Exogenous Cytokinin Induces “Out of Season” Flowering in *Protea* cv. Carnival

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ABSTRACT. The cytokinin concentration in the xylem sap of *Protea* L. cv. Carnival (*Protea compacta* R. Br. × *Protea neriifolia* R. Br.) 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. Benzyladenine (BA) at 50, 250, or 500 mg·L⁻¹ was applied to entire shoots on 22 Feb., 12 Apr., and 22 May 2001 (fall in the southern hemisphere) or only to terminal buds on 22 May 2001 at 500 mg·L⁻¹. Most of the terminal buds sprouted and initiated an inflorescence when BA application at 500 mg·L⁻¹ in May was directed only to terminal buds, whereas lower flowering percentages (0%–35%) were achieved when the entire shoot was treated. After whole shoots were treated with BA in Apr. 2001, between 5% and 45% floral reversion was observed. High flowering percentages of 87% to 93% were recorded when BA was applied at 500 mg·L⁻¹ to the terminal bud in the dormant state or up to the stage when sprouting buds reached the green point development stage. Later applications were less effective, inducing 42% to 43% inflorescence initiation. The flowering time of BA-induced inflorescences was advanced by more than 2 months compared with flowers that initiated naturally on the spring flush.

Members of the genera *Protea*, *Leucospermum* R. Br., and *Leucadendron* R. Br. are predominantly grown in South Africa for export to Europe. Manipulation of flowering time in these cut flowers is important in taking advantage of high-spring to midsummer (September–January) prices as well as to smooth supply and reduce periods of overproduction. *Leucospermum* (Malan and Jacobs, 1990) and *Leucadendron* (Hettasch and Jacobs, 2006) flowering times can be controlled by daylength manipulation. However, poor shoot qualities result under artificial long days applied in winter in the case of *Leucospermum* (Malan and Jacobs, 1994). Manual removal of the *Leucospermum* primary inflorescence that permits a secondary, and later maturing inflorescence to develop, is therefore practiced to smooth out overproduction during September and October (Jacobs and Minnaar, 1980).

A number of *Protea* species such as *P. magnifica* Link, *P. grandiceps* Trattinnick, *P. eximia* (Salisb. Ex Knight) Fourcade, and some ecotypes of *P. cynaroides* (L.) L. do flower in the desired window from spring to midsummer (September–January). However, many of the superior hybrid *Protea* cultivars flower from March to June. *Protea eximia* exhibits an ability to initiate flowers throughout the year and although hybrids of *P. eximia* such as ‘Sylvia’ (*P. eximia* × *P. susannae* Phill.) and ‘Cardinal’ (*P. eximia* × *P. susannae*) have inherited

this characteristic, most inflorescences predominantly develop on the spring flush with harvest dates then spread over January to April (Gerber, 2000). ‘Sylvia’ shoot growth is synchronized by pruning plants in winter (July or August) to effect a flowering peak 14 to 16 months later in the spring and early summer (October–December) (Gerber et al., 2001a).

Although effective in ‘Sylvia’ and also commercially applicable to ‘Cardinal’, this approach has been less successful on hybrid cultivars that initiate flowers almost exclusively on the spring flush such as ‘Pink Ice’ (*P. compacta* R. Br. × *P. susannae* Phill.) and ‘Carnival’ (Nieuwoudt, 2006).

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 before budbreak (Tromp and Ovaa, 1990; Van Staden and Davey, 1979). Furthermore, exogenous application of cytokinins can promote budbreak during late dormancy (Jones, 1973; O’Hare and Turnbull, 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).

The objectives of this study were to determine the concentration of zeatin riboside in the xylem sap of ‘Carnival’ before and after flower initiation in spring and to induce “out-of-season” flowers by treating shoots or terminal buds in autumn with BA.

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Materials and Methods

PLANT MATERIAL. Experiments on *Protea* cv. Carnival were carried out in commercial plantations of 6-year-old plants grown from cuttings in the Stellenbosch district, South Africa (lat. 33°55'S, long. 18°50'E). The climate is Mediterranean-like with cool, wet winters and dry, hot summers. Annual rainfall is 600 to 700 mm. Plants were spaced 1 m in the row and 4 m between rows. 'Carnival' plants were pruned during August (late winter) of the previous year of the experiment to effect biennial cropping as described by Gerber et al. (1995). Plants were not irrigated or fertilized. Pest control was applied according to commercial practices by risk analysis.

CYTOKININS IN XYLEM SAP OF 'CARNIVAL' SHOOTS. Xylem sap was collected at 3- to 4-week intervals from June 2001 (early winter) to Nov. 2001 (late spring). Shoots harvested before 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 shoots protrude from the brownish covering bud scale. Budbreak was recorded on 4 Sept. 2001 (early spring).

Entire shoots were cut 2 cm from their point of inception before 1000 HR, stripped of leaves, and xylem sap was extracted according to Belding and Young (1989). About 4 cm of bark was carefully removed from the stem adjacent to the basal cut and was rinsed with distilled water to eliminate possible contaminants. This stem section was inserted through a rubber stopper fitted into a side-arm vacuum flask. A 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, whereafter flow stopped due to constriction at the distal end of the stem section. Continuous cutting off 2 cm at short intervals from the distal end allowed the resumption of flow of xylem sap from the proximal 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 per extraction date were selected and the xylem sap of each three-flush 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 nanogram activity per 100 μL of xylem sap.

GROWTH REGULATOR TREATMENTS ON 'CARNIVAL'. 'Carnival' shoots consisting of three flushes from the point of inception were used. N-6-BA solutions were prepared by diluting with water ABG-3062 [2% BA (w/w); Abbott Laboratories, North Chicago, IL]. No additional wetting or penetrating agent was needed. Shoots were treated with BA at a concentration of 50, 250, or 500 $\text{mg}\cdot\text{L}^{-1}$ on 22 Feb. (late summer), 12 Apr. (midautumn) or 22 May 2001 (late autumn). The solutions were applied with a paint brush to the leaves and stem of the entire shoot. A treatment with 500 $\text{mg}\cdot\text{L}^{-1}$ BA was applied to the terminal bud only of the second summer flush on 22 May 2001 (late autumn). 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 end in an inflorescence was recorded for all shoots.

On 5 Dec. 2001 (early summer), when the first flowers were at the commercially "soft tip" harvestable stage (florets still enclosed in involucre 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 inflorescence dry mass (60°C , 72 h) 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 as revealed in this case by the differentiation of many involucre bracts.

VERIFICATION OF OPTIMUM BA CONCENTRATION FOR INFLORESCENCE INITIATION IN 'CARNIVAL'. An experiment to verify the optimum BA concentration for inducing budbreak and inflorescence initiation in *Protea* cv. Carnival when applied to the terminal buds alone was conducted on 6 May 2005 (late autumn). N-6-BA as contained in MaxCel™ [1.8% BA (w/w); Valent BioSciences, Libertyville, IL] was used. Only the terminal bud of three-flush shoots was treated at concentration levels of 50, 100, 250, or 500 $\text{mg}\cdot\text{L}^{-1}$ BA prepared by diluting MaxCel™ with water. Distilled water was used as the control treatment. The occurrence of an autumn flush as well as the ability of the autumn flush to end in an inflorescence was recorded for all shoots. Ten shoots were used per replicate and five replicates were in a randomized complete block design.

BA APPLICATION TO DIFFERENT DEVELOPMENTAL STAGES OF THE TERMINAL BUD IN 'CARNIVAL'. On 30 Apr. 2004 (midautumn), mature three-flush shoots were selected where the terminal bud exhibited various developmental stages. The various 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, Fig. 1A); swollen (bud noticeably swollen with bud scales beginning to fold back to reveal green leaves, Fig. 1B); green point (developing flush elongated up to 1.5 cm, representing an intact bulbous "torpedo" shape, Fig. 1C); elongation stage I (developing flush elongated up to 2.5 cm, with proximal leaves beginning to unfold, Fig. 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, Fig. 1E). On the day of selection, all shoots received a single treatment of BA at 500 $\text{mg}\cdot\text{L}^{-1}$ (MaxCel™) applied with a paint brush as described earlier. Five shoots were used per replicate and three replicates were in a randomized complete block design.

Axillary bud development that was stimulated following cytokinin-containing growth regulator applications to the entire shoot and to terminal buds of 'Carnival' were removed by hand at an early stage of growth.

STATISTICAL ANALYSIS. Categorical data of flowering incidences in 'Carnival' as induced by growth regulator treatments, including the assessment of the time of application in 'Carnival' was subjected to the maximum-likelihood (M-L) chi-square test using Statistica (version 7.1; Statsoft, Tulsa, OK). In the M-L chi-square tests, flowering incidences of controls were compared with that of growth regulator treatments, while comparisons within growth regulator treatments were also performed. Descriptive statistics for inflorescence characteristics were also obtained using Statistica (version 7.1). Analysis of variance was conducted using PROC GLM (version 9.1; SAS Institute, Cary, NC). Logit transformation was performed

Fig. 1. Morphological characteristics of various growth stages of terminal bud development in *Protea* cv. Carnival: (A) dormant, (B) swollen, (C) green point, (D) elongation stage I, (E) elongation stage II.

on the data describing the percentages bud sprouted and inflorescence initiation. Mean separation was conducted by least significant difference (LSD), where applicable.

Results

CYTOKININS IN XYLEM SAP OF 'CARNIVAL' SHOOTS. Levels of cytokinin activity in xylem sap increased from early winter (mid-June 2001) and peaked at a time coincidental with spring budbreak, before significantly declining by late spring (mid-November) (Fig. 2).

GROWTH REGULATOR TREATMENTS ON 'CARNIVAL'. No apical buds sprouted when the entire shoot length was treated with BA in February (late summer) or May (late autumn) (Table 1). However, terminal buds sprouted when the entire shoot was treated in April (midautumn) or when BA was applied in May (late autumn) to the terminal bud alone. In addition to the terminal buds, axillary buds also sprouted in the April (midautumn) treatment (data not presented). Application of BA (500 mg·L⁻¹) to terminal buds alone in May (late autumn) resulted in greater floral initiation compared with any of the other treatments.

Significant floral reversion (≥40%) occurred in shoots treated in their entirety with BA solutions of 250 or 500 mg·L⁻¹ (Table 1). This was indicated by the development of many involucre-like bracts at the base of the induced terminal spring flush (Fig. 3).

Inflorescence development was significantly advanced in BA-induced inflorescences compared with the normal inflorescence initiation phase on the spring flush (Table 2). Flowers borne on BA-induced flushes resulting from the April (midautumn) treatment reached commercially harvestable stage first

in early summer (5 Dec. 2001) compared with late-summer (February) for naturally induced flowering on the spring flush. Dry mass accumulation in inflorescences initiated on shoots treated with BA in May (late autumn) was ≈50% less than that of inflorescences initiated with the April (midautumn) treatment (Table 2).

VERIFICATION OF OPTIMUM BA CONCENTRATION FOR INFLORESCENCE INITIATION IN 'CARNIVAL'. Budbreak and flowering incidence following any BA application was significantly higher than in untreated control shoots (Table 3). Bud sprouted and inflorescence initiation increased linearly with an increase in BA concentration between 50 or 500 mg·L⁻¹ (Table 3). Expressing flowering as a percentage of buds sprouted revealed that BA treated shoots initiated more flowers than untreated control shoots and with an increase in BA concentration between 50 or 500 mg·L⁻¹ flowering increased linearly (Table 3).

BA APPLICATION TO VARIOUS DEVELOPMENTAL STAGES OF THE TERMINAL 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 other developmental stages. Shoot deformities were evident when BA applications were made to developing flushes at elongation stage II (Table 4).

Fig. 2. *t*-Zeatin riboside concentration in xylem sap of three flush shoots for *Protea* cv. Carnival before, during, and after spring budbreak (4 Sept. 2001). Values within parentheses represent the days relative to budbreak and the arrow indicates the point of budbreak. Data presented are means of five replicates ± SE.

Table 1. Effect of benzyladenine (BA) as ABG-3062 [2% BA (w/w); Abbott Laboratories, North Chicago, IL] on buds sprouted, flowering, and inflorescences reverted of three-flush shoots of *Protea* cv. Carnival. BA solutions were applied to the entire shoot in Apr. or May 2001 or to the terminal bud alone of the second summer flush in May 2001. Data were collected on 5 Dec. 2001 when first flowers were at the commercially harvestable “soft tip” stage.

BA (mg·L ⁻¹)	Application site: Entire shoot on 12 Apr. 2001			
	Buds sprouted (%)	Flowering (%)	Inflorescences reverted (%) ^z	Flowering and reverted inflorescences (%)
0	10 a ^y	5 a	0 a	5 a
50	75 b	35 b	5 a	40 b
250	95 b	25 b	45 b	70 c
500	100 b	35 b	40 b	75 c

	Application site: Entire shoot on 22 May 2001		Application site: Terminal bud on 22 May 2001	
	Buds sprouted (%)	Flowering (%)	Buds sprouted (%)	Flowering (%)
0	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

^zShoots with incomplete inflorescence differentiation that reverted to vegetative flush extension.

^yPercentages (n = 20) within parameters followed by different letters are significantly different at the 5% level via maximum-likelihood chi-square test.

Fig. 3. Floral reversion as observed in *Protea* cv. Carnival. Entire three flush shoots were treated with benzyladenine (BA) solutions as ABG-3062 [2% (w/w); Abbott Laboratories, North Chicago, IL] at 250 or 500 mg·L⁻¹ on 12 Apr. 2001. (A) Intercalation between autumn and spring flushes with no floral reversion observed. Bracts visible are bud scales that protect the terminal bud before flush extension. (B) Intercalation between autumn and spring flushes where floral reversion was recorded. Bracts visible resemble first involucre bracts of the inflorescence. (C) Abaxial view showing involucre bracts of the inflorescence. (D) Abaxial view at autumn and spring intercalation on floral reversion.

Discussion

The increase in cytokinin concentration before buds sprouted in spring of ‘Carnival’ xylem sap resembles similar patterns found in other woody perennials (Belding and Young, 1989; Hewett and Wareing, 1973), and the concentration is comparable with the results obtained with *Protea aurea* (Burm. F.) Rourke (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 (O’Hare and Turnbull, 2004; Steffens and Stutte, 1989; Turnbull et al., 1997).

Shoot growth of ‘Carnival’ occurs in distinct flushes with flowers initiating almost exclusively on a spring flush that develops terminally on overwintering 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 (Fig. 2). The rise in cytokinin concentration is most likely root derived because root growth is perceived to be most active soon after winter (Hanekom et al., 1973). It is possible that inadequate levels of cytokinin in competent shoots may partly account for the failure of ‘Carnival’ to initiate flowers on flushes at other times of the year when root growth is less. This view is supported by the successful inflorescence initiation achieved with exogenous cytokinin applications in autumn (Tables 1, 3, and 4). When only sprouted buds are considered, increasing concentrations of BA from 50 to 500 mg·L⁻¹ resulted in the percentage shoots that initiated flowers to increase from 62% to 87% (Table 3). Therefore, the effect of BA is not limited only to stimulating bud sprouting, but also to increasing the propensity of shoots to initiate inflorescences.

Cytokinin is considered one of several components in a multifactorial model of flowering control for angiosperms where various 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 some tropical and subtropical tree crops, such as mango (*Mangifera indica* L.) and lychee (*Litchi chinensis* Sonn.), where vegetative shoots exhibit periodic extension (flushing) ended 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

Table 2. Inflorescence diameter and dry mass of *Protea* cv. Carnival determined at harvest (5 Dec. 2001). Benzyladenine (BA) solutions (50, 250, and 500 mg·L⁻¹) as ABG-3062 [2% BA (w/w); Abbott Laboratories, North Chicago, IL] were applied to the entire shoot on 12 Apr. 2001 or to the terminal 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.

Treatment date with BA	Shoots (no.)	Inflorescence on spring flush	
		Inflorescence diam. [mean ± SE (mm)] ^z	Inflorescence dry mass [mean ± SE (g)]
Untreated control	76	19.4 ± 0.76	1.7 ± 0.15
Inflorescence on BA-induced autumn flush			
12 Apr. 2001 ^y	18	39.9 ± 1.35	23.5 ± 0.15
22 May 2001 ^y	19	37.6 ± 1.14	11.3 ± 1.18

^zMeasured at widest basal portion of the inflorescence.

^yData of BA concentrations (50, 250, and 500 mg·L⁻¹) were pooled.

Table 3. Effect of benzyladenine (BA) as MaxCel™ [1.8% BA (w/w); Valent BioSciences, Libertyville, IL] applied in late autumn to the terminal bud alone on three flush shoots of *Protea* cv. Carnival on buds sprouted and flowering.

BA (mg·L ⁻¹)	Buds sprouted (%) ^z	Flowering (%) ^z	Flowering of buds sprouted (%)
0 (control)	3	0	0
50	63	36	62
100	68	54	87
250	92	58	75
500	100	84	87
Significance ^y			
Control vs. BA	<0.0001	<0.0001	<0.001
BA linear	0.0047	0.0091	0.0124

^zLogit transformation of data were performed on all values expressed as percentages; n = 50 (five replications with 10 shoots per replication).

^ySAS (version 9.1; SAS Institute, Cary, NC) was used to fit single-df orthogonal polynomial contrasts to test for linear trends over BA rate.

Table 4. Effect of benzyladenine (BA; 500 mg·L⁻¹) as MaxCel™ [1.8% BA (w/w); Valent BioSciences, Libertyville, IL] on flowering (%) when applied (30 Apr. 2004) to various developmental stages of terminal buds on three flush shoots of *Protea* cv. Carnival.

Developmental stage of terminal bud	Flowering (%) ^z
Dormant	93.3 a ^y
Swollen	86.7 a
Green tip	91.7 a
Elongation stage I	43.3 b
Elongation stage II	42.2 b

^zn = 15 (three replications with five shoots per replication).

^yValues followed by different letters differ significantly at *P* = 0.05 via least significant difference test.

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 occur before shoot initiation, but that floral initiation only occurs after bud sprouting. Flower initiation in ‘Carnival’ occurs shortly after bud sprouting in spring (Gerber et al., 2001b). The cytokinin peak in ‘Carnival’ that coincides with the onset of bud sprouting in spring (Fig. 2) therefore also coincides with the time of flower initiation. Cytokinins may

therefore be directly involved in the initiation process as part of the floral signal.

Targeting the application of BA exclusively to the terminal bud of three-flush shoots of ‘Carnival’ appears important to optimize the efficacy of BA to induced inflorescences since shoots treated with BA over their entire length initiated fewer inflorescences and floral reversion occurred in some instances (Table 1, Fig. 3). Floral reversion was not observed where only the terminal bud was treated (Tables 1, 3, and 4).

Floral reversion is not frequently reported, as plants usually initiate flowers under conditions that will result in normal flower development. However, incomplete flowering may 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 *P. cynaroides*, under conditions where leaves were under heavy predation pressure. Witness to the reversion process in this study on ‘Carnival’ is the additional bract-like appendages (Fig. 3) that developed just above the upper intercalation on reverted shoots. 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 spatial and temporal regulation of cytokinin levels to facilitate flowering has been clearly demonstrated in transgenic *Arabidopsis thaliana* (L.) Heynh. Lawalrée mutants. Overexpression of the *hsp70ipt* gene that led to cytokinin enrichment of *A. thaliana* plants as a whole led neither to earlier nor enhanced flowering (Medford et al., 1989). It appears that localized stimulation of the terminal bud by BA will secure higher flowering percentages rather than a spray application to the whole plant or shoot (Table 1).

The developmental stage of the terminal bud 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 developmental stage 3 (green point stage). Inflorescence initiation was significantly less successful when BA was applied to the succeeding, more advanced vegetative developmental stages (elongation stage I or II) of the new developing flush (Table 4). Application of BA to the new developing flush past an elongation stage II 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 (data not presented).

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 millimeters in length, but not at later stages of shoot elongation (Batten and McConchie, 1995). Likewise, in avocado (*Persea americana* Mill.) and macadamia (*Macadamia integrifolia* Maiden & Betche $\times$ *Macadamia tetraphylla* L. Johnson) another member of the Proteaceae, 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 initiation only takes place after bud sprouting.

Inflorescence initiation on flushes of ‘Carnival’ induced by treatment with BA in April or May (autumn) advanced flowering from the “normal” time of March to May the following year to such an extent that flowers were ready for the Christmas market. It is possible that application of cytokinin to control flowering time in ‘Carnival’ may also be effective in related cultivars because 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 ‘Carnival’, without the exposure of the shoot to inductive winter conditions, is a first report for Proteaceae and has significant commercial potential for *Protea* as a cut flower crop.

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