Synchrony of Inflorescence Initiation and Shoot Growth in Selected Protea Cultivars

Audrey I. Gerber, Karen I. Theron,¹ and Gerard Jacobs²

Department of Horticultural Science, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

Additional Index Words. Proteaceae, budbreak, inflorescence development

Abstract. Protea L. sp. can be assigned to groups according to similar times of flower initiation and harvest. The stages occurring during flower initiation and their synchrony relative to shoot growth were investigated for three cultivars when flower initiation occurred on the spring growth flush. For all three cultivars, the spring flush was preformed and enclosed in the apical bud before spring budbreak. During elongation of the spring flush, the apical meristem produced floral primordia which differentiated into involucral bracts. After completion of the spring flush, meristic activity continued and produced floral bracts with florets in their axils. The different cultivars were characterized by differences or similarities in the time of budbreak, and the rates of shoot growth, appendage formation, and flower development. Insight into the time of flower initiation relative to vegetative growth could be useful in making management decisions, as well as forming a basis for manipulation of the flowering process.

Materials and Methods

Plant Material. Plants of ‘Carnival’, ‘Sylvia’, and ‘Lady Di’ used in this study were grown in commercial plantations in the Stellenbosch district (lat. 33°54′S; long. 18°40′E), South Africa. In 1995 ‘Carnival’ and ‘Lady Di’ plants were 5 years old, and ‘Sylvia’ plants were 6 years old. For studies of ‘Carnival’, two separate production blocks of the same age, pruned for biennial bearing, were used; one in the vegetative cycle of biennial bearing, and one in the flowering cycle (Gerber et al., 1995). The plants were spaced 1 m in the row and 4 m between rows, clean cultivated and were not fertilized or irrigated. The Stellenbosch climate is Mediterranean, with cool, wet winters (average maximum 17.6 °C and minimum 8.1 °C) and hot, dry summers (average maximum 27.2 °C and minimum 14.8 °C). Annual rainfall is 600 to 700 mm.

Successive Shoot Growth Flushes in ‘Carnival’. ‘Carnival’ plants in the vegetative cycle of biennial bearing (pruned 20 Aug. 1996) were used to study the manner in which shoot growth flushes developed. Pruning consisted of heading back all shoots to leave the basal 15 to 20 cm portion (bearer), as described previously (Gerber et al., 1995), on which new shoot growth

1Associate professor.
2Professor.
originated from axillary buds. At the first sign of budbreak in spring, similar sized bearers were tagged on a number of plants in the commercial plantation. When the first flush was complete, individual shoots of similar size were tagged to further reduce variation, and the production of subsequent summer and fall growth flushes studied.

At 2-week intervals, starting from bud sprouting in spring (18 Sept. 1996), five previously tagged buds or developing shoots were collected, shoot length measured, and the apical bud dissected using a stereomicroscope. The number of appendages (determined to develop into budscales, transitional leaves or true leaves) was found. This was continued until all three shoot growth flushes were completed (7 Aug. 1997). To enable comparison of these data with 'Carnival' plants in the reproductive cycle of biennial bearing, the sampling date was also calculated as the number of days from pruning, i.e., the start of shoot growth. At the completion of each growth flush, the number of appendages that constituted the flush became known. This number was subtracted from the total number of appendages present on the shoot and in the apical bud during development, to identify which appendages belonged to the following flush.

Appendage Formation During the Spring Growth Flush. During winter and before commencement of the spring growth flush in 1996, shoots were tagged on a number of plants in the commercial plantation. Shoots of 'Carnival' and 'Sylvia' were composed of three shoot growth flushes and 'Lady Di', two shoot growth flushes. 'Sylvia' shoots which were likely to flower on the spring, similar sized bearers were tagged on a number of plants in the field when the inflorescence bud was =10 mm in basal diameter, whereafter the diameter was measured at 2 week intervals. During inflorescence development, samples of apical buds of 'Carnival' were placed in FAA (formaldehyde–acetic acid–50% ethanol, 1:1:18 by volume). These apical buds were dissected to view the morphological changes and the number of days from pruning, i.e., the start of shoot growth). Legend in A applies to B, and the legend in C applies to D.

Inflorescence and flowering shoot characteristics. Five flowering shoots of 'Carnival', composed of the following shoot growth flushes: Spring 1995, Summer 1995, Fall 1996, and Spring 1996, which subtended an inflorescence, were cut from different plants 24 Feb. 1997 (summer). Simi-
taking place in the bud during inflorescence development, by scanning electron microscope (SEM). After removal from the FAA, the buds were ethanol-dehydrated, critical-point-dried with CO₂, and sputter-coated with gold at 1 kV for 5 min in an ioncoater (Auto 306; Edwards, Crawley, Sussex, United Kingdom). The buds were viewed on a scanning electron microscope (JSM 6100; Joel, Watchmead Garden City, Hertfordshire, United Kingdom) at an accelerating voltage of 5 kV. Micrographs were taken with a camera (HR 80018; Joel) using 120 mm, 100 ASA film (Agfa-Gevaert AG, D-51301 Leverkulsen, Germany).

### Results

**Successive Shoot Growth Flushes in 'Carnival':** Pruning ‘Carnival’ shoots released rudimentary buds in upper axillary positions on the bearer from correlative inhibition, allowing meristematic activity in the buds to resume. The first visual signs of bud expansion were noticed 29 d after pruning, on 18 Sept. 1996. Bud expansion continued for a further 21 d before flush extension started in early October. During this 50-d period from pruning, the meristem produced a total number of 28 appendages, which constituted the first (spring) growth flush (Fig. 1A). Only after all the appendages had been produced did the spring flush start elongating.

During elongation of the first spring flush (1996) (Fig. 1B), the meristem produced the elements necessary for the subsequent (summer) flush (Fig. 1A). Almost the full complement of appendages necessary for the summer flush was contained in the apical bud when expansion of the spring flush was complete in early December. A lag of ≈2 weeks separated the end of spring flush growth and the start of enlargement of the terminal bud to produce the summer flush.

The aforementioned developmental pattern was repeated for the fall growth flush. While the summer flush was elongating (Fig. 1B), the meristem formed the appendages that would comprise the fall flush (Fig. 1A). When elongation of the summer flush was complete, the majority of the fall flush appendages had been formed, and there was a lag of 1 week before growth of the fall flush began. Fall flush elongation was accompanied by formation of appendages for the spring flush. When elongation of the fall flush was complete (at the end of April) the apical bud contained ≈70% of the appendages for the second spring flush. Meristematic activity continued through winter to produce the full complement of appendages for the spring flush (Spring 1998, on plants in the reproductive cycle) (Fig. 1C). Before spring budbreak, in early September, the entire spring flush was pre-formed and enclosed in the apical bud. During spring budbreak,
between. Beginning and end of elongation, with a phase of rapid growth in
(Fig. 1B and D). Slow rates of growth were apparent at the 'Carnival', especially the spring growth flush, was sigmoidal
quent elongation of internodes, leaf development continued. Dur-
closed by scalelike bracts surrounding the bud. During subse-
apical bud differentiated and began development while still
before elongation of the spring flush, the preformed leaves in the
apical bud differentiated and began development while still
by scalelike bracts surrounding the bud. During subse-
neither appendage formation, nor flush elongation occurred at
constant rate (Fig. 1). Extension growth of the flushes of
'Carnival', especially the spring growth flush, was sigmoidal
(Fig. 1B and D). Slow rates of growth were apparent at the
beginning and end of elongation, with a phase of rapid growth in
between.

APPENDAGE FORMATION ON FLOWERING SPRING GROWTH FLUSHES.
At the time when terminal budbreak occurred in spring, terminal
buds contained 67 ± 7.8, 78 ± 14.0 and 66 ± 3.8 appendages for
'Carnival', 'Sylvia', and 'Lady Di', respectively. These numbers
equaled or exceeded the average final number of appendages that
constituted the spring growth flush subtending an inflorescence
for the three cultivars (Table 1). The appendages for the spring
growth flush were thus preformed before elongation began. Sub-
tracting this number from the total number of appendages
present in the developing bud and apical meristem yielded the
progression in the formation of involucral bract primordia during
spring flush elongation (Fig 1C). It is evident for all three cultivars
that the involucral bract primordia were formed during extension
growth of the spring shoot growth flush (Fig. 2). The number of
appendages present in the apical bud at the time of completion
of the spring flush was comparable to the number of involucral
bracts present on the mature inflorescence (Table 2).

The relationship between length of the spring flush and num-
ber of appendages present in the apical bud of 'Carnival' and
'Sylvia' showed an exponential rise to a maximum (Fig. 2). Dur-
ing the early stages of extension growth, many appendages
were formed but fewer formed during the end of elongation of
the spring flush. The relationship between length of the spring flush
and number of appendages present in the apical bud of 'Lady Di',
however, was linear.

INFLORESCENCE AND FLOWERING SHOOT CHARACTERISTICS. The
characteristics of flowering shoots of 'Carnival', 'Sylvia', and
'Lady Di' are presented in Tables 1 and 2. The characteristics of the spring shoot growth flush, which subtended the inflorescence,
differed from flushes lower down on the stem in the follow-
ing ways. For all three cultivars, the number of leaves, transitional
leaves and budscales, and, therefore, the total number of append-
ages of the spring flush was greater than preceding flushes (Table
1). The spring flush of 'Carnival' and 'Lady Di', but not 'Sylvia',
was longer than preceding flushes. In all three cultivars, starting
with the most basal flush, the flushes preceding the spring flush
of 1996 progressively contained a greater number of appendages.
In the case of 'Carnival' and 'Lady Di' this was due to an increase
in the number of budscales, and for 'Sylvia' an increase in both
budscales and leaves.

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

INFLORESCENCE DEVELOPMENT. The rate of inflorescence de-
velopment, as approximated by the increase in diameter, was
similar for the three cultivars (Fig. 3). 'Sylvia' and 'Carnival',
with similar sized inflorescences, completed development within
the same time period. Development of 'Lady Di' inflorescences
continued for longer and anthesis occurred ≈10 weeks later than
'Carnival' and 'Sylvia'.

The stages of apical meristem development during floral
initiation in 'Carnival' are shown in Fig. 4. In the vegetative state,
the apical meristem is dome shaped (Fig. 4A), becoming flat and
broad for the production of involucral bracts (Fig. 4B). The
transition from production of involucral bracts to the production
of floral bracts is obvious by a change in phyllotaxy (Fig. 4C). A
single floret develops in the axil of each floral bract, with
development proceeding acropetally (Fig. 4E). The tips of these
florets are hirsute which rapidly obscures development of indi-
vidual florets (Fig. 4F).

**Discussion**

Shoot growth in *Protea* sp. occurs by elongation of successive
growth flushes. With the exception of the flush originating from a
rudimentary axillary bud, a flush develops from a preformed shoot
in the terminal bud. The leaf primordia in the terminal bud are
initiated during elongation of the previous flush and the full comple-
ment is present at budbreak. Shoot growth is due to extension
of preformed internodes and development of preformed leaf primor-
dia. This is the same pattern of development that describes rhythmic
growth in oak (*Quercus* L. sp.) (Crabbé, 1987)

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

The inflorescence bearing spring growth flush differed from other flushes in the number of appendages formed (Table 1). An increase in the number of budscales and transitional leaves with successive growth flushes and a large increase in the number of leaves on the flush subtending the inflorescence were common features for all three cultivars. It is unclear if the increase in appendage number is a prerequisite for flowering. The correlation between a decrease in plastochnon and the transition from the vegetative to floral state in apple [Malus sylvestris (L.) Mill. Var domestica (Borkh.) Mansf.] has received much attention (Fulford, 1966; Verheij, 1996). The plastochnon was not measured in these investigations and little can be concluded regarding the interrelationship between meristem activity and its fate, although it is apparent from SEM that phyllotaxy does change during the progression from production of leaf primordia through the different stages of inflorescence initiation. The flattening and broadening of the apical meristem prior to production of involucral bracts is similar to meristem changes seen in Leucospermum during production of peduncular bracts, as is the return to the domed, or conical, state during the initiation of floral bracts (Malan et al., 1994). Floral bracts and individual florets initiate and develop acropetally, as is seen in inflorescence development in Banksia (Fuss and Sedgley, 1990).

With respect to inflorescence initiation on the spring growth flush, it is clear that, for the three cultivars studied, the initial stages of inflorescence initiation and differentiation coincided with elongation of the spring growth flush. Inflorescence development had advanced to the stage where all the involucral bracts had initiated by the time elongation of the spring growth flush was complete, and initiation of floral bracts had begun. Floret initiation and differentiation occurred after completion of the spring growth flush. Dupee and Goodwin (1990) stated that flower initiation in P. nerifolia ‘Salmon Pink’ occurs after spring flush growth in late October or early November (summer), and in P. cynaroides (L.) L. there are two short periods during which flower initiation can occur, i.e., May (fall) and December (summer). In neither instance were the stages of initiation discussed, and the macroscopic appearance of the bud was taken as an indication that initiation had occurred. Heinsohn and Pammeter (1988) noted the presence of visible flower buds in late October on P. nerifolia in South Africa, and concluded that the ‘flowering signal’ was received and acted upon earlier in the growing season. That flower initiation had indeed occurred is unequivocal, yet macroscopic evidence of flowering provides no information as to the time or progression of initiation and development. In P. aristata Phill. up to a year can elapse between flower initiation and development, during which axillary shoot growth continues. This is also found, although to a lesser extent, in P. repens (L.) L. and P. lanceolata E. Mey. Ex Meisn., and was suggested to be an adaptive mechanism, allowing extra time for shoot diameter growth to occur, both for increased mechanical strength and production of additional conducting tissue to support flower development (Le Maitre and Midgley, 1991). In ‘Carnival’, ‘Sylvia’, and ‘Lady Di’ three phases of inflorescence development can be distinguished: a phase of involucral bract formation, which occurs during extension growth of the shoot flush that subtends the inflorescence; a phase of floret initiation, which occurs after completion of the subtending shoot growth flush; followed directly by an inflorescence enlargement phase.

The characteristics of ‘Carnival’ and ‘Sylvia’ inflorescences, when subtended by the spring flush, were similar. Rate of development was similar and they were harvested within the same time period. The start of spring budbreak of ‘Lady Di’, signalling commencement of inflorescence initiation, was 8 weeks later than ‘Carnival’ and ‘Sylvia’. This, together with the longer period

Fig. 4. Changes occurring during floral meristem development in Protea ‘Carnival’ (scale bar indicates 500 µm). (A) Vegetative meristem prior to flowering is domed and producing leaf primordia. (B) Apical meristem is flat and expanded and producing involucral bracts (ib). (C) Rate of meristem activity increases with rapid production of floral bract initials (i). (D) Later stage of floral bract (fb) initiation, with the surrounding involucral bracts removed. (E) At completion of floral bract initiation the meristem returns to the domed shape. Development of individual florets begins in the outer floral bracts first, which become obscured by hairs on the floret tips. The involucral bracts have been removed. (F) Detail of floret initiation showing gradual stages of development towards the apex (a). A floret primordium (p) develops within each floral bract (fb).
of inflorescence development, resulted in a much later harvest
time (May and June), compared with ‘Carnival’ and ‘Sylvia’,
which were harvested in February and March, despite also being
initiated on the spring flush. In Banksia the 3- to 7-month later
flowering time of B. hookeriana, compared with B. baxteri R. Br.,
was due to both a slightly later time of initiation (November to
January, compared with September to October, respectively) and
a slower initial rate of inflorescence development (Röhl et al.,
1994). Floral initiation of both B. coccinea and B. menziesii R. Br.
occurred in late spring (October to November), but subsequent
inflorescence development was more rapid in B. menziesii, lead-
ing to earlier anthesis (Fuss and Sedgley, 1990). The reason for
later spring budbreak in ‘Lady Di’ is unclear, but may be due to
a photoperiodic requirement which is only fulfilled by the long
days occurring after the September equinox (Southern hemi-
sphere). Vegetative growth in Protea ‘Ivy’ was inhibited under
short day conditions in winter and was stimulated by daylength
continuation with an artificial light source (unpublished results).
Other environmental factors, such as increasing solar radiation
and temperature, could also play a role.

In conclusion, three sinks are active during development of a
shoot growth flush in Protea sp.: growth of preformed leaves,
extension growth of preformed internodes, and formation of new
appendages in the apical meristem. The appendages give rise
either to new leaves for the next flush of shoot growth, or
involucral bracts of the inflorescence. All three cultivars studied
had similar strategies of inflorescence initiation and development
when borne on the spring flush.

**Literature Cited**

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

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

Fulford, R.M. 1966. The morphogenesis of apple buds. III. The inception
of flowers. Ann. Bot. 30:207–219.

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

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

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

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.

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

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

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

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

Malan, D.G., J.G.M. Cutting, and G. Jacobs. 1994. Correlative inhibition
of inflorescence development in Leucospermum ‘Red Sunset’. J. S. Afr.
Soc. Hort. Sci. 4:26–31.

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.

Rieger, M.A. and M. Sedgley. 1996. Effect of daylength and temperature
on flowering of the cut flower species Banksia coccinea and Banksia
hookeriana. Austral. J. Expt. Agr. 36:747–753.

Röhl, L.J., A.M. Fuss, J.A. Dhaliwal, M.G. Webb and B.B. Lamont. 1994.
Investigation of flowering in Banksia baxteri R. Br. and B. hookeriana
Meissner for improving pruning practices. Austral. J. Expt. Agr. 34:1209–
1216.

Verheij, F.A. 1996. Morphological and physiological aspects of the early
phases of flower bud formation in apple. PhD diss. Agr. Univ. Wageningen,
The Netherlands.