	**
3Flush Temperature vs Days	-0.93089	0.007	**

Figure 2. A. The number of days required to green point as induced by ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) application on two-and three-flush shoots of 12-year old plants of *Protea* cv. Carnival on six application dates (2003). The average temperature (°C) recorded from growth regulator application to green point for each respective treatment date is indicated on the secondary y-axis. B. Relationship between number of days required from growth regulator application to reach green point and the average temperature (°C) recorded for that period.

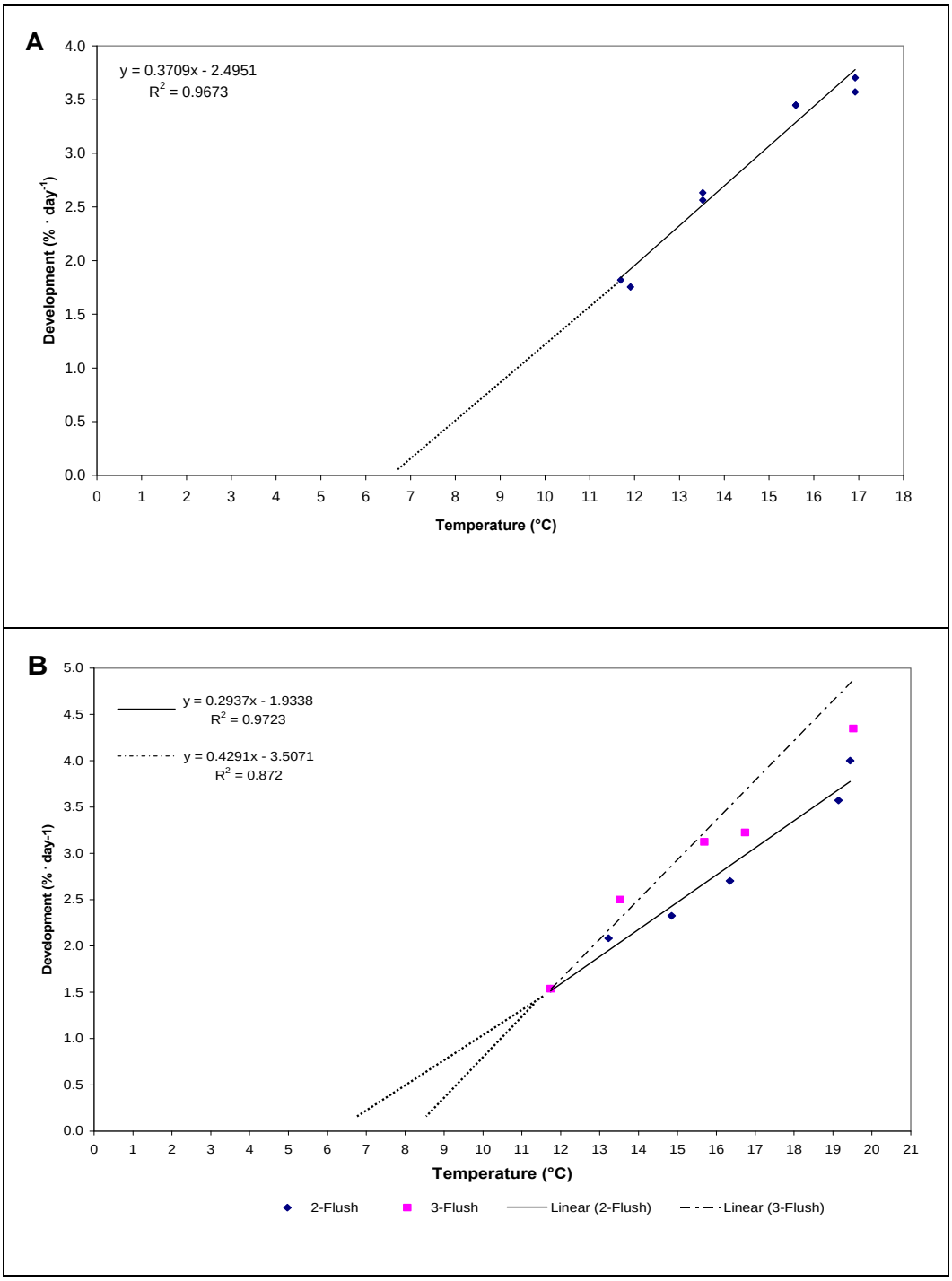
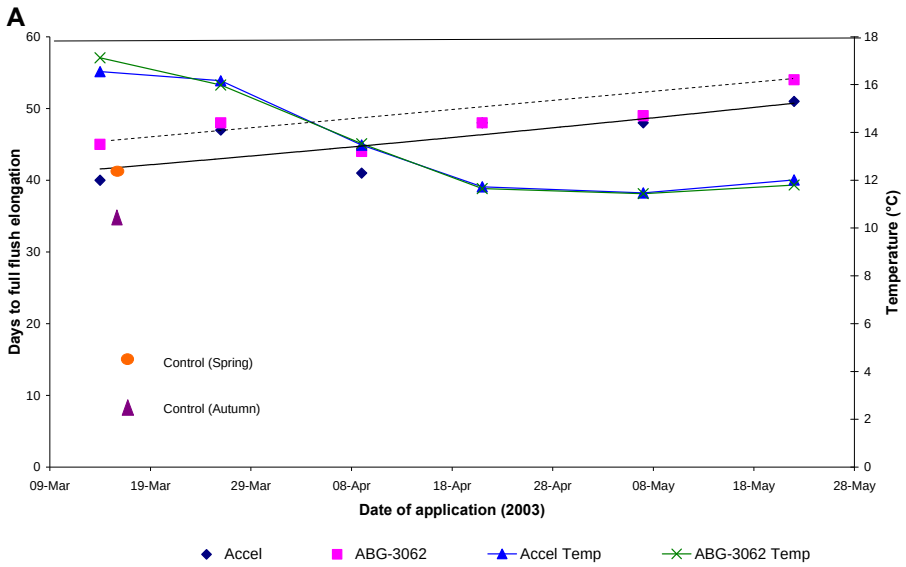
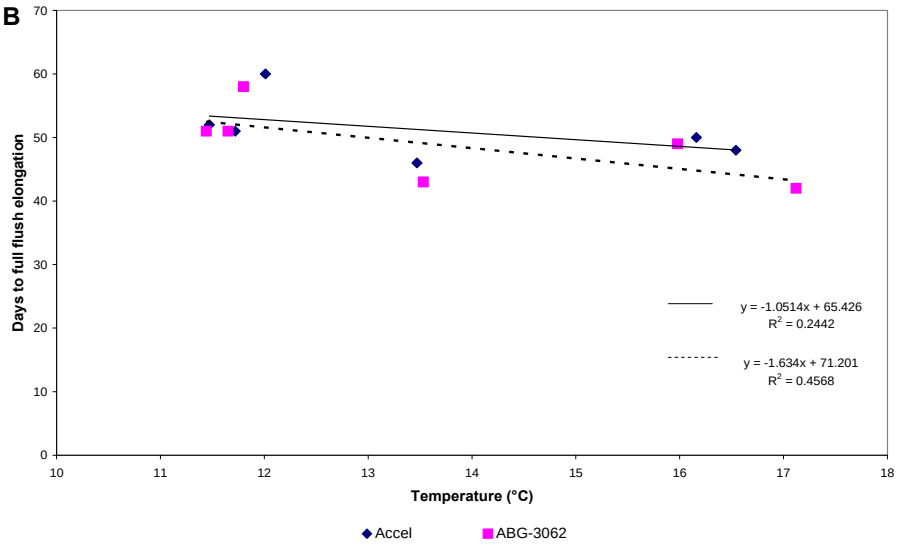


Figure 3. A. Relationship between mean daily temperature (°C) and rate of development (%·day^{-1}) to green point as induced by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on three-flush shoots of eight-year old *Protea* cv. Carnival plants. B. Relationship between mean daily temperature (°C) and rate of development (%·day^{-1}) to green point of two-and three-flush shoots of 12-year old *Protea* cv. Carnival plants. Dotted lines indicate extrapolated values.





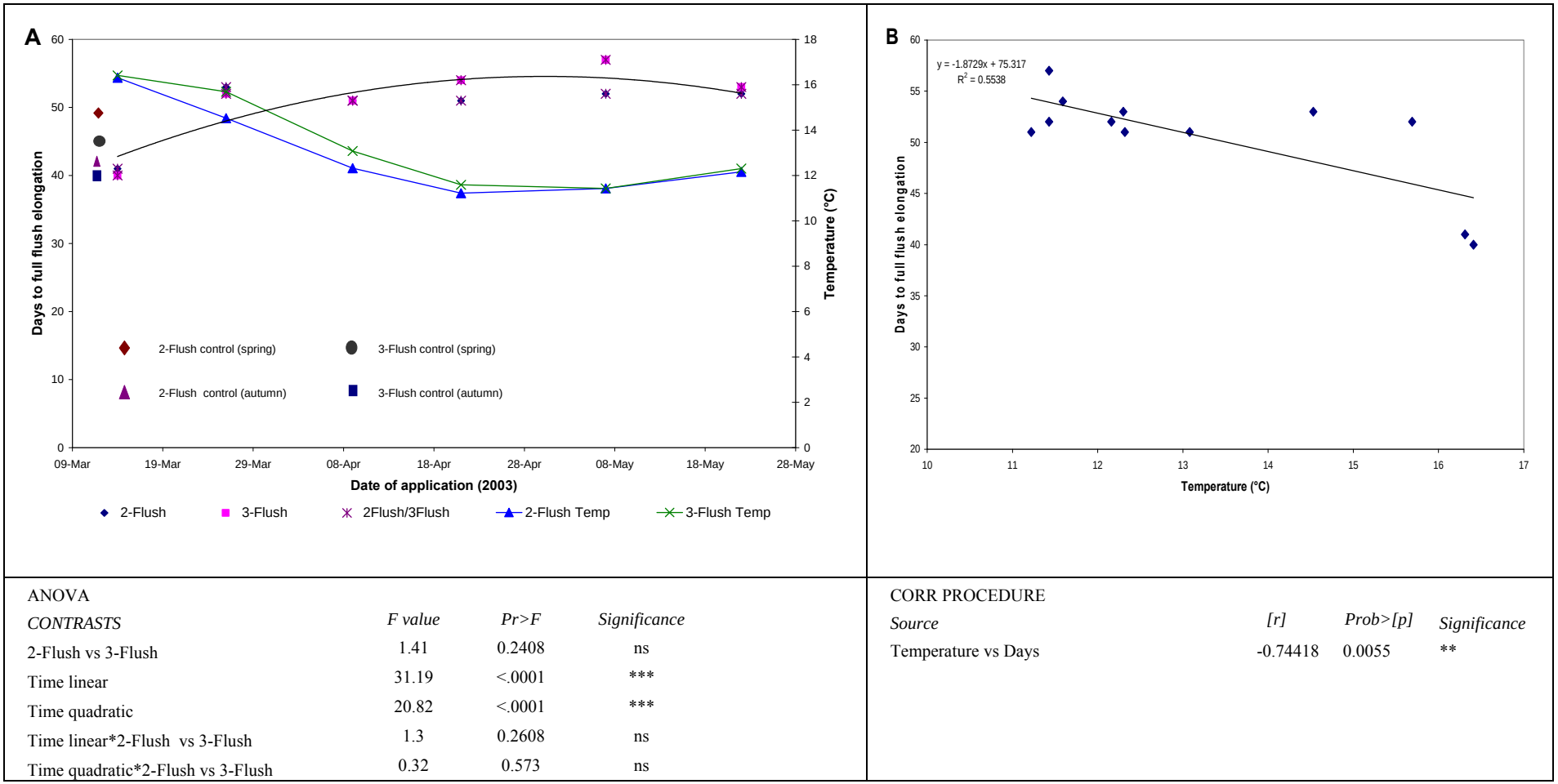
ANOVA

CONTRASTS	*F value*	*Pr>F*	*Significance*
Accel® vs BA	4.72	0.0348	*
Time linear	84.81	<.0001	***
Time quadratic	12.76	0.0008	***
Time linear*Accel® vs BA	1.29	0.2612	ns
Time quadratic*Accel® vs BA	3.05	0.087	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
Accel ® Temperature vs Days	-0.67585	0.1406	ns
BA Temperature vs Days	-0.49413	0.3191	ns

Figure 4. A. The number of days required from green point to cessation of flush elongation as induced by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) applications on three-flush shoots of eight-year old plants of *Protea* cv. Carnival plants on six application dates. The average temperature (°C) recorded for each treatment from green point to cessation of flush elongation is indicated on the secondary y-axis. B. Relationship between number of days required from green point to cessation of flush elongation and the average temperature (°C) recorded for that period.



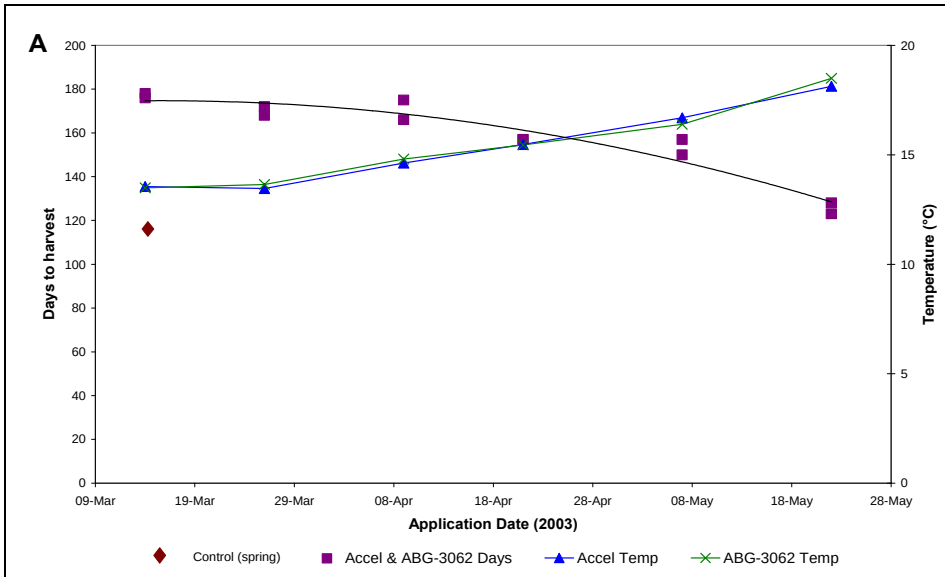
ANOVA

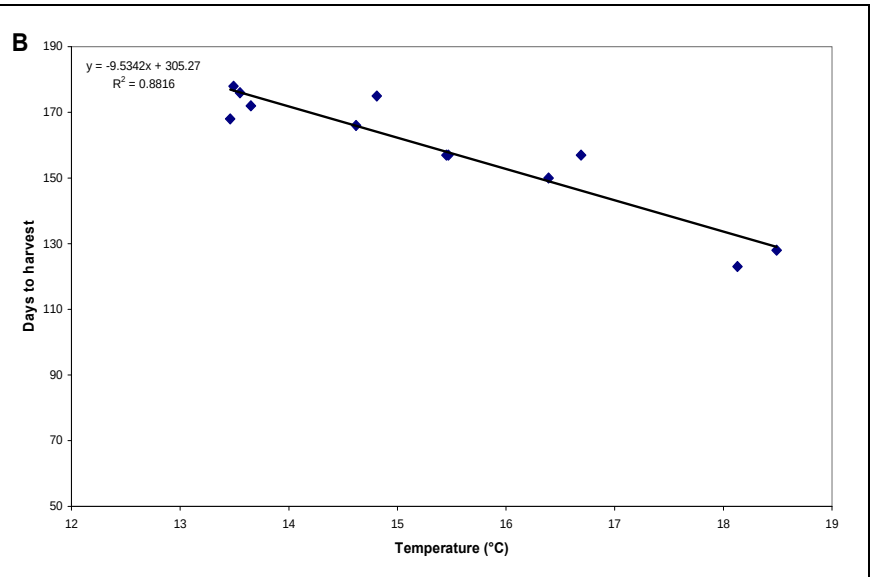
CONTRASTS	*F value*	*Pr>F*	*Significance*
2-Flush vs 3-Flush	1.41	0.2408	ns
Time linear	31.19	<.0001	***
Time quadratic	20.82	<.0001	***
Time linear*2-Flush vs 3-Flush	1.3	0.2608	ns
Time quadratic*2-Flush vs 3-Flush	0.32	0.573	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
Temperature vs Days	-0.74418	0.0055	**

Figure 5. A. The number of days required from green point to cessation of flush elongation as induced by ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) in two- and three-flush shoots of 12-year old plants of *Protea* cv. Carnival on six application dates (2003). The average temperature (°C) recorded from green point to cessation of flush elongation as induced by BA is indicated on the secondary y-axis. B. Relationship between number of days required from green point to cessation of flush elongation and the average temperature recorded for that period.





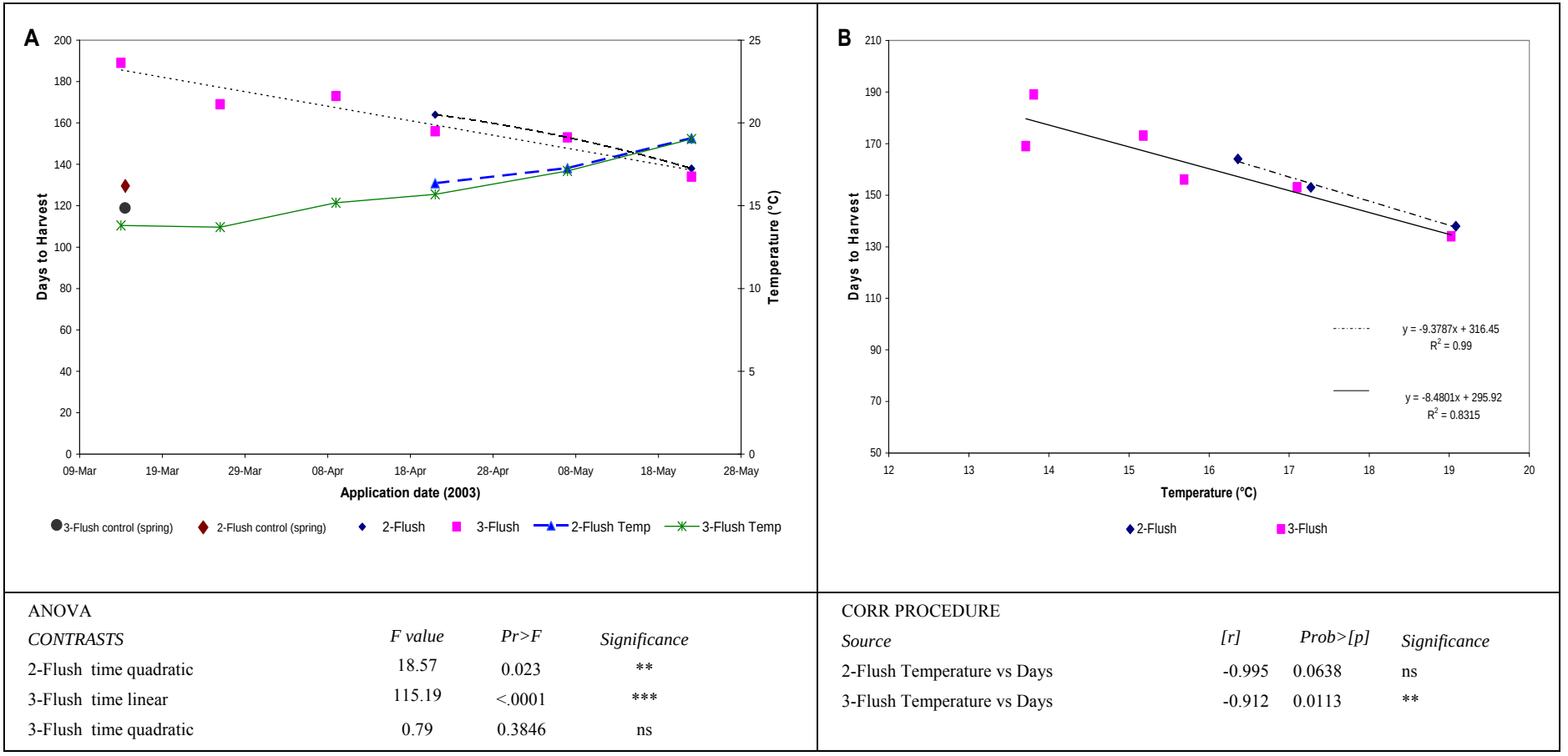
ANOVA

CONTRASTS	*F value*	*Pr>F*	*Significance*
Accel® vs BA	2	0.1643	ns
Time linear	341.86	<.0001	***
Time quadratic	43.01	<.0001	***
Time linear*Accel ® vs BA	0.27	0.6042	ns
Time quadratic*Accel ®vs BA	0.11	0.7453	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
Temperature vs Days	-0.93892	<.0001	***

Figure 6. A. The number of days required from cessation of flush elongation to reach the harvest stage of inflorescences induced on six application dates (March-May 2003) by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on three-flush shoots of eight-year old plants of *Protea* cv. Carnival. The average temperature (°C) recorded from cessation of flush elongation to harvest is collectively indicated on the secondary y-axis. B. Relationship between the number of days required from cessation of flush elongation to harvest and the average temperature (°C) recorded for that period.



ANOVA

CONTRASTS	*F value*	*Pr>F*	*Significance*
2-Flush time quadratic	18.57	0.023	**
3-Flush time linear	115.19	<.0001	***
3-Flush time quadratic	0.79	0.3846	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Significance*
2-Flush Temperature vs Days	-0.995	0.0638	ns
3-Flush Temperature vs Days	-0.912	0.0113	**

Figure 7. A. The number of days required from cessation of flush elongation to harvest for inflorescences induced by ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on two- and three-flush shoots of 12-year old plants of *Protea* cv. Carnival. The average temperature (°C) for each treatment as recorded from cessation of flush elongation to harvest is indicated by the secondary y-axis. B. Relationship between the numbers of days required from cessation of flush elongation to harvest and the average temperature recorded for that period.

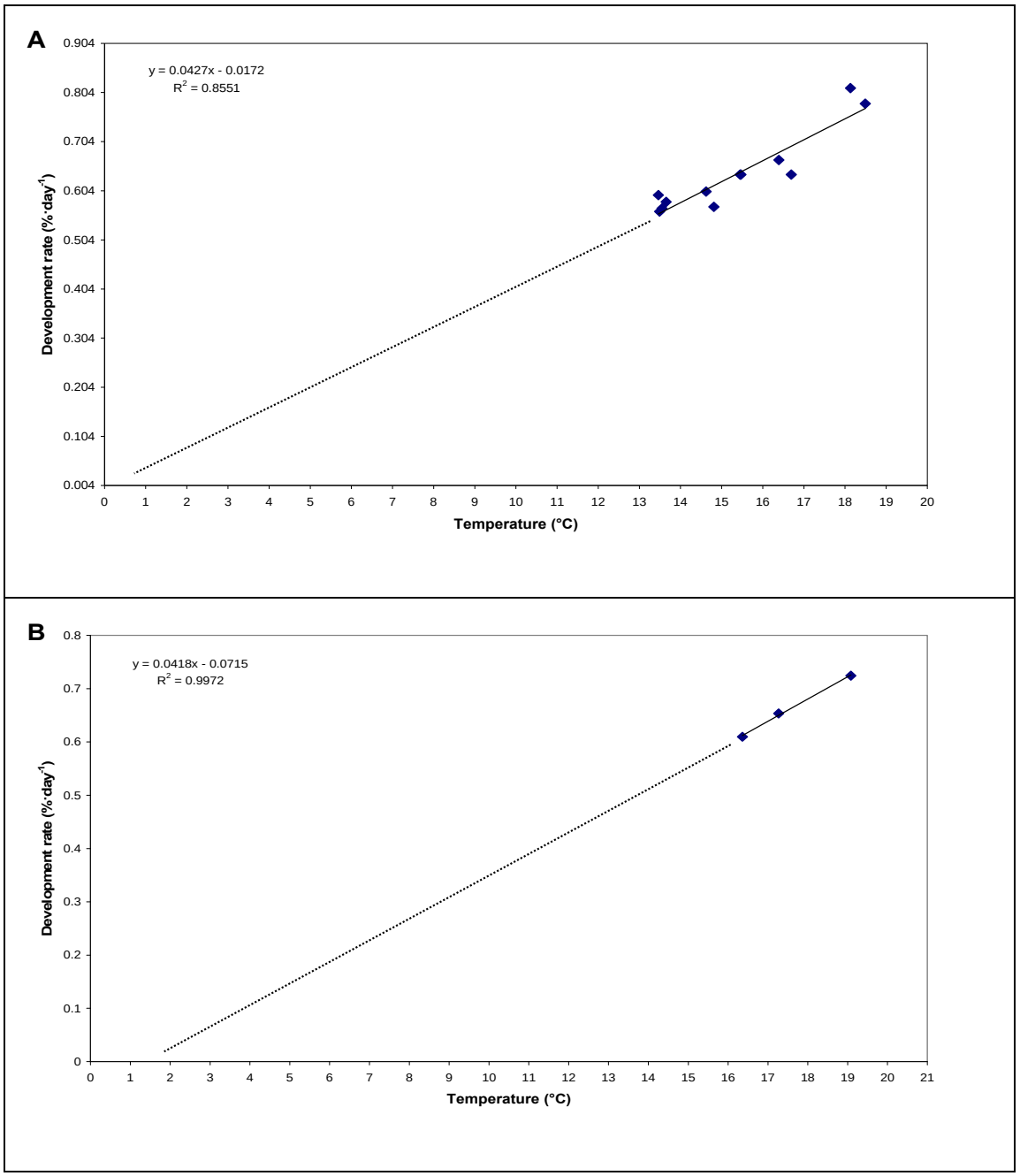


Figure 8. A. Relationship between mean daily temperatures (°C) and rate of daily average development (%·day^{-1}) from cessation of induced flush elongation to harvest of inflorescences induced by Accel® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 0.18% $^{w}/_{w}$) and ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$) on three-flush shoots of eight-year old *Protea* cv. 'Carnival' plants. B. Relationship between mean daily temperatures (°C) and rate of development (%·day^{-1}) from cessation of induced flush elongation to harvest of inflorescences induced ABG-3062 on two-and three-flush shoot of 12-year old *Protea* cv. Carnival plants. Dotted lines indicate extrapolated values.

5. PAPER IV. Leaves, flush maturity and plant age affect the responsiveness of *Protea* cv. Carnival shoots to benzyladenine induction of 'out of season' flowering.

Leaves, flush maturity and plant age affect the responsiveness of *Protea* cv. Carnival shoots to benzyladenine induction of 'out of season' flowering.

Abstract

Terminal buds on mature three-flush shoots of *Protea* cv. Carnival were treated in autumn with 500 mg·L^{-1} BA (MaxCel^{TM}, active ingredients: BA 1.9% $^{w}/_{w}$). Defoliating different sectors of the shoot immediately prior to treatment revealed that when the flush that subtended the BA-treated terminal bud is defoliated, the efficacy of BA to induce 'out of season' flowering was compromised. Percentages budbreak and inflorescence initiation following BA applications over an eight week period were assessed with increasing maturity of the terminal 2nd summer flush on vigorous, vegetatively orientated three-year old plants in their first year of bearing compared to that of shoots of more mature, commercially productive six-year old plants. BA applications induced budbreak at percentages of 96-100% throughout the 2nd summer flush maturation, irrespective of plant age or treatment date. Shoots on young (three-year old) plants were more vigorous than those on six-year old plants as was evident from their greater ability to flush unaided in autumn. BA treatment of three-flush shoots on three-year old plants where the terminal flush was not 'hardened off' (mature), resulted in no or poor flowering (20%) 'out of season'. Flowering increased *ca.* 80% when BA applications were delayed to permit 'hardening off' to take place. In contrast high percentages of between 70-90% of three-flush shoots on six-year old plants initiated flowers 'out of season', irrespective of the stage of maturation.

The vegetative phenology of *Protea* follows a pattern where shoot growth take place as periodic ephemeral flushes emerging successively from apical buds. In active growing shoots these flushes exhibit strong apical dominance (Malan and Le Roux, 1995). Between

flushing episodes, the apical and axillary buds of a shoot are dormant, with the apical bud containing the preformed leaf primordia of the next flush (Gerber *et al.*, 2001a). New flush growth is initiated with budbreak, followed by internode extension and the expansion and increase of dry mass of preformed leaves. Inflorescences are terminally borne on a shoot consisting of two or more growth flushes (Greenfield *et al.*, 1994). For most hybrids and species selections currently cultivated in Mediterranean areas, the flush subtending the inflorescence predominantly resumes after an extended period of rest. This rest period, where no visible bud activity or shoot extension growth can be observed, stretches from mid autumn (early to late March, local conditions) until budbreak in early spring (end of August).

The number of shoot flushes per growth period is thought to be species or cultivar specific (Coetzee and Littlejohn, 2001). Growth vigour of *Protea* cv. Pink Ice (*P*. neriifolia × *P*. *compacta*) allows for production of as many as seven flushes per year, whilst *P*. *magnifica* may habitually produce no more than two flushes per year (Jacobs, pers. comm., 2001). Young vigorous plants of *Protea* have also been observed to produce more shoot growth flushes per year than older more complex plants (Malan and Le Roux, 1995). The vigour of shoot growth was studied for a variety of cultivars not subjected to pruning. Shoot growth vigour was found to differ considerably within a plant (Malan and Le Roux, 1995). Not all shoots observed to flush during a period necessarily flowered, which resulted in unsynchronized flowering of shoots on a particular plant.

The windows during which shoot flush extension takes place in *Protea* appear to be partly under environmental control. Comparative studies on *Protea neriifolia* growth habits when cultivated in a summer rainfall area versus its natural Mediterranean habitat, showed a markedly earlier shift of phenophases occurring in the summer rainfall cultivated plants (Heinsohn and Pammenter, 1988). Flower initiation closely followed shoot growth pattern changes so that the flowering window for the summer cultivated plants shifted several

months earlier to peak in late summer to autumn, compared to the flowering period of autumn to early winter for plants cultivated in a winter rainfall area. This indicated a link between the vegetative growth phenology and the reproductive phase.

The presence of mature leaves is regarded as essential to the flowering process in *Protea* (Gerber *et al.*, 2001b; 2002). A common feature in all three *Protea* cultivars studied by Gerber *et al.* (2001a), was a progressive increase in the number of leaves with each successive flush leading up to inflorescence initiation. Capacity to support the flowering process in *Protea* is believed to be derived directly from current photosynthesis and not from the reserve carbohydrate status in the wood which is low throughout the year (Greenfield *et al.*, 1995). This view was supported by Smart (2005) using tracer stable isotopes on *Protea* cv. Sylvia. Furthermore, Smart (2005) suggested supplementation of the shoot's current photosynthesis by possible translocation of carbon from vegetative to flowering shoots and/or that the utilization of stored reserves may occur to support developing sinks, such as flowers or young leaves.

Apart from the essential role leaves of *Protea* are thought to play in the perception of floral induction (Gerber *et al.*, 2002), mature over-wintering leaves in 'Lady Di' proteas were also considered crucial to the early stages of inflorescence initiation and differentiation (Gerber *et al.*, 2001b). Removal of leaves prior to the completion of spring growth flush elongation prevented flowering. Shoots subsequently either remained vegetative or produced inflorescences that aborted. During elongation of the spring flush, photosynthates were found to be provided by the mature leaves of the over-wintering shoot. Soon after the cessation of the spring flush elongation, leaves of the new unfolded spring flush, started to serve as the main source for the developing inflorescence (Gerber *et al.*, 2001b).

Recent findings demonstrated that in *Protea* cv. Carnival (*P. neriifolia* × *P. compacta*) application of cytokinin to terminal buds of resting shoots may lead to subsequent budbreak

and induced flowering (Paper I, table 4, pg57; figure 4, pg. 62). This novel approach could provide growers with a tool to initiate inflorescences 'out of season'. Application of benzyladenine (BA) to shoots of 'Carnival' in late summer to early autumn induced inflorescences to reach anthesis in November and December (Paper II, table 1, pg.87), compared to the normal flowering time of February to April (Gerber, 2000). Such an advancement of flowering time with BA application would enable South African producers to take maximum advantage of the European marketing period that peaks before or around Christmas (25 December).

However, BA shows considerable variation in its efficacy to induce flowering in treated shoots. The reason(s) for the inconsistencies in flowering incidence with BA treatment are not clear. The difference in temperatures and photoperiod as possible inductive signals (Paper III, pg.110-114) occurring in February and May might account for the difference in inflorescence initiation occurrence (Paper II, figure 3A, pg.95, figure 4A, pg. 96). However, in addition to the possible changing of environmental cues, field observations suggest that inflorescence incidence with BA induction may also be linked to the vegetative maturity status of the terminal flush subtending to the treated bud. The visual appearance of the terminal 2nd summer flush immediately after completion of extension growth in February, is distinctly different from that observed in May when the shoot is considered to be fully mature (Hoffman, pers. obs.). In the immediate period following the cessation of flush extension, the flush appearance is hairy, light-green in colour with both the leaves and stem relatively soft. The flush then gradually 'hardens off' under conditions where the terminal bud remains dormant and is transformed by the end of May into a rigid, woody stem with dark green leaves of a leathery texture.

The objectives of this study were to investigate the vegetative status of a shoot as a possible indicator of the ability of the shoot to respond to BA in terms of bud sprouting and inflorescence initiation.

Inflorescence initiation occurrence in 'Carnival' by means of BA induction is assessed with increasing shoot maturity of the terminal 2nd summer flush over eight weeks on vigorous, vegetative-orientated, three-year old plants in their first year of bearing compared to shoots of more mature and commercially productive, six-year old plants.

The number and dry mass of leaves as well as the position of the leaves (after maturation) to the terminal bud for inflorescence initiation was assessed through defoliation treatments immediately prior to BA application.

It is proposed that inflorescence initiation under BA induction for *Protea* cv. Carnival occurs under the influence of complex intra-plant interactions, including plant- and shoot maturity and vigour, in combination with specific environmental inductive signals.

Materials and Methods

Plant material. Experiments on *Protea* cv. Carnival (*P. nerifolia* × *P. compacta*) were carried out in commercial plantations grown from cuttings in the Stellenbosch district (lat. 33°55′S; long. 18°50′E), South Africa. The climate is Mediterranean-like with cool wet winters and dry hot summers. The annual rainfall is 600-700 mm, concentrated during the winter months. All plants, unless mentioned otherwise, were pruned back to bearers in August 2003 to effect biennial cropping as described by Gerber *et al.* (1995). Plants were spaced 1 m in the row and 4 m between rows. Plants were not irrigated or fertilized. Pest control focused on the control of thin-line leaf miner (*Phyllocnistis* sp.) and the speckled protea borer (*Orophia* spp.) and were applied by risk analysis which included the emergence and extension of the immature flush and during early inflorescence development.

Autumn application of benzyladenine (BA) and defoliation. Flush maturation as a variable possibly affecting inflorescence initiation was kept constant whilst the leaf number and the position of the leaves relative to the terminal bud were assessed for their importance in floral initiation under BA induction. Fully matured (rigid, woody stem with dark green leaves of a leathery texture) three-flush shoots of six-year old 'Carnival' plants of comparative diameter (F value=392; Pr>F=0.69956) of approximately 8 mm were selected on 8 May 2004 for treatment with an aqueous solution of MaxCel^{TM} (Valent Biosciences, Libertyville, IL, USA; active ingredients: BA 1.9% $^{w}/_{w}$) at 500 mg·L^{-1} to the terminal bud. No surfactants were needed to facilitate applications. Three-flush shoots consisted of the basal flush originating from an axillary position on the bearer, followed terminally by two additional flushes, produced in November (1st summer flush) and January (2nd summer flush) respectively. The stem diameter was measured with vernier digital callipers just below the apex and at the upper position of the intercalation between the distal two flushes. Immediately prior to BA application, leaves were removed at their point of inception from different combination of flushes to represent eight defoliation treatments as indicated in Table 1. Ten shoots were used per treatment. Treatments were replicated 5 times in a randomized complete block design. In addition, ten shoots resembling the treatment shoots were harvested and analyzed to quantify shoot characteristics in terms of leaf number, leaf area and leaf dry weight. Leaf area was determined by a portable Li-COR leaf area meter (model LI-3000 LI-COR Biosciences, Nebraska, USA) and leaf dry mass determined after drying the leaves (separated from the stem) in a draught oven (60°C, 72 h).

Autumn application of benzyladenine and flush maturity on six-year old plants. Three hundred three-flush shoots of six-year old plants were selected for uniformity immediately after the unfolding of all 2nd summer flush leaves. Directly after all shoots were tagged on 3 March 2004, 50 shoots received a single treatment of BA at 500 mg·L^{-1} ($6T_1$). Subsequently,

four sets of 50 shoots each received single BA (MaxCel^{TM}, Valent Biosciences, Libertyville, IL, USA) treatments, on 17 March ($6T_2$), 31 March ($6T_3$), 14 April ($6T_4$) and 28 April ($6T_5$) 2004 respectively. The BA solution was applied to the terminal bud only using a paint brush. Terminal buds of a further 50 shoots were treated with distilled water on the first treatment date (3 March 2004) and served as the control treatment. A randomized complete block design of five blocks was used.

Autumn application of benzyladenine and flush maturity on three-year old plants. A second trial identical in design and methodology to the trial described above was also performed on three-year old 'Carnival' shoots, again directly after the completion of the unfolding of the leaves on the 2nd summer flush. The three-year old plants completed the extension of the 2nd summer flush 3 weeks later than the six-year old plants and therefore application dates for this trial were later, on 24 March ($3T_1$), 7 April ($3T_2$), 21 April ($3T_3$), 5 May ($3T_4$) and 19 May ($3T_5$) respectively, with the control application on 24 March 2004. Each treatment date (T_n) of the three-and six-year old plants represented shoots of the same phenological and physiological stage.

Shoot characteristics of three-flush shoots of three- and six-year old plants at the first treatment date are given in Table 2.

With each treatment date (nT_n) ten representative shoots of each plant age category were harvested and the following characteristics determined: total shoot length (mm); number of true leaves; leaf area (cm^2) as determined by a portable Li-COR leaf area meter (model LI-3000; LICOR Biosciences, Nebraska, USA); stem diameter (mm) as recorded by a digital vernier calliper at the upper position of the intercalation between flushes; leaf and stem dry mass (g) after drying in a draught oven (60°C ,72 h). Subsequently, these above-mentioned characteristics were determined for the maturing terminal flush (2nd summer flush) at each BA application date (T_n).

All shoots in the sample set that produced an autumn flush spontaneously in its natural flushing cycle were documented and were treated with BA up to the green point stage (torpedo shaped, terminal bud with new leaves intact, but unfolded). Shoots that progressed beyond the green point stage were removed from the data set (Paper I, table 4, pg.57).

Photosynthesis determinations. Photosynthesis measurements were performed on the same shoots on 18 March, 6 April and 2 May 2004. On these dates the terminal flushes of the three-flush six-year old 'Carnival' plants represented a soft (leaves appear hairy, light-green in colour with both the leaves and stem relatively soft); partially mature; and fully mature (rigid, woody stem with dark green leaves of a leathery texture) physiological state respectively. Light intensity was determined using a LICOR quantum sensor (model LI-190SA, LI-COR Biosciences, Nebraska, USA) for 50 shoots at midday, holding the light sensor horizontal against the stem at flush midpoint. Photosynthesis was determined using a LI-6400 portable photosynthesis system (LI-COR Biosciences, Nebraska, USA) between 10:00 and 14:00. The photosynthetic active radiation (PAR) settings for the different flushes were set to: 1500 $\mu mol \cdot m^{-2} \cdot min^{-1}$ for the 2nd summer flush (top flush), 1000 $\mu mol \cdot m^{-2} \cdot min^{-1}$ for the 1st summer flush (middle flush) and 500 $\mu mol\ m^{-2} \cdot min^{-1}$ for the basal spring flush. Number of leaves and leaf area values for each flush were obtained from the ten representative shoots harvested on the day of measurement. Photosynthesis readings ($\mu mol\ CO^2.m^{-2}.sec^{-1}$) were taken between 10h00-12h00 on clear days from a leaf of each of the three flushes. Measurements were made on comparable positions within a flush. Five shoots were used per maturity class. Correcting the photosynthetic rate expressed on a m^{-2} bases for the respective leaf areas per flush produced the photosynthetic rate per flush. The photosynthetic contribution of each flush to that of the whole shoot was also expressed as a percentage.

Autumn application of benzyladenine or Promalin® to shoots from three-and fifteen-year old plants. All plants in this trial were pruned back to bearers in August 2001 as part of a biennial cropping system (Gerber *et al.*, 1995). Three-flush shoots with mature terminal flushes on three- and fifteen-year old plants were selected. The terminal buds were given a single treatment on 12 April 2002 with either BA (ABG-3062: active ingredient: BA 2% $^{w}/_{w}$; Abbott Laboratories, North Chicago, USA) at 250 or 500 mg·L^{-1} or Promalin® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 1.8% $^{w}/_{w}$; Abbott Laboratories, North Chicago, USA) at 500 mg·L^{-1}. Thirteen shoots were used per treatment in both age categories.

Budbreak attainment that followed the growth regulator application, together with the flowering incidences on the induced flush, was recorded.

Statistical analysis. Standard analysis of variance was performed on data using the General Linear Model Procedure generated by the SAS® program (SAS Institute, 2000). LSMeans and LSD values were calculated at the 5% significance level. The SAS® program was also used to fit correlations (Pearson correlation coefficient) and orthogonal contrasts. Logit transformation of data was performed on all values expressed as percentages. Descriptive statistics for shoot characteristics were obtained using Statistica version 7.1 (Stasoft, 2005).

Results

Autumn application of benzyladenine and defoliation. The absence of leaves from the flush subtending the BA-induced flush was detrimental to inflorescence initiation following BA (as MaxCel™) application (Table 1). Inflorescence initiation occurrence was significantly lower in shoots where all leaves were removed from the terminal 2nd summer flush compared to non-defoliated, control shoots. The negative effect of 2nd summer flush defoliation on inflorescence initiation was intensified when defoliated in combination with either 1st

summer or with both 1st summer and spring flushes (Table 1). Defoliation of only half of the terminal (2nd summer) flush, whether basal or proximal, or the entire 1st summer flush only or the entire basal spring flush, did not result in inflorescence occurrence to differ from that of the non-defoliated (control) shoot.

Autumn application of benzyladenine and flush maturity on three- and six-year-old plants. MaxCel™ application to terminal buds induced budbreak percentage of between 96-100% throughout 2nd summer flush maturation, irrespective of plant age or treatment date (Figures 1A, 2A). No linear or quadratic trend over time was observed at the 5% confidence level for any of the treatment groups. Budbreak as induced by BA in both the three- and six-year old plants was greater than the natural budbreak incidence of respective control shoots (Figures 1A, 2A). Natural budbreak incidence in control shoots of three-year old plants (50%) was considerably higher than that of the control shoots of six-year-old plants (20%) (Figures 1A, 2A).

Flowering occurrence as initiated by BA application on the immature 2nd summer flushes of six-year-old plants started at 70% and increased linearly with time and flush maturation to 91% when BA application was made to the fully matured terminal flush (Figure 1A). Induction of flowering by BA application was significantly greater at the 5% confidence level than flowering occurrence (2%) of control shoots.

Few shoots initiated an inflorescence in three-year old plants over the first two treatment dates where BA application was made to the very immature 2nd summer flush. There was a significant increase in flowering incidence from the application date of 21 April onwards compared to that of the control. Flowering increased in a quadratic relationship with each consecutive treatment date (Figure 2A).

Dry mass of the terminal flush and the complete shoot increased significantly over time in both three- and six-year old plants (Figure 1A, 2A). Inflorescence initiation occurrence

was positively correlated with dry mass increase of both the complete shoot and flush subtending the BA treated terminal bud in both three- and six-year old plants (Figure 1B, 2B).

Characteristics of the 2nd summer flush for both three-and six-year old plants (Table 3) indicate that, together with the dry mass increase, an increase either in a linear or a quadratic relationship, with respect to all measured parameters, followed over the maturation period.

Tables 4 and 5 disclose the correlative relationships between the respective characteristics of the 2nd summer flush of three- and six-year old plants.

Photosynthetic contributions, as calculated for the individual flushes on three-flush shoot of six-year old plants, indicated that when the terminal flush was still soft and immature, approximately 50% of all photosynthates of a three-flush shoot were delivered from the mature middle (1st summer flush) (Figure 3). At this early stage of the shoot's phenology, the basal spring flush was calculated to be even more important at 26.7% as a source of photosynthates than the immature terminal flush at 23.4%. However, as the shoot matured, the importance of the terminal flush as a source of photosynthates increased relative to the middle and basal flushes. In the fully mature, 'hardened off' shoot, the terminal flush was found to be the main supply of photosynthates, followed by the middle and basal flushes respectively (Figure 3).

Autumn application of benzyladenine or Promalin® to three-flush shoots on three- and fifteen-year old plants. Fifteen-year old plants initiated greater number of inflorescences on both BA and Promalin®-induced flushes than for the three-year old plants (Table 6). No flowers were recorded on control shoots of either three- or fifteen-year old plants (Table 6). The shoot quality in terms of stem diameter (7.17±0.07 mm) at the intercalation between the treated and subtending flush did not vary between growth regulator treatments across the respectively plant ages ($F_{(4, 108)}$= 0.78451, p= 0.53766). BA treated shoots initiated a greater

number of inflorescences than Promalin® (GA:BA; 1:1) treated shoots in both plant age categories (Table 6).

Discussion

Leaves have been shown to be essential for inflorescence initiation on the spring flush (Gerber *et al.*, 2001b; 2002). This is also the case for 'out of season' floral initiation induced by an autumn application of BA. Few shoots initiated flowers when shoots were defoliated completely as compared to all shoots initiating an inflorescence when all leaves were left intact (Table 1). Under a BA induction system, the location of the leaves relative to the terminal bud is important to achieve inflorescence initiation. To accomplish a high propensity for floral initiation, leaves should be present on the flush that subtends the terminal bud to be treated with BA.

In Paper II (pg. 77-80) it was suggested that the efficacy of BA to induce inflorescence initiation, when applied in autumn over a range of treatment dates, may be affected by shoot characteristics such as the 'degree of hardening off' (maturation) of the terminal flush. Characteristics of shoots on three- and six-year old plants differed in a number of important ways (Tables 2, 3). To attain a comparable phenological stage of the 2nd summer flush on three and six-year old plants, the first application date for BA on the three-year old plants had to be delayed by three weeks compared to the six-year old plants (Table 3). Since 50% of non-treated control shoots of three-year old plants produced an autumn flush as compared to only 20% in the case of six-year old plants it is concluded that shoots of three-year old plants were more vigorous than shoots of six-year old plants. Quantitative differences such as total dry mass, shoot diameter and leaf area that existed between shoots on three- and six-year-old plants at the time of the first BA application were also still evident at later application dates (Tables 2, 3). It was therefore possible to firstly assess the effect of differences in shoot characteristics caused as a result of the stage of shoot maturation and

secondly to evaluate the efficacy of BA to induce flowering in plants showing differences in plant vigour.

BA application to terminal buds of three-flush shoots induced bud sprouting in more than 95% of the shoots, irrespective of plant age or treatment date (Figures 1A, 2A). Lack of bud sprouting was therefore not the reason for differences in flowering percentages between treatments.

In a number of subtropical crops such as lychee, mango and citrus, floral management strategies for 'out of season' flowering centre around prevention of new shoot initiation before resting stems have reached sufficient maturity to induce flowering shoots (Núňez-Elisea and Davenport 1995; Davenport 2003). A greater stem maturity has consistently been shown to increase the likelihood of the succeeding flush to be a reproductive flush. Davenport (2003) however did not quantify the changes in dry weight or other constituents that accompanied shoot maturation.

In this study, the dry weight that accumulated on the 2nd summer flush after the eight-week period of maturation was 2.18 or 2.68 times more for three- and six-year–old plants respectively than that measured at the first treatment date (Table 3). Part of the increase in dry weight was caused by an increase in flush dimensions. Photosynthetic capacity of the maturing flush was increased by an increase in leaf area, not leaf number as the shoots of *Protea* are known to originate from preformed flushes in terminal buds (Gerber *et al*., 2001a). Leaf area increased by approximately 50% and leaf dry mass had increased > 2.5 times by the end of the eight-week maturation period. Stem length of the 2nd summer flush increased by approximately 40%, whilst the dry weight of the stem of the maturing flush more than doubled. However, it is the diameter of the distal end of the stem of the 2nd summer flush that proportionately increased the most, *viz.* 1.58 and 1.85 times for three-and six-year old plants respectively (Table 3). It is therefore clear that maturation of a flush in

'Carnival' consists of both an increase in flush dimensions as well as in dry weight. Proportionately dry weight increased more than flush dimensions. Similarly, on a physiological level, the photosynthesis output of the maturing flush increased with the increase in flush dimension and in relation to the subtending flushes (Figure 3).

For both three- and six-year old plants the percentage shoots that initiated an inflorescence increased with each progressive date of BA application. For three-year-old plants, the relationship was quadratic with few shoots initiating an inflorescence for the first two application dates. Percentages rapidly increased for later dates reaching a high of 78% for the last date (Table 3). In contrast, for the six-year-old plants, 70% of the shoots initiated an inflorescence after treatment on the earliest date of BA application, while inflorescence initiation incidence increased linearly to reach a high of 91% for the last application date.

The next question to consider is whether there is a relationship between the characteristics of the 2nd summer flush as they change during maturation and the propensity to initiate an inflorescence following treatment with BA. For both three- and six-year old plants there were significant correlations between percentage flowering and the dry weight of the 2nd summer flush as well as with its components leaf and stem dry mass (Tables 4, 5). Likewise, there were significant correlations between percentage flowering and flush dimensions such as stem length, apex stem diameter and leaf area for the six-year-old plants and apex stem diameter for the three-year old plants. It therefore appears that there is a relationship between maturity of the terminal flush and the propensity to initiate inflorescences after BA application.

However**,** although the propensity of shoots to initiate flowers could be related to the increase of the maturity of the 2nd summer flush when three and six-year old plants were considered separately, the difference in percentage flowering between vigorous shoots on

three-year old and less vigorous shoots on six-year old plants can not be explained by flush maturity or any of the other flush characteristics for the following reasons.

Firstly, shoots on six-year old plants were less dependent on flush maturity to initiate an inflorescence than shoots on three-year old plants. This is apparent from the high percentage of shoots that initiated an inflorescence after BA treatment on very immature 2nd summer flushes of six-year old plants, whereas treatment of comparable shoots on three-year old plants failed to initiate an inflorescence. A comparable flowering percentage of 70% obtained with six-year old plants on the first treatment date, was only achieved on the last treatment date in the case of three-year-old plants. At that stage maturity of the 2nd summer flush of three-year old plants was far more advanced than that of the six-year old plants as indicated by both the dry weight and other parameters used to quantify flush maturity. It is therefore clear that explanations other than differences in shoot maturity of the 2nd summer flush should be considered to explain the differences in flowering outcome between the three- and six-year old plants.

Secondly, the positive relationship between shoot thickness and flowering has been reported in Paper II (Figure 5, pg. 97), whilst in paper V evidence will be presented to demonstrate the lower propensity of thin, mature shoots to initiate flowers in response to an autumn application of BA compared to thick shoots. However, differences in flush diameter during the maturation phase of the 2nd summer flush between three- and six-year old plants failed to account for the differences in percentage flowering. The significance of leaves and therefore possibly photosynthesis in flower initiation were dealt with earlier. As with shoot diameter, differences in leaf area during the maturation phase of the 2nd summer flush between three- and six-year old plants also failed to explain the differences in percentage flowering.

Thirdly, it is acknowledged that the climatic conditions perceived to be favourable for flower induction after BA application improves with later applications in autumn (Paper I: figure 4, pg. 62; Paper II: figure 3A, pg. 95; figure 4A, pg. 96). Phenological development of the 2nd summer flush on three-year old plants was three weeks behind that of six-year old plants with the result that BA was applied three weeks later, under environmental conditions that were supposedly better for flower initiation. However, flower initiation was found to be much poorer on the three-year old plants compared to six-year old plants.

Inherent vegetative plant vigour or management practices promoting vegetative vigour such as excess nitrogen fertilization, severe pruning or the selection of a vigorous rootstock have been known to discourage flower bud formation or to delay flowering in woody angiosperms (Scholefield *et al.*, 1986; Bubán and Faust, 1982; Green, 1996). Practices to reduce vegetative growth, such as bending of shoots, girdling or root pruning, often have the opposite effect, in that floral initiation is stimulated (Bangerth, 1997). As plant vigour decreases with age, a flowering gradient where the sensitivity of plants to floral signals increases, has been shown in a number of plants both *in vitro* (Dickens and Van Staden, 1988) and under natural conditions (Bernier, 1988). Since the shoot growth of three-year old plants was more vigorous than that of six-year old plants, the difference in floral initiation between the respective groups may be ascribed to shoot vigour. Such a gradient is apparent in 'Carnival' where fifteen-year old plants were more responsive to BA compared to very young plants, when BA was applied at the same level of shoot maturity (Table 6). Gibberellins (GA) have often been implicated as vegetative promoters and as inhibitors of flowering in a number of temperate and tropical fruit tree species (Davenport, 2000). Application of GA (mostly GA_3) has been repeatedly shown to significantly suppress flower initiation in apple, plums, sour cherries, apricots and peaches (Bubán, 2003). Conversely, inhibitors of GA synthesis such as the triazole growth retardants, paclobutrazol and

uniconazole reduced vegetative growth and increased flowering in apples, pears, peaches, sour cherry (Meilan, 1997) and mango (Núñez-Elisea *et al.*, 1993).

In lychee and macadamia, resumption of bud development (soon where-after inflorescence differentiation is believed to occur) was promoted by removal of the immature leaves of the most recent flush. This effect was ascribed to the weakening of an inhibitory signal from the maturing leaves of that flush (Olesen, 2005). Although the nature of the signal remains unclear, high concentrations of indole-acetic acid and gibberellins have been implicated rather than the sink strength of the immature leaves. Similarly in mango, the presence of mature leaves was considered essential during floral induction and floral transition of developing shoots by facilitating the expression of a putative flowering stimulus (Nunez-Elisea and Davenport, 1992).

In Table 6 data is presented for *Protea* cv. Carnival showing Promalin®, a growth regulator containing both BA and GA in an equal ratio, to be less effective in floral induction than when BA was applied alone. Data presented for *Protea* cv. Pink Ice concur with these findings (Paper I: table 5, pg. 58). This lends credence to the idea that higher GA levels in vigorous shoots on three-year old plants will result in poorer flowering of shoots as compared to less vigorous shoots on older six-year old plants.

In conclusion, the efficacy of BA, applied in autumn to terminal buds of 'Carnival' shoots, to attain inflorescence initiation, was less effective on shoots of over young, vigorous plants. When subject to BA applications, an increased maturity of the flush subtending the terminal bud, resulted in a higher propensity for a shoot to flower, especially for shoots on vigorous plants.

Literature Cited

Bangerth, F. 1997. Can regulatory mechanism in fruit growth and development be elucidated through the study of endogenous hormone concentrations? Acta Hort. 463:77-87.

Bernier, G. 1988. The control of floral evocation and morphogenesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39:175-219.

Bubán, T. 2003. Hormonal aspects of flower formation and fruit set. In: P. Kozma, J. Nyéki, M. Soltész, and Z. Szabó. Floral Biology, pollination and fertilisation in temperate zone fruit species and grape. Akadémiai Kiadó, Budapest, Hungary. pp.3-24

Bubán, T. and M. Faust. 1982. Flower bud induction in apple trees: Internal control and differentiation. Hort. Rev. 4:174-203.

Coetzee, J.H. and G.M. Littlejohn. 2001. *Protea*: A Floricultural Crop from the Cape Floristic Kingdom. Hort. Rev. 26:1-48.

Davenport, T.L. 2000. Processes influencing floral initiation and bloom: The role of phytohormones in a conceptual flowering model. HortTechnol. 10:733-739.

Davenport, T.L. 2003. Management of flowering in three tropical and subtropical fruit tree species. Hortscience 38:1331-1335.

Dickens, C.W.S. and J. Van Staden. 1988. The induction and evocation of flowering *in vitro*. S. Afr. J. Bot. 54:325-344.

Gerber, A.I. 2000. Inflorescence initiation and development, and the manipulation thereof, in selected cultivars of the genus *Protea*. Ph.D. diss. Univ. of Stellenbosch, Stellenbosch, South Africa.

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

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001a. Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars. J. Amer. Soc. Hort. Sci 126:182-187.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001b. The role of leaves and carbohydrates in flowering of *Protea* cv. 'Lady Di'. HortScience 36:905-908.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2002. Defoliation alters spring growth flush characteristics and inhibits flowering in *Protea* cv. Carnival. Sci. Hort. 94:345-350.

Green, D.W. 1996. Flower development. In: M. Marl, P. Andrew, G. Lang, and K. Mullinex (ed.). Tree fruit physiology: Growth and development. Good fruit Grower, Yahima, Washington. pp. 91-98.

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

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. 5:25-28.

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

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.

Meilan, R. 1997. Floral induction in woody angiosperms. New Forests 14:179-202.

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

Núñez-Elisea, R., T.L. Davenport, and M.L. Caldeira. 1993. Bud initiation and morphogenesis in 'Tommy Atkins' mango as affected by temperature and triazole growth retardants. Acta Hort. 341:192-198.

Núñez-Elisea, R. and T.L. Davenport. 1995. Effect of leaf age, duration of cool temperature, and photoperiod on bud dormancy release and floral initiation in mango. Sci. Hort. 62:63-73.

Olesen, T. 2005. The timing of flush development affects the flowering of avocado (*Persea americana*) and macadamia (*Macadamia integrifolia* x *tetraphylla*). Aust. J. Agric. Res. 56:723-729.

Scholefield, P.B., D.R. Oag, and M. Sedgley. 1986. The relationship between vegetative and reproductive development in the Mango in Northern Australia. Aust. J. Agric. Res. 37:425-433.

Smart, M. 2005. Physiology of floral induction in *Protea* spp. M.Sc. Thesis. Univ. of Stellenbosch, Stellenbosch, South Africa.

Statistical Analysis Systems Institute Inc. 2000. SAS®/STAT User's Guide, Version 9.1, SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

Statsoft Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com

Table 1. Percentage flowering outcome resulting from MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) application on three-flush shoots of *Protea* cv. Carnival subjected to a range of defoliation (DF) treatments. Means (n=50) within columns followed by the same letter are not significantly different according to LSD values (5%) for logit transformed data of flowering incidences. Differences in the shoot characteristics of retained leaf number, leaf area (cm^2) and leaf dry mass (g) resulting from the respective defoliation treatments are indicated as means (n=10) followed by ± standard error (SE) of the mean.

Defoliation (DF) Treatment	% Flowering	Leaf number	Leaf area	Leaf dry mass
Control (no defoliation)	100^{a}	74 ± 1.5	1270 ± 28.0	33.0 ± 1.29
Half DF of 2nd summer flush adjacent the terminal bud	85^{a}	60 ± 1.3	1022 ± 24.7	25.9 ± 1.01
Half DF of 2nd summer flush adjacent the intercalation with 2nd summer flush	92^{a}	60 ± 1.3	1022 ± 24.7	25.9 ± 1.01
Full DF of 2nd summer flush only	56^{b}	47 ± 1.1	774 ± 22.1	18.8 ± 0.75
Full DF of 1st summer flush only	94^{a}	49 ± 0.9	800 ± 16.1	20.7 ± 0.74
Full DF of combined 2nd summer flush and 1st summer flush	17^{c}	22 ± 0.5	304 ± 12.9	6.5 ± 0.24
Full DF of basal 1st spring flush only	92^{a}	51 ± 1.1	967 ± 23.9	26.5 ± 1.12
Total defoliation (all flushes)	7^{c}	0 ± 0.0	0 ± 0.0	0 ± 0.0

Table 2. Accumulative shoot characteristics of all flushes of *Protea* cv. Carnival of three-flush shoots on three- and six-year-old plants respectively when immature in March 2004. Means (n=10) are followed by ± standard error (SE) of the mean.

Shoot characteristic	3-flush shoot, 3-year old plant	3-flush shoot, 6-year old plant
Shoot length (mm)	659 ± 11.57	621 ± 29.76
Number of leaves	78 ± 2	85± 2
Leaf area (cm^2)	856 ± 50.79	899 ± 44.27
Leaf dry mass (g)	15.31 ± 0.88	18.2 ± 1.12
Stem dry mass (g)	7.10 ± 0.37	8.89 ± 0.49
Total dry mass (g)	22.41 ± 1.21	27.10 ± 1.59

Table 3. Shoot characteristics of the 2nd summer flush of three-flush shoots (n=10) at time of MaxCel™ (active ingredients: BA 1.9% $^{w}/_{w}$) application (500 mg·L^{-1}) to terminal buds of three- and six-year old plants. First treatment date (nT_1) corresponded to a point where all 2nd summer flush leaves were unfolded. Shoot characteristics were followed over an eight week period, through to maturation of the terminal flush.

BA application on 2nd summer flush of three-flush shoots		% Flowering		Total dry mass (g)		Flush length (mm)		Leaf area (cm^2)		Intercalation stem diameter (mm)		Apex stem diameter (mm)		Stem dry mass (g)		Dry mass per leaf (g)	
3yr-old	6yr-old	3-yr	6-yr	3-yr	6-yr	3-yr	6-yr	3-yr	6-yr	3-yr	6-yr	3-yr	6-yr	3-yr	6-yr	3-yr	6-yr
24 Mar	3 Mar	4	70	4.87	5.99	176	171	220	257	5.97	6.55	4.55	4.31	1.39	1.64	0.120	0.126
7 Apr	17 Mar	0	84	5.71	11.38	209	227	273	404	5.78	6.89	5.03	5.74	1.65	2.94	0.150	0.264
21 Apr	31 Mar	20	83	8.39	12.98	236	232	333	436	7.10	6.89	6.55	6.54	2.23	3.19	0.235	0.316
5 May	14 Apr	43	86	9.10	13.70	241	235	310	450	6.54	7.22	6.77	7.38	2.36	3.13	0.261	0.360
19 May	28 Apr	78	91	10.65	15.76	244	245	329	415	7.00	7.93	7.21	8.00	2.86	3.89	0.294	0.388
CONTRASTS		Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F	Pr>F
Time Linear		<.0001	0.0047	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Time Quadratic		0.0078	0.8656	0.6950	0.0029	0.0004	<.0001	0.007	<.0001	0.3008	0.0742	0.0530	0.1103	0.9531	0.0329	0.0356	<.0001

Table 4. Correlative relationships of shoot characteristics of the terminal flush of three-flush shoots on three-year old plants of *Protea* cv. Carnival. MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) at 500 mg·L^{-1} was applied to the maturing shoot over five application dates spaced at two-weekly intervals in the autumn 2004. Correlations are expressed as the Pearson coefficient accompanied by the *p*-value.

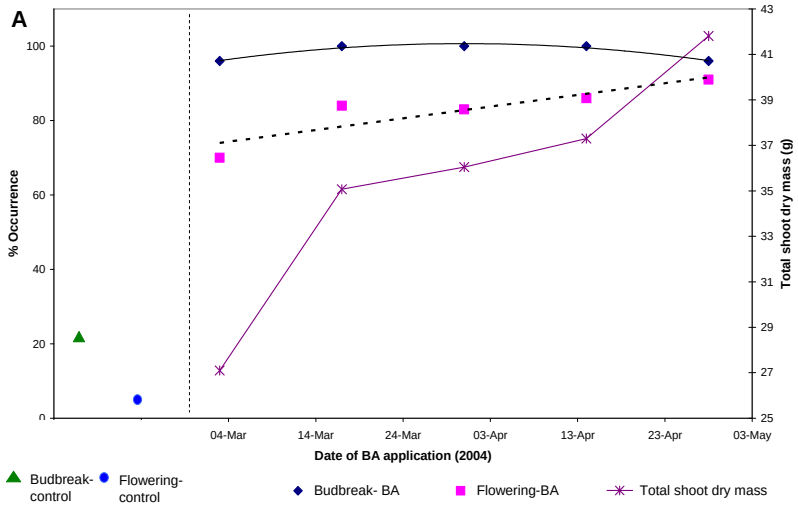
Shoot characteristics	% Flowering	Leaf number	Leaf area	Leaf dry mass	Stem length	Stem dry mass	Intercalation stem diameter	Apex stem diameter	Total dry mass
% Flowering	*	-0.50046	0.57571	0.92094	0.73974	0.93625	0.70407	0.86565	0.92571
	*	0.3906	0.3098	0.0264	0.1530	0.0191	0.1844	0.0579	0.0240
Leaf number	-0.50046	*	-0.90576	-0.79221	-0.91274	-0.74015	-0.77485	-0.86007	-0.78068
	0.3906	*	0.0342	0.1101	0.0305	0.1527	0.1238	0.0615	0.1192
Leaf area	0.57571	-0.90576	*	0.89031	0.97255	0.88552	0.85471	0.92932	0.89032
	0.3098	0.0342	*	0.0429	0.0054	0.0457	0.0650	0.0223	0.0429
Leaf dry mass	0.92094	-0.79221	0.89031	*	0.93060	0.99331	0.85936	0.99224	0.99961
	0.0264	0.1101	0.0429	*	0.0217	0.0007	0.0620	0.0008	<.0001
Stem length	0.73974	-0.91274	0.97255	0.93060	*	0.91910	0.78660	0.95812	0.92907
	0.1530	0.0305	0.0054	0.0217	*	0.0273	0.1145	0.0102	0.0224
Stem dry mass	0.93625	-0.74015	0.88552	0.99331	0.91910	*	0.84158	0.97748	0.99615
	0.0191	0.1527	0.0457	0.0007	0.0273	*	0.0739	0.0040	0.0003
Intercalation stem diameter	0.70407	-0.77485	0.85471	0.85936	0.78660	0.84158	*	0.88294	0.85602
	0.1844	0.1238	0.0650	0.0620	0.1145	0.0739	*	0.0472	0.0641
Apex stem diameter	0.86565	-0.86007	0.92932	0.85936	0.95812	0.97748	0.88294	*	0.98991
	0.0579	0.0615	0.0223	0.0620	0.0102	0.0040	0.0472	*	0.0012
Total dry mass	0.92571	-0.78068	0.89032	0.99961	0.92907	0.99615	0.85602	0.98991	*
	0.0240	0.1192	0.0429	<.0001	0.0224	0.0003	0.0641	0.0012	*

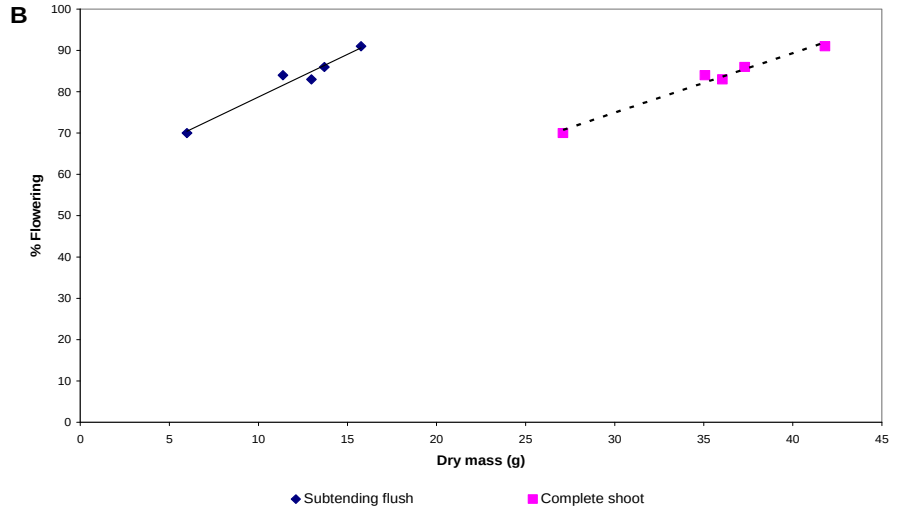
Table 5. Correlative relationships of shoot characteristics of the terminal flush of three-flush shoots on six-year-old plants of *Protea* cv. Carnival with MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) applications at 500 mg·L^{-1} to the maturing shoot over five application dates spaced with two-weekly intervals in the autumn 2004. Correlations are expressed as the Pearson coefficient accompanied by the *p*-value.

Shoot characteristics	% Flowering	Leaf number	Leaf area	Leaf dry mass	Stem length	Stem dry mass	Intercalation stem diameter	Apex stem diameter	Total dry mass
% Flowering	*	-0.09076	0.87187	0.87687	0.97721	0.98176	0.85511	0.93857	0.97943
	*	0.8846	0.0509	0.0509	0.0041	0.0029	0.0647	0.0181	0.0035
Leaf number	-0.09076	*	-0.20910	-0.32900	-0.09667	-0.08192	-0.22310	-0.40147	-0.84742
	0.8846	*	0.7357	0.5888	0.8771	0.8958	0.7183	0.5029	0.0699
Leaf area	0.87687	-0.20910	*	0.91569	0.95229	0.85978	0.54428	0.83246	0.89895
	0.0509	0.7357	*	0.0290	0.0124	0.0617	0.3430	0.0802	0.0380
Leaf dry mass	0.87687	-0.32900	0.91569	*	0.96518	0.95818	0.82650	0.98418	0.99090
	0.0509	0.5888	0.0290	*	0.0078	0.0102	0.0845	0.0024	0.0010
Stem length	0.97721	-0.09667	0.95229	0.96518	*	0.97038	0.74427	0.91249	0.97654
	0.0041	0.8771	0.0124	0.0078	*	0.0061	0.1491	0.0307	0.0043
Stem dry mass	0.98176	-0.08192	0.85978	0.95818	0.97038	*	0.85944	0.94125	0.98771
	0.0029	0.8958	0.0617	0.0102	0.0061	*	0.0619	0.0169	0.0016
Intercalation stem diameter	0.85511	-0.22310	0.54428	0.82650	0.74427	0.85944	*	0.83246	0.84893
	0.0647	0.7183	0.3430	0.0845	0.1491	0.0619	*	0.0802	0.0689
Apex stem diameter	0.93857	-0.40147	0.83246	0.98418	0.91249	0.94125	0.83246	*	0.97499
	0.0181	0.5029	0.0802	0.0024	0.0307	0.0169	0.0802	*	0.0047
Total dry mass	0.97943	-0.84742	0.89895	0.99090	0.97654	0.98771	0.84893	0.97499	*
	0.0035	0.0699	0.0380	0.0010	0.0043	0.0016	0.0689	0.0047	*

Table 6. Flowering proportion in *Protea* cv. Carnival as induced in three-year and fifteen-year old plants respectively when treated with ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$ active ingredient) at 250- and 500 mg·L^{-1} and Promalin® (active ingredients: BA 1.8% $^{w}/_{w}$; gibberellins A_4A_7 1.8% $^{w}/_{w}$) at 500 mg.L^{-1}. Growth regulators were applied only once to the terminal bud on 12 April 2002. Data is presented as real observations out of 13 treated or control shoots respectively.

Growth regulator	Concentration (mg·L^{-1})	Three-year old plants	Fifteen-year old plants
Control	0	0/13	0/13
BA	250	3/13	10/13
BA	500	4/13	10/13
Promalin®	500	0/13	6/13





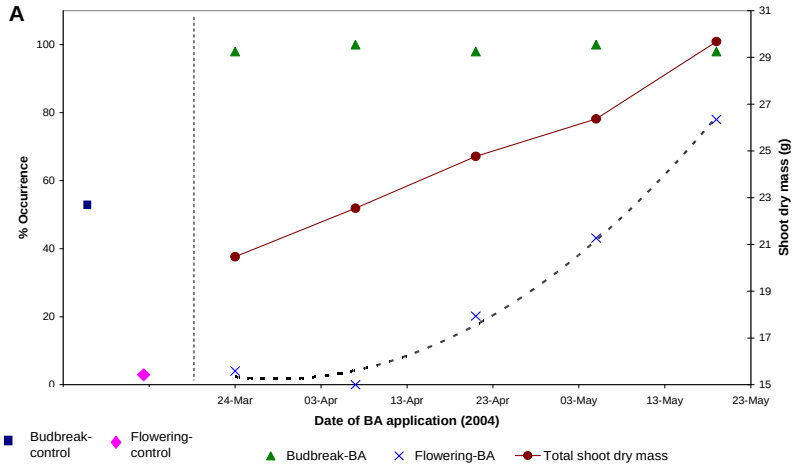
ANOVA

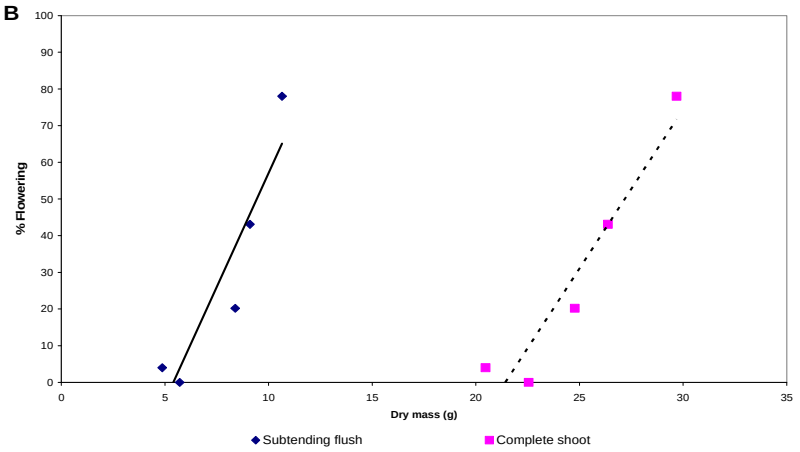
CONTRAST	*F value*	*Pr>F*	*Significance*
Ctrl vs. Trt (budbreak)	238.63	<.0001	***
Time Lin (budbreak)	0.00	1.0000	ns
Time Qua (budbreak)	4.09	0.0566	ns
Ctrl vs. Trt (flowering)	136.62	<.0001	***
Time Lin (flowering)	10.11	0.0047	**
Time Qua (flowering)	0.03	0.8656	ns

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Signficance*
Dry mass (subtending flush) vs % Flowering	0.98903	0.0014	**
Dry mass (complete shoot) vs % Flowering	0.97943	0.0035	**

Figure 1. A. Percentage budbreak and flowering incidence in *Protea* cv. Carnival induced by a single MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) application at 500 mg·L^{-1} on six-year-old plants immediately after the cessation of the 2nd summer flush, followed by consecutive fortnightly treatments over an eight week period until terminal flushes were fully mature. B. The relationship between % flowering incidence under BA induction and the dry mass as present in the complete shoot or in the subtending flush to the terminal bud respectively, immediately prior to BA application.



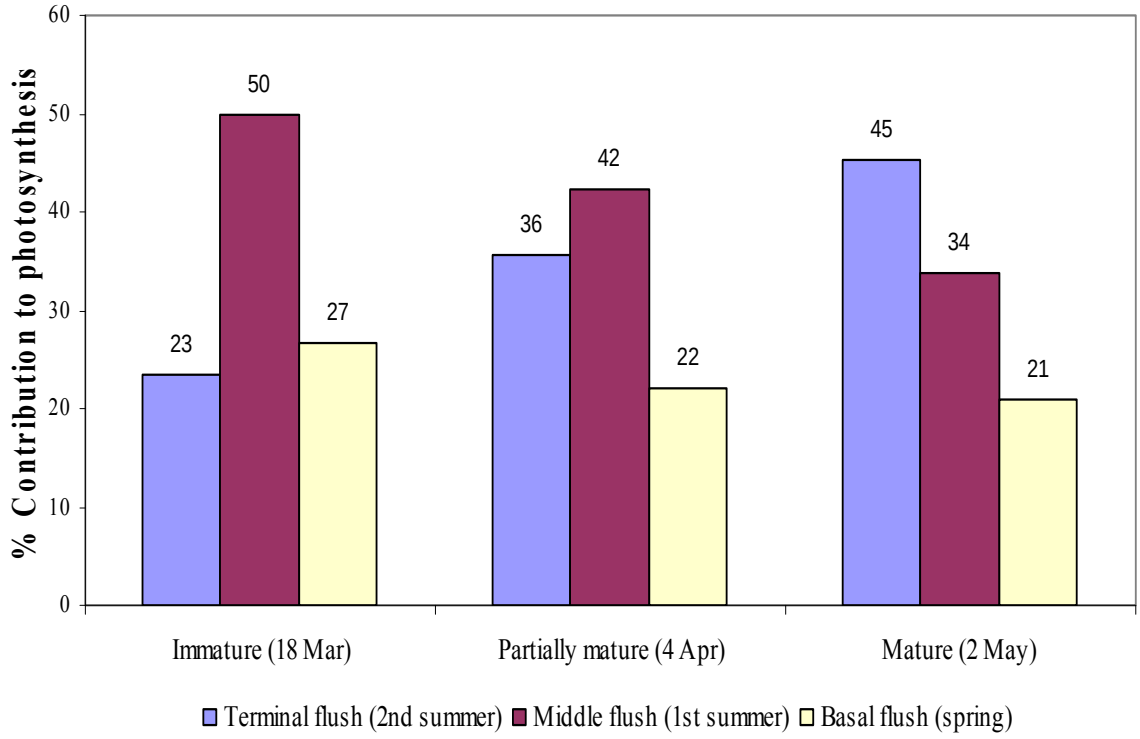


CONTRAST	*F value*	*Pr>F*	*Significance*
Ctrl vs. Trt (budbreak)	173.98	<.0001	***
Time Lin (budbreak)	0.00	1.00	ns
Time Qua (budbreak)	0.40	0.5358	ns
Ctrl vs. Trt (flowering)	32.19	<.0001	***
Time Lin (flowering)	132.92	<.0001	***
Time Qua (flowering)	8.73	0.0078	***

CORR PROCEDURE

Source	*[r]*	*Prob>[p]*	*Signficance*
% Flowering vs Dry mass (subtending flush)	0.92666	0.0236	**
% Flowering vs Dry mass (complete shoot)	0.95497	0.0114	**

Figure 2. A. Percentage budbreak and flowering incidence in *Protea* cv. Carnival as induced by a single MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) application at 500 mg·L^{-1} on three-year-old plants immediately after the cessation of the 2nd summer flush, followed by consecutive fortnightly treatments over an eight week period until terminal flushes were fully mature. B. The relationship between % flowering incidence under BA induction and dry mass accumulation as present in the complete shoot or in the subtending flush to the terminal bud respectively, immediately prior to BA application.



Date of assessment	Flush position to terminal bud	Leaf photosynthesis (µmol $CO_2.m^{-1}.sec^{-1}$)	Number of leaves	Leaf area (cm^2)	Flush photosynthesis (µmol $CO_2.sec^{-1}$)
18-Mar-04	Basal flush	9.76 ± 0.19	22 ± 0.9	273.5 ± 18.9	26.69
	Middle Flush	13.52 ± 0.71	23 ± 0.7	369.3 ± 21.6	49.93
	Terminal flush	9.10 ± 0.51	26 ± 0.8	256.7 ± 14.3	23.35
06-Apr-04	Basal flush	6.16 ± 1.05	22 ± 0.5	291.3 ± 17.4	17.94
	Middle Flush	8.59 ± 0.82	23 ± 0.7	401.0 ± 18.5	34.44
	Terminal flush	6.65 ± 0.75	26 ± 0.8	436.2 ± 19.8	29.02
02-May-04	Basal flush	9.10 ± 0.9	23 ± 0.8	300.5 ± 13.1	27.33
	Middle Flush	11.54 ± 0.6	22 ± 0.8	381.7 ± 20.3	44.05
	Terminal flush	14.24 ± 0.3	25 ± 1.0	415.0 ± 22.8	59.10

Figure 3. Photosynthetic rate and proportional contribution to over all shoot photosynthesis as affected by flush position on a three-flush shoot of *Protea* cv. Carnival at different stages of maturity of the terminal flush in the autumn 2004 Values are means of readings on five shoots ± SE. Values for leaf number and leaf area per flush are means of 10 shoots ± SE

7. PAPER VI. Developing inflorescences of *Protea* cv. Carnival reduce the responsiveness of non-flowering shoots to benzyladenine induction of flowers 'out of season'.

Developing inflorescences of *Protea* cv. Carnival reduce the responsiveness of non-flowering shoots to benzyladenine induction of flowers 'out of season'.

Abstract

The responsiveness of non-flowering shoots on eight and 12-year old plants of *Protea* cv. Carnival in terms of budbreak and flowering incidence following a 500 mg·L^{-1} benzyladenine (BA) application (ABG-3062; active ingredient: BA 2% $^{w}/_{w}$), eight months after pruning in April 2003, was compared to that of shoots subject to BA treatment as MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) at either 500, 1000 or 2000 mg·L^{-1} on the same plants, 20 months after pruning. Plants treated in April 2004 differed from the April 2003 treatment in that developing inflorescences were present on the plants in 2004. BA application enhanced budbreak in all shoots, irrespective of plant age, BA concentration or treatment year. For both age groups of plants more shoots initiated an inflorescence when treated with BA eight months after pruning as compared to shoots treated 20 months after pruning. The quality of shoots treated 20 months after pruning were comparable or better than shoots treated eight months after pruning. Apparently the presence of developing inflorescences on the same plant when non-flowering shoots in autumn are treated with BA reduces the probability of flower initiation taking place.

Protea cv. Carnival is commercially managed in a biennial pruning system of approximately 18-20 months, implying that a flowering crop is harvested only every second year. The first year is dedicated to the vegetative development of three- or four flush shoots (the 'off' year), with the production of a second spring flush and an inflorescence in the following year (the 'on' year) (Gerber *et al.*, 1995; Hettasch *et al.*, 1997).

The vegetative cycle starts when both flowering and non-flowering shoots are pruned in late-winter (July-August) to an approximate 15 cm portion of the basal stem which serves as

bearer for the subsequent growth (Gerber *et al.*, 1995). Spindly shoots are removed at their base by thinning cuts. New growth commences in the spring of the pruning year (late August/early September) from axillary buds on the bearer. As flowers rarely initiate on spring flushes that originate from rudimentary axillary buds (Greenfield *et al.*, 1994), shoot growth continues terminally with the addition of two summer flushes, the first flush beginning in early summer (December) of the pruning year, with the second flush produced in the late summer (February) of the following year (Gerber *et al.*, 1995). Depending on vigour, some shoots may produce an additional autumn flush in April/May. Terminal buds on shoots remain dormant throughout winter. The reproductive cycle commences with budbreak of the preformed terminal buds in spring (August/September), as soon thereafter, inflorescence initiation is known to occur (Gerber *et al.* 2001). During elongation of the spring flush, the apical meristem produces floral primordia that differentiate into involucral bracts. Floral bracts with florets in their axils are produced only after the completion of the spring flush (Gerber *et al.*, 2001). Spring-initiated inflorescences are harvested in late summer to autumn (February-May), 6 to 9 months after spring budbreak. After the harvest is completed, the non-flowering shoots and the stumps left after harvesting are pruned back again in August to repeat the production cycle.

Exogenous applications of BA to three-flush shoots of 'Carnival' scheduled in the biennial pruning regime described above proved to effect the induction of budbreak and initiation of inflorescences 'out of season' in late summer and autumn (March-May) (Paper I, Table 1, pg.54; Paper II, figures 3-4; pg. 95-96).

In this paper we report on the lack of responsiveness to BA application of non-flowering shoots to induce inflorescences, 20 months after pruning in a biennial production system.

Materials and Methods

Plant material. Experiments on *Protea* cv. Carnival were carried out in commercial plantations grown from cuttings in the Stellenbosch district (33°55'S; 18°50'E), South Africa. The climate is Mediterranean-like with cool, wet winters and hot, dry summers. The annual rainfall is 600-700 mm. Plants were spaced 1 m in the row and 4 m between rows. Plants were not irrigated or fertilized. Pest control focused on the control of thin-line leaf miner (*Phyllocnistis* sp.) and the speckled protea borer (*Orophia* spp.) and were applied by risk analysis which included the emergence and extension of the immature flush and during early inflorescence development.

Benzyladenine(BA) applications to eight-year old plants. All plants were pruned back to bearers in August 2002 to effect biennial cropping as described by Gerber *et al.* (1995).

On 25 April 2003 (mid-autumn), three-flush shoots were selected. Shoots were treated on the terminal bud of the mature 2nd summer flushes with BA at 500 mg·L^{-1} as ABG-3062 (active ingredient: BA 2% $^{w}/_{w}$; Abbott Laboratories, North Chicago, USA). Distilled water was used as control treatment. Ten shoots were used per treatment, replicated five times in a randomised complete block design. Stem diameters were measured at the intercalation of terminal and sub-terminal flushes as a non-destructive parameter indicative of shoot quality (Paper V). Immediately prior to BA application, ten representative shoots were harvested, separated into the respective flushes, with the following characteristics determined: length (mm), number of true leaves, leaf area (cm^2) (using a portable Li-COR leaf area meter (model LI-3000 LI-COR Biosciences, Nebraska, USA) together with both leaf and stem dry mass (60°C, 72 h).

On 20 April 2004, a further 200 shoots were selected from 50 plants which had not initiated inflorescences on the 2003 spring flush. These non-flowering shoots remained on plants subsequent to the harvesting of approximately 25-30 flowering stems induced either

by the BA treatment of April 2003 or those formed naturally on the spring flushes initiated in September 2003 within the biennial cycle. A single BA treatment at a concentration of 500, 1000 or 2000 $mg \cdot L^{-1}$ respectively was applied with a paintbrush to the terminal bud alone. Distilled water was used as control treatment. Ten shoots were used per treatment replicated five times in a randomised complete block design. Intercalation stem diameters between the spring and subtending flushes of BA-treated shoots were determined. Budbreak and inflorescence initiation subsequent to the BA application was recorded for each shoot. At the time of application ten representative shoots were harvested and shoot characteristics as previously described were determined.

BA applications to 12-year old plants. The experimental layout as described for the eight-year old plants above was repeated on 12-year old plants of 'Carnival'. BA applications were made to the 2nd summer flush of non-flowering, vegetative shoots on 25 April 2003 or to shoots on plants still in flower on 20 April 2004.

Statistical analysis. Data were analyzed by using the General Linear Method, SAS (SAS Institute, 2000). Mean separation was accomplished by using least significant difference (LSD) at the 5% significance level. Logit transformation of data was performed on all values expressed as percentages. Descriptive statistics were obtained using Statitica version 7.1 (StatSoft Inc., 2005).

Results

Comparative shoot characteristics of the flush subtending the treated bud (Table 1) as well as that of the combined three flushes (Table 2) disclose that shoots treated in April 2004 were comparable to shoots treated in April 2003 with respect to shoot characteristics perceived to promote inflorescence initiation under BA induction (Paper IV: tables 4-5, pg. 156-157). Terminal flush length and leaf area were greater in shoots treated in April 2004

(20 months after pruning) compared to shoots treated in April 2003 (eight months after pruning).

BA application significantly enhanced budbreak in all shoots irrespective of plant age, BA concentration or treatment year (Table 3). Budbreak in April 2003 of control shoots was as high as 24%, but with the exception of a few isolated shoots floral initiation did not occur on the naturally-initiated autumn flush.

Compared to control shoots the percentage shoots initiating an inflorescence was significantly higher for shoots treated with BA in April 2003 for both the eight-year old and 12-year old plants. However, treating shoots with BA in April 2004 on the same plants that were treated in 2003 failed to increase percentage flowering over the control for the 12-year old plants. In the case of the eight-year old plants, BA treatment of shoots in April 2004 on the same plants treated in 2003, caused significantly more shoots to flower than untreated control shoots. Flowering percentages increased from 30% for shoots treated with 500 $mg \cdot L^{-1}$ BA to 48% for the higher concentrations, but is much lower than the 94% flowering achieved on the same plants when treated in 2003 (Table 3).

Discussion

No flowers were present on plants of both the eight –and twelve-year-old plants when three-flush shoots were treated with BA in April 2003, as plants were pruned back to bearers in late winter of 2002. In contrast, in April 2004, when non-flowering three-flush shoots were treated, flowers (initiated on the spring flush of 2003) were still present on the experimental plants. The insensitivity to the BA treatment in April 2004 in terms of inflorescence initiation compared to April 2003 could not be due to differences in percentages of budbreak as budbreak incidences were comparable (Table 3). Also, it is unlikely that differences between treatment years (2003 vs. 2004) could the cause since a classification tree analysis showed that the year of treatment does not greatly affect the

efficacy of BA to induce flowers in 'Carnival' (Paper V: figure 5, pg. 180). When considering shoot characteristics as a possible cause for the poor flowering following BA treatment in April 2004, it is clear that the characteristics of the shoots such as leaf number, stem diameter and leaf or total dry mass when treated in 2004 were comparable to that of shoots treated in 2003. In fact, shoots in 2004 were of a better quality with regards to a number of characteristics that have been shown to correlate with increased responsiveness to BA to induce flowers, such as flush length and leaf area (Paper IV: tables 4-5, pg. 156-157 Paper V: table 2, pg. 175). Apparently the presence of developing inflorescences reduces the likelihood of non-flowering shoots on the same plant initiating an inflorescence in response to BA treatment and this effect could not effectively be overcome by increasing the concentration of the BA solution used to treat shoots in autumn (Table 3).

A similar effect was reported by Nieuwoudt (2006) for the cultivar 'Pink Ice'. After pruning plants to bearers in May, shoots that attained a length of 100 cm or more by the following May, were reported to readily initiate flowers unaided on an autumn flush. However, when there were developing flowers on the plant, shoots of comparable length rarely flowered on an autumn flush.

The phenomenon of developing reproductive structures inhibiting flower initiation has been reported. Young developing fruit inhibit flower initiation in apple. Apparently flower initiation is inhibited by gibberellins that are produced by the developing apple seeds (Tromp, 1992; Bangerth, 1997).

Developing flowers inhibiting further flower initiation has also been reported in *Chamaelaucium uncinatum* (Shillo, 1985) and *Clerodendrum speciosum* (Shillo and Engel, 1985). Continuous floral initiation was achieved in *Chamaelaucium uncinatum* grown under inductive short days by either disbudding flower buds or removal of flowering branches at a very early stage of flower development (Shillo, 1985).

Successful management of mango flowering in the tropics includes tip pruning of shoots to ensure a uniform flush of growth throughout the canopy. More importantly, it is believed that growth- and flower inhibiting factors in stems derived from the previous season's flowering and fruiting panicles is removed by pruning (Núñez–Elisea and Davenport, 1995; Davenport, 2003).

The success of 'out of season' flowering by the application of BA is highly reliant on the management of the vegetative shoot prior to treatment. Synchronisation of shoot growth by pruning 'Carnival' plants in late winter appear to be a necessary first step to ensure high percentages inflorescence initiation in autumn. Permitting shoot growth to take place in the absence of developing flowers on the same plants appears to be a second requirement. Lastly, shoots should have acquired the desired dimensions (minimum stem diameter and leaf area) and characteristics such as a degree of maturation to achieve inflorescence initiation on a high percentage of shoots treated with BA.

Literature Cited

Bangerth, F.K. 1997. Can regulatory mechanism in fruit growth and development be elucidated through the study of endogenous hormone concentrations? Acta Hort. 463:77-87.

Davenport, T.L. 2003. Management of flowering in three tropical and subtropical fruit tree species. HortScience 38:1331-1335.

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

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001. Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars. J. Amer. Soc. Hort. Sci. 126:182-187.

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

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

Nieuwoudt, G. 2006. The effect of pruning on the growth and development of *Protea* cv. Pink Ice (*P. compacta* R. Brown × *P. susannae* Phill). M.Sc. thesis, Univ. of Stellenbosch, Stellenbosch, S. Africa.

Núñez-Elisea, R. and T.L. Davenport. 1995. Effect of leaf age, duration of cool temperature treatment, and photoperiod on bud dormancy release and floral initiation in mango. Scientia Hort. 62:63-73.

Shillo, R. 1985. *Chamaelaucium uncinatum*. In: A.H. Halevy (ed.). CRC Handbook of Flowering. Vol II. CRC Press, Boca Raton, FL. pp. 185-189.

Shillo, R. and R. Engel. 1985. *Clerodendrum speciosum*. In: A.H. Halevy, CRC Handbook of Flowering. Vol II. CRC Press, Boca Raton, FL. pp. 302-307.

Statistical Analysis Systems (SAS) Institute Inc., 2000. SAS®/STAT User's Guide, Version 9.1, SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

Statsoft Inc. 2005. STATISTICA (data analysis software system), version 7.1. Table 1. Percentage budbreak and flowering on application of benzyladenine (BA) in April to non-flowering shoots on eight- or twelve-year plants of *Protea* cv. Carnival, 8 months or 20 months after pruning in August of 2003. www.statsoft.com

Tromp, J. 1992. Flower bud formation in apple as affected by various gibberellins. J. Hort. Sci. 57:277-282.

Table 1. Characteristics of the terminal flush in April 2003 on three-flush shoots on eight- and 12-year old plants of *Protea* cv. Carnival, 8 months after pruning or in April 2004, 20 months after pruning in August 2002. Values represent the means of ten shoots ± SE.

Shoot Type	Intercalation stem diameter (mm)	Flush length (mm)	Leaf number	Leaf area (cm^2)	Leaf dry mass (g)
8-year old plants					
April 2003	6.73 ± 0.16	235 ± 6.2	27 ± 0.8	218.2 ± 10.9	9.48 ± 0.37
April 2004	6.42 ± 0.22	329 ± 9.4	29 ± 0.7	389.0 ± 42.9	9.51 ± 0.32
12-year old plants					
April 2003	6.17 ± 0.25	157 ± 9.7	24 ± 1.0	178.5 ± 17.6	6.10 ± 0.38
April 2004	6.16 ± 0.15	307 ± 7.8	30 ± 0.5	379.5 ± 31.0	9.71 ± 0.40

Table 2. Characteristics of the combined three flushes shoots on eight- and 12-year old plants of *Protea* cv. Carnival with a terminal 2nd summer flush (April 2003) or non-flowering spring flush (April 2004) respectively. Values represent the means of ten shoots ± SE.

Shoot type	Flush length (mm)	Leaf number	Leaf area (cm^2)	Leaf dry mass (g)	Shoot dry mass (g)
8-year old plants					
April 2003	657 ± 16.7	72 ± 1.9	544.0 ± 26.7	23.60 ± 1.13	33.11 ± 1.61
April 2004	769 ± 9.57	69 ± 0.8	899.1 ± 47.3	21.54 ± 0.52	30.98 ± 0.71
12-year old plants					
April 2003	577 ± 16.4	69 ± 1.8	570.9 ± 52.5	18.59 ± 0.94	26.46 ± 1.13
April 2004	686.70 ± 23.02	68 ± 1.8	837.3 ± 49.9	21.45 ± 1.02	30.31 ± 1.45

Table 3. Percentage budbreak and flowering on application of benzyladenine (BA) in April to non-flowering shoots on eight- or 12-year plants of *Protea* cv. Carnival, 8 months or 20 months after pruning in August of 2003.

Date of application	BA ($mg \cdot L^{-1}$)	Shoot diameter (mm)	% Budbreak	% Flowering
		8-year old plants		
25 Apr 2003	0	6.98^{b}	24^{b}	2^{b}
	500	7.32^{a}	100^{a}	94^{a}
20 Apr 2004	0	7.16^{a}	0^{b}	0^{c}
	500	7.21^{a}	91^{a}	30^{b}
	1000	7.37^{a}	100^{a}	48^{a}
	2000	7.16^{a}	100^{a}	48^{a}
		12-year old plants		
25 Apr 2003	0	6.98^{b}	6^{b}	0^{b}
	500	7.32^{a}	96^{a}	87^{a}
20 Apr 2004	0	6.75^{a}	14^{b}	2^{a}
	500	7.73^{a}	98^{a}	2^{a}
	1000	6.63^{a}	100^{a}	9^{a}
	2000	6.82^{a}	98^{a}	0a

Means within column per treatment date followed by superscripts with the same letter are not significantly different at LSD $_{(p=0.05)}$ level.

6. PAPER V. Shoot thickness- a useful predictor of the responsiveness of shoots of *Protea* cv. Carnival to autumn-applied benzyladenine induction of flowering.

Shoot thickness- a useful predictor of the responsiveness of shoots of *Protea* cv. Carnival to autumn-applied benzyladenine induction of flowering.

Abstract

Terminal buds on three-flush shoots of *Protea* cv. Carnival with a mean stem diameter of 6.9 ± 0.07 mm as well as two-flush shoots of three subjective thickness classes of thin, medium and thick with mean stem diameters (mm) of 4.4 ± 0.05, 5.4 ± 0.05 and 6.0 ± 0.07 respectively, were painted in autumn with 500 or 1000 mg·L^{-1} MaxCel^{TM} (active ingredients: benzyladenine (BA) 1.9% $^{w}/_{w}$). Comparable shoots were harvested and a number of indicator shoot characteristics determined. Shoot and leaf dry weight as well as leaf- number and area increased from thin to thick two-flush shoots, but were significantly less than for three-flush shoots. The percentage shoots initiating an inflorescence on two-flush shoots increased from 0 to 29 to 76% for thin, medium and thick shoots respectively, but was significantly less than the 96% flowering achieved for three-flush shoots. Shoot thickness was positively correlated with leaf area, shoot- and leaf dry mass and percentage flowering following BA treatment and appears to be a functional non-destructive parameter for selecting shoots likely to flower with BA treatment in autumn. Analysis of an expanded data set revealed that shoot diameter is by far the most determining factor of the inductive potential of shoots treated in autumn with BA, compared to plant age, month of treatment in autumn, year of treatment or number of flushes that constitute a shoot.

Producer experience and field observations have shown that the thickest shoots on mature plants of *Protea* have a higher propensity to flower than thin shoots (De Swardt, 1989). The importance of the vegetative status of the shoot, particularly that of the flush

subtending to the inflorescence, for successful and continued floral induction and inflorescence initiation, as described by Gerber *et al.* (2001a, 2001b, 2002) was confirmed for *Protea* cv. Carnival in Paper II and IV.

Pruning studies on 'Carnival' showed that flowering shoots consisted of at least two flushes of which the terminal flush was a spring flush produced on an over-wintering shoot (Greenfield *et al.*, 1994). Total defoliation of 'Carnival', 40 days before spring budbreak, or earlier, had a significant effect on the characteristics of the spring flush and prevented flowering in all treatments (Gerber *et al.*, 2002). Later defoliation had a less severe impact on both stem length and number of leaves on the following spring growth flush, and all shoots initiated flowers.

It has been suggested that the sink-source capacity of the *Protea* stem may play a role in inflorescence initiation. 'Sylvia', a *Protea* cultivar with an open flowering window, appears to initiate inflorescences independent of environmental cues as an inflorescence is apparently produced on a stem when sufficient carbohydrates are available (Gerber *et al.,* 2001c). Stem length and thickness in *Protea* cv. 'Ivy' contributed significantly to the ability of a stem to flower (De Swardt, 1989). In 'Carnival', a greater percentage of shoots form flowers when plants are pruned for biennial bearing, a regime which significantly increases both vegetative stem length and thickness (Gerber *et al.*, 1995). 'Carnival' plants flower predominantly on the spring flush with flowers rarely initiated on either the 2nd summer or autumn flushes (Greenfield *et al.*, 1994). Floral initiation before winter is only possible on a very limited number of 'Carnival' shoots exhibiting a higher, but unspecified stem diameter, than the majority of non-flowering shoots (G. Jacobs, pers. comm., 2001).

A significant correlation was obtained between stem diameter and percentage inflorescence initiation in three-flush shoots treated with benzyladenine (BA) in late summer to autumn (Paper II, figure 5A, pg. 97). Furthermore, apex stem diameter was also

significantly correlated with other shoot characteristics such as stem length, leaf- and total dry mass, all of which themselves are well correlated with inflorescence initiation (Paper IV, tables 4-5, pg. 156-157).

Comparison of the efficacy of BA to induce inflorescences on two- and three-flush shoots, indicated that the thinner two-flush shoots had significantly lower percentages budbreak and inflorescence initiation than the thicker, three-flush shoots (Paper II, figure 5B, pg.97). In addition, an interaction between number of flushes and time of application emerged. Two-flush shoots failed to initiate inflorescences with earlier treatment dates although limited inflorescence initiation occurred with later treatment dates.

This study examines the possible correlation between stem diameter and inflorescence initiation under benzyladenine (BA) induction in *Protea* cv. Carnival. In particular, the possible existence of a threshold stem diameter above which flowering is likely to occur, is assessed. Furthermore, the importance of stem diameter for inflorescence initiation in conjunction with other possible contributing factors, such as flush number, plant age, month and year of BA application, is investigated. Application of elevated concentration levels of BA, to enable inflorescence initiation on shoots with stem diameters considered marginal for natural inflorescence initiation, is explored.

Materials and Methods

Plant material. Experiments on *Protea* cv. Carnival were carried out in a commercial plantation grown from clonal cuttings on the farm Protea Heights located in the Stellenbosch district (33°15'S; 18°50'E), South Africa. The climate is Mediterranean-like with cool, wet winters and dry, hot summers. The annual rainfall is 600-700mm, concentrated in winter. Plants were spaced 1 m in the row and 4 m between rows. Plants were not irrigated or fertilized. Pest control focused on the control of thin-line leaf miner (*Phyllocnistis* sp.) and the speckled protea borer (*Orophia* spp.) and were applied by risk analysis which included

the emergence and extension of the immature flush and during early inflorescence development. All plants were subject to a pruning regime in July 2003 to effect a biennial bearing regime (Gerber *et al.*, 1995).

Stem thickness and benzyladenine concentration. Two-flush shoots on 17-year old plants were selected and classified subjective into three stem diameter categories namely thin, medium and thick, with an additional 50 shoots included in the category: thick, three-flush shoots. The terminal bud of shoots were painted with BA as contained in MaxCel™ (active ingredients: BA 1.9% $^{w}/_{w}$; Valent BioSciences Corporation, Libertyville, USA) at a concentration of either 500 or 1000 mg·L^{-1} BA on 11 May 2004. Ten shoots were used per treatment and treatments were replicated five times in a randomized complete block design.

The following shoot characteristics were determined from ten representative shoots of each shoot category harvested immediately prior to BA application: number of leaves, leaf area (cm^2), stem diameter (mm) and dry mass of stem and leaves (g). Stem diameter was measured at the upper position of the intercalation of the terminal and sub-terminal flushes. Stem diameters were adequately represented in a range from 3.7 to 8.95 mm (Figure 1). Subsequent budbreak and flowering incidence was recorded for each shoot. Ten shoots per treatment were used and treatments were repeated five times.

Expanded data set. The stem intercalation diameter between the terminal and sub-terminal was determined on 1800 two- and three-flush shoots on plants ranging in age from three to 17-year old treated with BA (500 or 1000 mg·L^{-1}) either in March, April or May of both 2003 and 2004. Intercalation diameter was measured for each shoot immediately prior to BA application and the flowering response recorded.

Statistical analysis. Logit transformation of data was performed on all values expressed as percentages. Standard analysis of variance was performed, using the General Linear Method generated by the SAS® program (SAS Institute, 2000). The data was also

analyzed using classificiation trees of the CART analysis from Statistica version 7.1 (Statsoft Inc., 2005). Data was randomly split in ratio of 60:40 into a train and test where the CART model used the training set to evaluate the independent test data set. This procedure serves as an assessment to ensure that trends reported from the analysis were not due to a random trend in the data set (Appendix 1). Only results from the test set are reported and discussed.

Results and Discussion

Budbreak occurred in all treatments (>90%) with no significant difference between different treatments (Table 1).

There was no interaction (F value: 2.28; Pr>F: 0.1014) between BA concentration and shoot type treated with respect to percentage flowering. However, both main effects, BA concentration (F value: 17.34; Pr>F: 0.0003) and shoot type (F value: 212.70; Pr>F: <.0001), were significant. Fifty-seven percent shoots initiated an inflorescence when treated with BA at 500 $mg \cdot L^{-1}$. This result was significantly better (5% level F test) than the 43% achieved when BA was applied at 1000 $mg \cdot L^{-1}$ (Figure 2). The percentage shoots that flowered following BA treatment were 0%, 29%, 76% and 92% for thin, medium, thick two-flush and three-flush shoots respectively. Each value differed significantly from the others at $LSD_{(p=0.05)}$ level (Table 1).

Gerber *et al.* (2001b) found that terminal buds of 'Carnival' shoots are preformed and hence, contain the full complement of leaves of the next shoot growth flush which are differentiated during the extension growth of the flush that subtends the terminal bud. Shoot diameter was positively correlated with leaf number, leaf area and shoot dry weight (Table 2). Therefore, thick shoots are thus characterized by more leaves, a larger leaf area and greater dry weight, all of which apparently increases the responsiveness of the shoot to BA with respect to flower initiation, compared to thin shoots.

Shoot characteristics were also correlated with the propensity of shoots to initiate an inflorescence in *Banksia*, an Australian member of the Proteaceace. A strong positive correlation was documented between the number of leaves on a shoot and the shoot's length and basal diameter (Fuss *et al*., 1992). For *Banksia*, 28% of *B. coccinea* and 9% of *B. menziesii* shoots flowered in their first year (Fuss *et al*., 1992). Shoots that produced an inflorescence in the first year were longer and thicker than non-flowering shoots (Röhl *et al.*, 1994). Shoots which after one year's growth had a basal diameter <4.5 mm for *B. coccinea*, <6.0 mm for *B. menziesii* and <11 mm for *B. baxteri* were unlikely to flower (Sedgley and Fuss, 1992).

The link between the vegetative status of the shoots, stem diameter and flowering has also been accepted in other members of the Proteaceace family, apart from *Protea* and *Banksia*. In *Telopea*, competition for light in densely packed natural stands led to the production of thin shoots with only 1% flowering. In comparison, in commercial stands where higher light intensities and increased plant growth were prevalent, flowering occurred in most of the shoots (Faragher, 1989).

The ability of a shoot to flower was also correlated with shoot diameter and leaf number in *Leucospermum* (Jacobs and Minnaar, 1980). Leaf removal and heavy shading applied during summer reduced the number of stems forming an inflorescence, although long stems were less responsive to the inhibition of flowering at low light intensities (Jacobs, 1983). BA was found indirectly promotive of flowering in *Leucospermum* by increasing stem thickness, but did not affect the flowering time of this qualitative short-day plant (Malan and Jacobs, 1990).

Paper IV (Figure 3, pg. 61) reported on the importance of leaves for inflorescence initiation by BA induction, while Gerber *et al.* (2002) have also shown the importance of leaves for natural inflorescence initiation on the spring flush of 'Carnival'. A greater supply

of photosynthates due to a greater leaf area and better carbohydrate reserve status as indicated by the higher dry weight, may account for thick shoots initiating flowers more readily in response to BA treatment than thin shoots.

Another explanation for the inability of thinner shoots to initiate inflorescences with BA application may be that the size of the apical dome itself may control the ability to initiate inflorescences in response to BA. In a number of species, floral initiation was correlated with the size of the apical dome. The onset of floret initiation in the quantitative short-day plant, *Chrysanthemum morifolium*, bearing a capitulum-type of inflorescence, was associated with a narrow range of apical volumes (Cockshull, 1985). A critical size of the apical dome, below which only leaf initiation occurred and above which bract and receptacle formation began was reported for *Chrysanthemum* cv. Polaris (Horridge and Cockshull, 1979) and confirmed in *Chrysanthemum* cv. Bittersweet (Cockshull and Horridge, 1980). Other plants for which the transition to reproductive development is associated with a particular size of apex include *Amaranthus* (Koller *et al.*, 1977) and *Lolium* (Evans, 1960). Atherton *et al.* (1998) reported shoot apical diameter to be linearly related to leaf number in *Cineraria*. This may also be the case for 'Carnival' since leaf number in this study was also correlated with inflorescence initiation (Table 2).

A higher concentration of BA could not compensate for deficiencies in shoot characteristics since treating shoots with a 1000 $mg \cdot L^{-1}$ BA inhibited rather than promoted flowering, compared to the 500 $mg \cdot L^{-1}$ BA treatment (Figure 2). Inhibition of floral initiation at supra-optimal concentrations of BA has been reported (Richards, 1985). It appears that when conditions are not optimal, a thicker shoot is required to achieve a comparable result. This is clearly illustrated by the results in Figure 3 where 68% of shoots thicker than 5.1 mm flowered when treated with 500 $mg \cdot L^{-1}$ BA, whereas to achieve a

comparable flowering percentage with 1000 mg·L^{-1} BA shoots of ≥5.9mm diameter were required.

The shoot apex diameter at which the floral transition occurred in *Chrysanthemum* was also larger under non-inductive long-day conditions than under the promotive short-day conditions (Horridge and Cockshull, 1979). Floral initiation in response to BA on two-flush shoots was found to be highly dependent on stem diameter (Figure 4). Percentage flowering in two-flush shoots increased from 0 to 72% with the increase in stem diameter from 4.5 to 7mm respectively. However, for three-flush shoots, with diameters of ≥5.5 mm, floral initiation was equally high and appears to be unaffected by shoot diameter. It is therefore to be concluded that shoot characteristics are key factors in determining the efficacy of autumn BA application to induce flowering in 'Carnival'.

Shoot diameter appears to be a useful non-destructive measure to determine the propensity of a shoot to initiate an inflorescence. In an analysis of a data set of 1800 shoots treated with BA and examining plant age, treatment date during autumn and year (Figure 5), shoot diameter was identified by the CART statistical analysis as the single most important variable influencing inflorescence initiation. The classification tree of independent variables predicted that inflorescence initiation is 71% likely to occur in shoots with an intercalation stem diameters of ≥7.085 mm compared to a probability of 75% non-flowering when the stem diameter with BA application is lower than 7.085 mm (Figure 6).

In addition to stem diameter, results from this study (Figure 5) also support our earlier findings that date of treatment during autumn (Paper I: table 1, pg.54; Paper II: figures 3-4, pg. 95-96), plant age (Paper IV: table 6, pg. 158) and year (varying environmental conditions) may affect the efficacy of BA to cause floral initiation 'out of season' during autumn.

In conclusion, BA application at concentration of 500 $mg \cdot L^{-1}$ to the terminal bud of three-flush shoots with an intercalation stem diameter >7mm can be considered an optimal treatment combination to improve the chance of inflorescence initiation in *Protea* cv. Carnival. Accurate shoot selection for treatment with BA may be of critical importance as shoots that do not initiate an inflorescence on a BA-induced autumn flush, may flower later than untreated shoots (Paper II: tables 5-6; pg. 91-92). The natural flowering time of 'Carnival' when managed in a biennial pruning regime, deliver part of the crop just in time for Valentine's Day (14 February) and a later flowering date would be undesirable. Information obtained from this study is valuable in the development of management strategies to ensure that only shoots meeting the required criteria for initiation are commercially treated with BA to advance flowering time.

Literature Cited

Atherton, J.G., D.M. Yeh, J. Craigon, and G.A. Tucker. 1998. Leaf initiation and shoot apical diameter in relation to phase transition in cineraria. J. Hort. Sci. Biotechnol. 73:45-51.

Brieman, L., J.H. Friedman, R.A. Olshen, and C.J. Stone. 1993. Classification and regression trees. New York. Chapman & Hall.

Cockshull, K.E. 1985. *Chrysanthemum morifolium*. In: A.H. Halevy (ed.) Handbook of Flowering. Vol. VI, CRC Press. Boca Raton, FL. pp. 238-257

Cockshull, K.E. and J.S. Horridge. 1980. Further evidence of a relationship between size of the *Chrysanthemum* shoot apex and inflorescence development. Ann. Bot. 46: 125-127.

De Swardt, D.C. 1989. Aspekte van die vegetatiewe en reproduktiewe ontwikkeling by geselekteerde Proteaceace cultivars. M.Sc. Thesis, Univ. of Stellenbosch, Stellenbosch, S. Africa.

Evans, L.T. 1960. The influence of environmental conditions on inflorescence development in some long day grasses. New Phytol. 59:163-174.

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

Fuss, A.M., S. J. Patterson, 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.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001a. The role of leaves and carbohydrates in flowering of *Protea* cv. Lady Di. Hortscience 36:905-908.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001b. Synchrony of inflorescence initiation and shoot growth in selected *Protea* cultivars. J. Amer. Soc. Hort. Sci. 126:182-187.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2001c. Manipulation of flowering time by pruning of *Protea* cv. Sylvia (*P. eximia* × *P. susannae*). Hortscience 36:909-912.

Gerber, A.I., K.I. Theron, and G. Jacobs. 2002. Defoliation alters spring growth flush characteristics and inhibits flowering in *Protea* cv. Carnival. Scientia Hort 94:345-350.

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. 4:42-46.

Horridge, J.S. and K.E. Cockshull. 1979. Size of the *Chrysanthemum* shoot apex in relation to inflorescence initiation and development. Ann. Bot. 44: 547-556.

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

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

Koller, D., J. Kigel, and S. Ovadiah. 1977. A kinetic analysis of the facultative photoperiodic response in *Amaranthus retroflexus* L. Planta 136:13-19.

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

Richards, D. 1985. Effect of cytokinin application and environment on growth and flowering of *Boronia heterophylla* F. Meull. Scientia Hort 27:325-334.

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. Austr. J. Exp. Agric. 34:1209-1216.

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

Statistical Analysis Systems Institute Inc. 2000. SAS®/STAT User's Guide, Version 9.1, SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

Statsoft Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com

Table 1. Percentages budbreak and flowering as induced by MaxCel™ (active ingredients: BA 1.9% $^{w}/_{w}$) application on three-flush shoots and three subjective stem thickness categories (thin, medium and thick) of two-flush shoots of 17-year old *Protea* cv. Carnival plants. Shoot characteristics (n=10) is described by intercalation stem diameter (mm), total leaf number, leaf area (cm^2) and dry mass of both leaves and total shoot (g), as determined immediately prior to BA application as (11 May 2004).

Shoot category	% Budbreak[z]	% Flowering[z]	Intercalation[y] stem diameter	Leaf number[y]	Leaf area[y]	Leaf dry mass[y]	Total shoot dry mass[y]
Three-flush shoot	93[a]	91.5[a]	6.9[a]	71[a]	968.4[a]	27.32[a]	35.75[a]
Two-flush shoot							
'Thin'	92[a]	0.0[d]	4.5[d]	37[c]	327.2[d]	7.35[d]	9.09[d]
'Medium'	97[a]	29.0[c]	5.4[c]	41[c]	447.9[c]	11.55[c]	14.34[c]
'Thick'	95[a]	75.6[b]	6.0[b]	54[b]	768.9[b]	20.27[b]	25.93[b]

[z] Ten shoot replicates, repeated five times.

[y] Means are values of 10 shoot replicates

Values in the same column followed by superscripts with the same letter are not significantly different at $LSD_{(p=0.05)}$ level.

Table 2. Correlative relationships of shoot characteristics and percentage flowering for *Protea* cv Carnival. Correlations are expressed as the Pearson coefficient accompanied by the p-value.

Shoot characteristics	% Flowering	Intercalation stem diameter	Leaf number	Leaf area	Leaf dry mass	Total shoot dry mass
% Flowering	*	0.96846	0.93851	0.98630	0.98185	0.97826
	*	0.0315	0.0615	0.0137	0.0182	0.0217
Intercalation stem diameter	0.96846	*	0.96152	0.97126	0.98048	0.97891
	0.0315	*	0.0385	0.0287	0.0195	0.0211
Leaf number	0.93851	0.96152	*	0.98075	0.98695	0.99851
	0.0615	0.0385	*	0.0193	0.0131	0.015
Leaf area	0.98630	0.97126	0.98075	*		0.99797
	0.0137	0.0287	0.0193	*		0.0020
Leaf dry mass	0.98185	0.98048	0.98695	0.98695	*	0.99983
	0.0182	0.0195	0.0131	0.0131	*	0.0002
Shoot dry mass	0.97826	0.97891	0.99851	0.99797	0.99983	*
	0.0217	0.0211	0.0015	0.0020	0.002	*

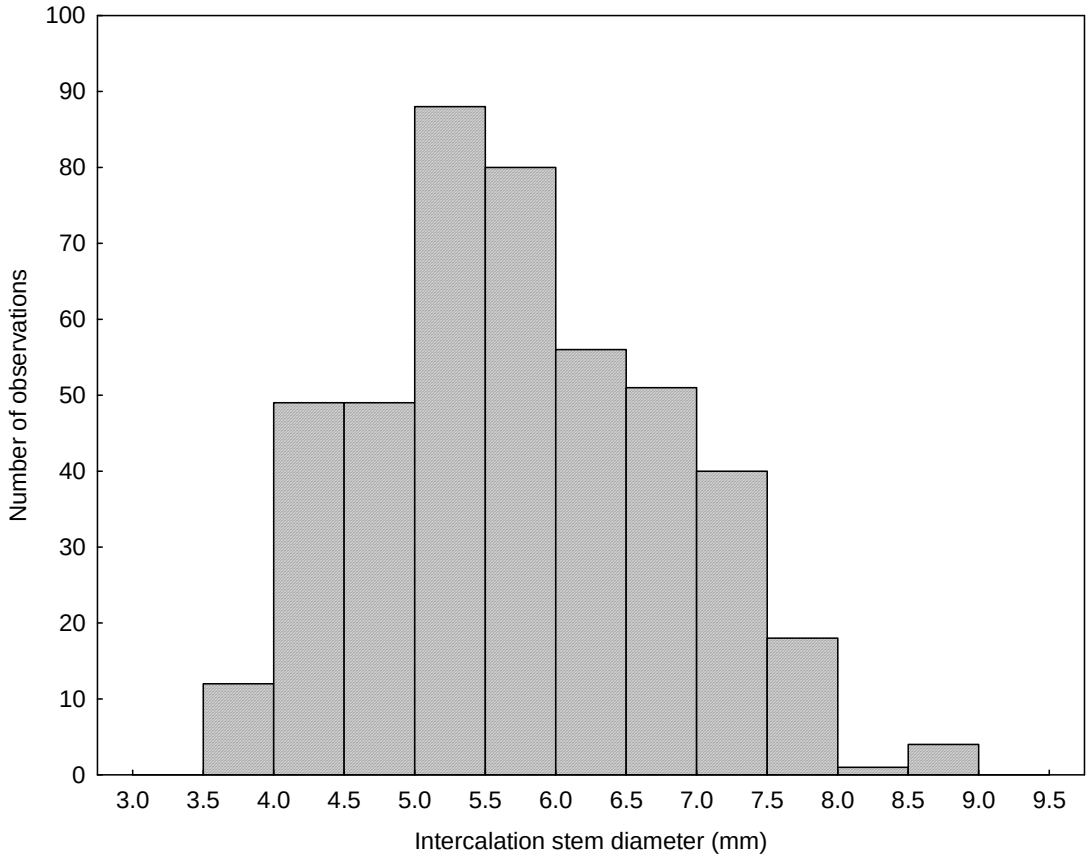


Figure 1. Intercalation stem diameter (mm) distribution of 450 shoots consisting of two- and three-flush shoots selected for MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) on 11 May 2004. Diameter was measured at the upper position of the intercalation between terminal and sub-terminal flushes.

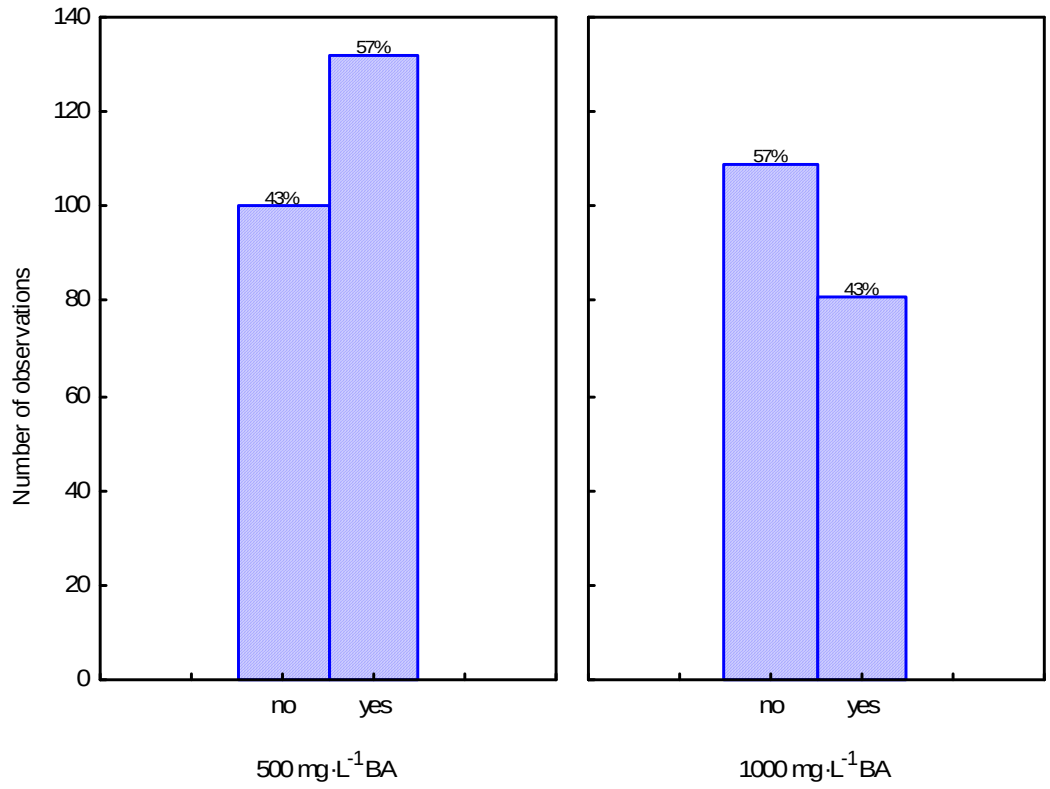


Figure 2. Classification of *Protea* cv. Carnival shoots as flowering (yes) or vegetative (no) based on MaxCel™ (active ingredients: BA 1.9% $^{w}/_{w}$) application to shoots at 500 mg·L^{-1} or 1000 mg·L^{-1}. Data of both two-flush- and three-flush and shoots of all stem diameters were pooled.

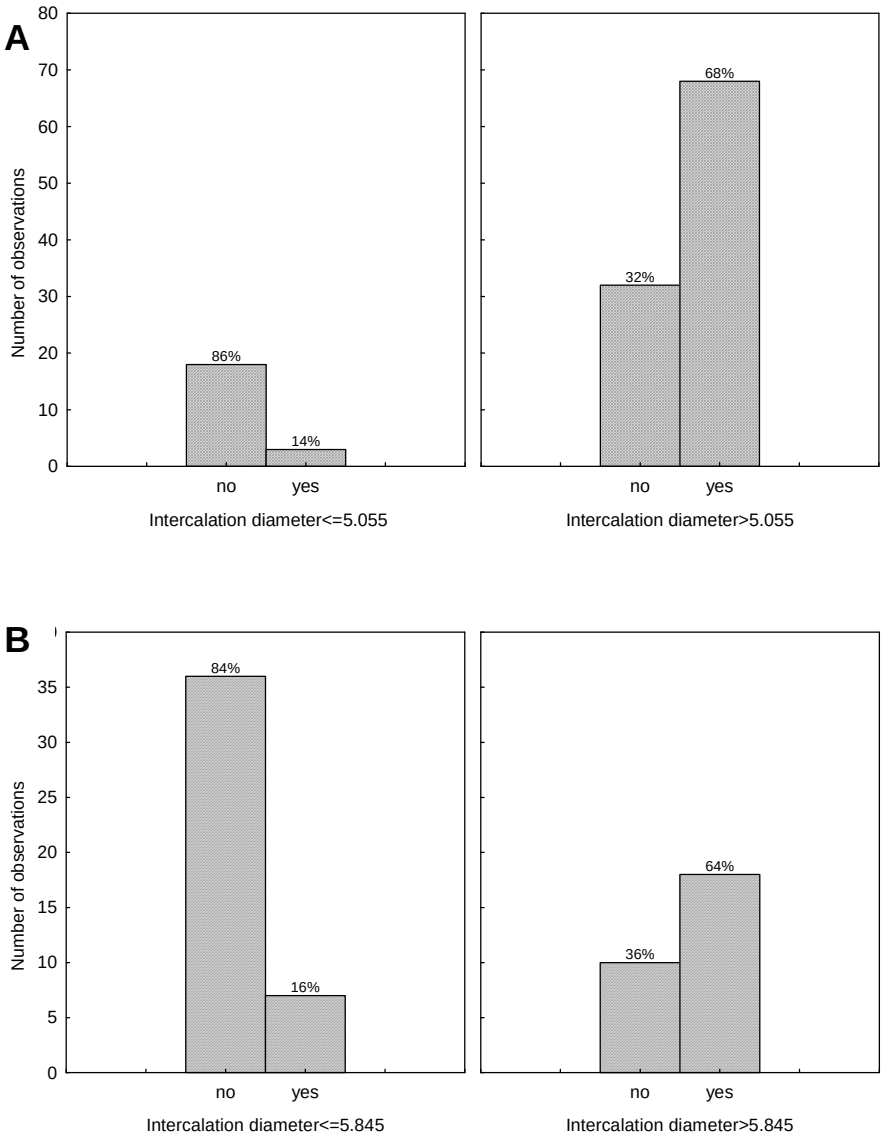


Figure 3. Classification of *Protea* cv. Carnival shoots as flowering (yes) or vegetative (no) based on stem diameter and treatment with MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) at A. 500 mg·L^{-1} or B. 1000 mg·L^{-1}. Data of two and three-flush shoots pooled. Stem diameter was measured at the intercalation between the terminal and sub-terminal flushes.

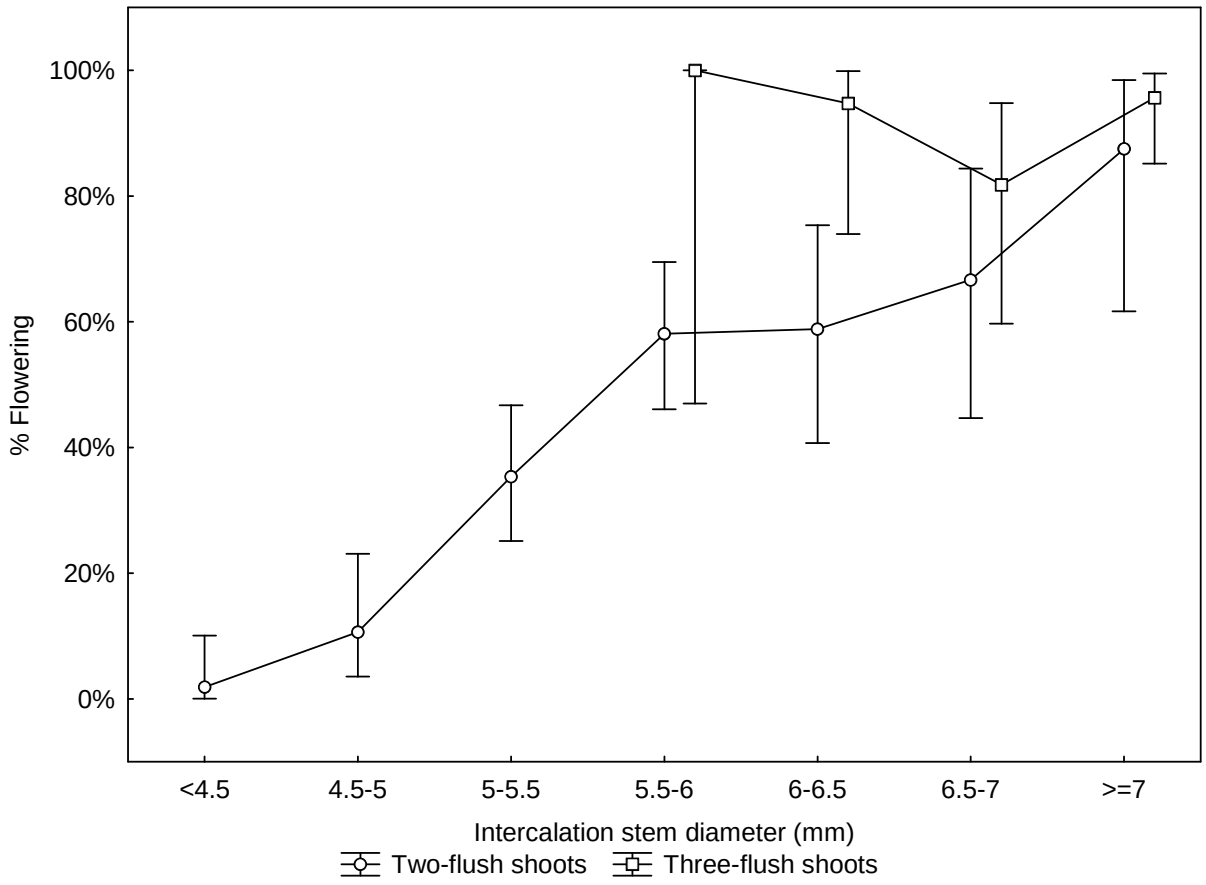


Figure 4. Effect of intercalation stem diameter (mm) ± SE at application of MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) on the percentage flowering of two- and three-flush shoots of *Protea* cv. Carnival. Diameter was measured at the intercalation between the terminal and sub-terminal flushes.

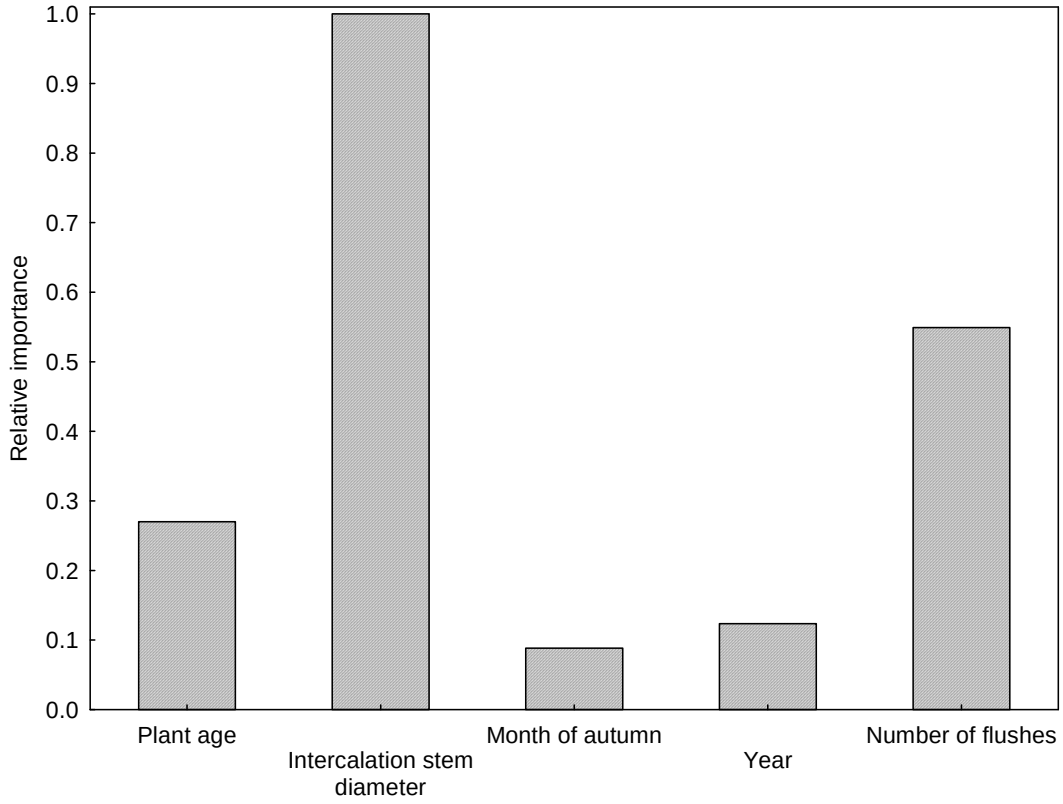


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Figure 5. Relative importance of the contribution of plant age, intercalation stem diameter, autumn month of application, year of application, number of flushes and benzyladenine concentration to flower initiation in *Protea* cv. Carnival. The most important variable is allocated a number of one and the other variables were weighted relative to one to obtain their relative of importance.

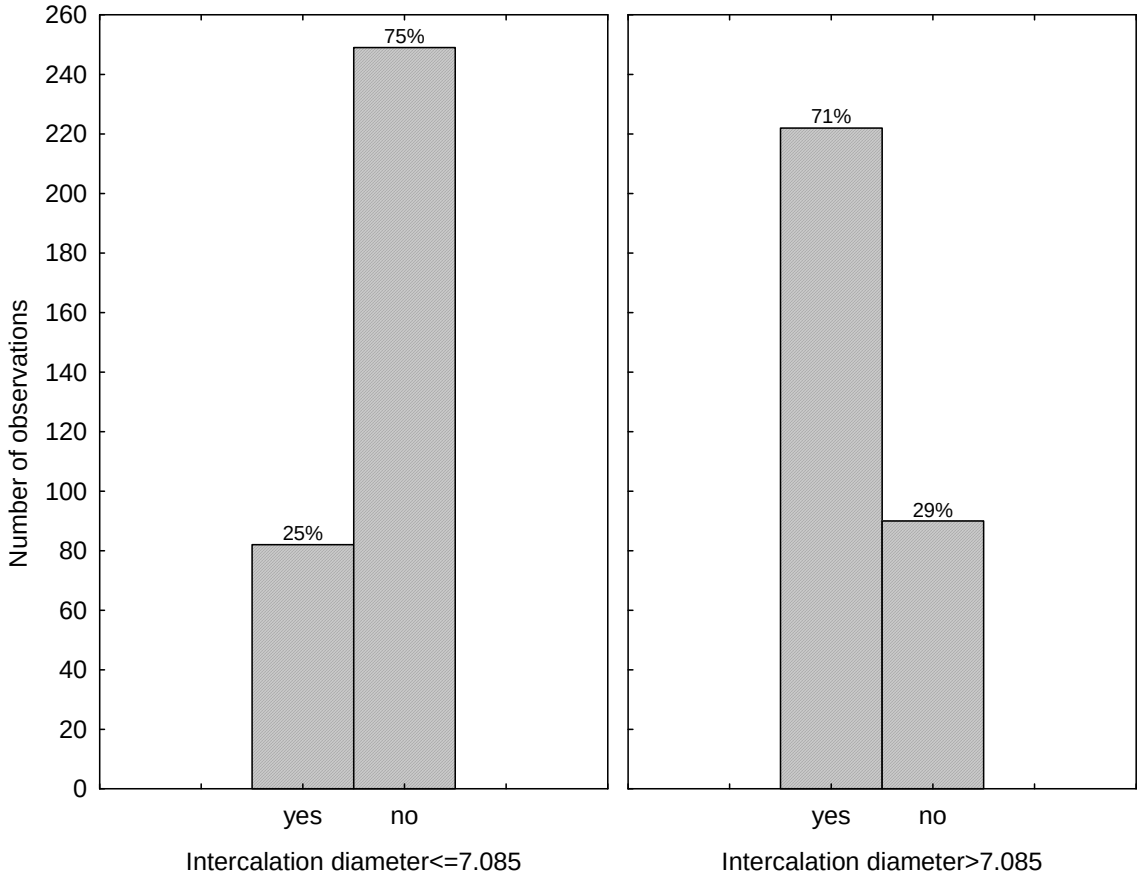

Figure 6. Classification of *Protea* cv. Carnival shoots as flowering (yes) or vegetative (no) based on stems with diameters <= 7.085 or >7.085 mm. Diameter was measured at the intercalation between the terminal and sub-terminal flushes. Data of two and three flush shoots as well as shoots treated with MaxCel^{TM} (active ingredients: BA 1.9% $^{w}/_{w}$) at 500 mg·L^{-1} or 1000 mg·L^{-1} were pooled.

Appendix 1 (Brieman *et al.*, 1993)

Classification tree methodology. In the case of classification trees the dependent (response) variable y is a discrete variable consisting of 2 or more classes (eg yes/no, present/not present, low/medium/high). (For continuous response variables, a similar technique called regression trees can be used.)

The concept of entropy. The concept of entropy (chaos) is used as basis for constructing classification trees. To explain entropy in the framework of classification trees, consider a response variable with 2 classes namely yes/no. If a data set consists of 50% yes and 50% no responses, then the entropy of that data set is a maximum because the data will have only a 50% chance of correctly predicting the class of the response variable. As the proportion of one of the classes tends to 100%, the lower the entropy becomes, and it reaches a minimum when a data set consists of 100% of one class. In this case the data will have a 100% chance of correctly predicting the class of the response variable.

Entropy can be calculated from a data set using various methods of which the Gini measure is probably the most common in classification trees.

The aim of a classification tree is to divide the data set into subsets such that the subsets have a lower entropy than the full data set. Thus it strives to group the classes together into subsets as best possible based on the independent or predictor variables. This is achieved as follows.

Case 1: One continuous independent variable(x). The method selects a point x_p between the minimum and maximum of x that splits the data into two sets (or nodes in a tree). All cases for which $x \leq x_p$ go to the left node and conversely all cases where $x > x_p$ go to the right node. The point where the split is made is the point that decreases the entropy from the parent node to the

child nodes the most. The above procedure above is then repeated for each of the two nodes. Thus, a binary split is made on each node using the criteria mentioned above.

Stopping rules are used to decide when the splitting process should stop. For example: a minimum number of cases per node can be specified, and if that minimum number is reached, the node will split no further.

Case 2: One categorical independent variable. In the case of a categorical independent variable, all combinations of binary splits of the levels of the variable are considered and the combination that most successfully decreases the entropy are used as splitting criteria. For example, if a variable has three levels namely a, b and c then the following combinations of splits will be considered:

Left node	Right node
a	b,c
a,b	c
a,c	b

Case 3: More than one independent variable (combination of continuous and discrete). The procedure described above is applied independently to each variable. Variables are then compared and the one that provides the best split over all the variables is used as the splitting variable.

Variable importance. A variable importance factor in terms of its effect on the response variable can be derived once the tree has been built. This variable importance factor is calculated using the number of times the variable was used as splitting variable and how well it separated response variable classes.

8. GENERAL CONCLUSION.

General Conclusion.

Manipulation of flowering time of *Protea* to fall within the premium price bracket for export to Europe (October to December) has been successful for the two *P. eximia* hybrids ('Sylvia' and 'Cardinal') exhibiting an open flowering window (Gerber *et al.*, 2001). This was accomplished by means of a pruning regime. For 'Pink Ice' (*P. compacta* × *P. susannae*) where flower initiation can occur on either an autumn or spring shoot growth flush, a pruning regime that synchronized shoot growth advanced flowering time favourably to extend from December to May (Nieuwoudt, 2006). However, application of this technique appears to be less successful for the majority of commercial cultivars that flower almost exclusively on the spring flush. For 'Carnival', although pruning could advance flowering time by 4-6 weeks, peak harvests essentially still only followed after February (Greenfield *et al.* 1994). Therefore, in *Protea* cultivars with spring-initiated inflorescences, manipulations of flowering time using techniques other than pruning is required to advance harvest times to fall within the pre-Christmas marketing period.

Observations of increased endogenous cytokinin levels in xylem sap of *Protea* cv. Carnival immediately prior to spring budbreak and during the time when the switch to reproductive growth is known to occur, led to the development of a novel approach for *Protea* where growth regulator application with high cytokinin activity can induce budbreak 'out of season' in late summer and autumn. The release of budbreak by benzyladenine (BA) application to dormant shoots, leads to shoot elongation, the essential phenological stage during which inflorescence initiation takes place. Even though benzyladenine (BA) induces budbreak, not all cytokinin-induced shoots will terminate in an inflorescence.

Vegetative synchronization of all shoots prior to BA application is important as the presence of developing inflorescences within the same plant at the time of BA application is greatly inhibitory to inflorescence initiation. Furthermore, the efficacy of BA to facilitate inflorescence initiation following budbreak and flush elongation in 'Carnival' is affected by intra-plant factors describing shoot quality and maturity. Shoot diameter appeared to be a useful non-destructive predictor of shoot quality and the propensity of a shoot to initiate with BA application. Shoot diameter itself is positively correlated with leaf number, leaf area and shoot dry weight, all of which apparently increases the responsiveness of the shoot to BA with respect to flower initiation. Increased maturity of the flush subtending the terminal bud subjected to BA application resulted in a higher propensity for a shoot to flower, especially for those shoots exhibiting high vigour typical of younger plants. In addition, the timing of budbreak and early elongation appears to be of critical importance as shoots that have not been induced either before or during early elongation will remain vegetative.

Product quality is not compromised by the exogenous cytokinin application. Inflorescences resulting from BA induction on the autumn flush have a more complex appearance with an increased number of involucral bracts and florets and a more intense colour (when initiated in early autumn) compared than the inflorescences borne on the spring flush. The flowering time of 'Carnival' as induced by BA on the autumn flush is advanced by approximately 3 months compared to the normal harvest time. Harvest dates of BA-induced inflorescences which can be as early as mid-November is desirable as it falls within the margins for Christmas (25 December). Furthermore, these earlier flowers can take advantage of traveling slower, but considerably cheaper by sea freight, as opposed to air freight, and still be marketed within the prime window.

The most favorable composition of the cytokinin-containing growth-regulator, effective concentration ranges and application methodologies of the growth-regulator to ensure inflorescence initiation and development has been optimized at 500 $mg \cdot L^{-1}$ for 'Carnival' but needs to be tested and adapted for other *Protea* species and cultivars in order to secure high inflorescence initiation percentages within required marketing windows. In addition, the proposed requirement for low temperatures and possibly a specific photoperiod that is promotive for floral initiation in 'Carnival' require confirmation and refinement under controlled experimental conditions. Evaluation of the prevalence of the post-harvest disorder, leaf blackening, on the induced flush compared to the spring flush is of commercial importance and requires elucidation.

BA-induced inflorescence initiation is a valuable experimental tool to provide insight into the process of floral evocation in *Protea*. However, the mechanism by which this exogenous cytokinin-induced inflorescence initiation operates is not known. Nevertheless, the use of benzyladenine as a management tool in a commercial production system to manipulate flowering times to for better market opportunities seems promising.

Literature cited

Gerber, A.I., K.I. Theron and G. Jacbos. 2001. Manipulation of flowering time by pruning of Protea cv. Sylvia (*P. eximia* × *P. susannae*). Hortscience 36:909-912.

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

Nieuwoudt, G. 2006. The effect of pruning on the growth and development of *Protea* cv. Pink Ice (*P. compacta* R. Brown × *P. susannae* Phill). M.Sc. thesis, Univ. of Stellenbosch, Stellenbosch, S. Africa.