The Role of Leaves and Carbohydrates in Flowering of *Protea* cv. Lady Di

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Abstract. Inflorescence initiation in *Protea* cv. Lady Di (*P. magnifica* Link x *P. compacta* R. Br.) occurs predominantly on the spring growth flush when it is subtended by one or more previous growth flushes. Mature, overwintering leaves are essential for induction of flowering in 'Lady Di', and are also crucial to the early stages of inflorescence initiation and differentiation. Defoliation before elongation of the spring growth flush was complete prevented flowering, and shoots either remained vegetative or produced inflorescences that aborted. Levels of carbohydrates in the stem and leaves of overwintering shoots were low, and early growth and development of both the spring flush and inflorescence were, therefore, supported by current photosynthates from the mature leaves on the overwintering shoot. Likewise, reserve carbohydrates available in the flowering shoot were insufficient to account for the rapid increase in dry weight during the major portion of growth of the spring flush and inflorescence. This increase occurred after elongation of the spring flush was complete and was supported by current photosynthates from the leaves of the spring flush. Defoliation treatments that did not prevent inflorescence initiation had no effect on inflorescence development or on flowering time.

Mature leaves on an overwintering shoot are essential for induction of flowering in *Protea* cv. Carnival (*P. compacta* R. Br. x *P. neriifolia* R. Br.) (Gerber, 2000). Induction is complete in late winter, 6–7 weeks before budbreak in spring. After induction the shoot is committed to flowering, although initiation commences only with spring budbreak. Defoliation before induction prevents flowering.

Initiation of inflorescences in 'Carnival' and 'Lady Di' occurs predominantly on the spring flush, when subtended by one or more previous flushes. The spring flush is preformed in the apical bud prior to spring budbreak (Gerber et al., 2001). Growth of the spring flush occurs by elongation of preformed internodes, together with differentiation and growth of leaves. During this phase of rapid growth the apical meristem on an induced shoot produces involucre bracts. Floral bracts and florets are produced after elongation is complete, followed by growth and development of the inflorescence.

Production of new tissues in spring in deciduous plants is supported by the mobilization of reserve carbohydrates stored in the permanent, woody structures until new leaves have developed sufficiently and become net exporters of photosynthates (Priestley, 1962). Carbohydrates in the 2-year-old branches of 'Carnival' were low throughout the year, indicating a poor supply of stored reserves (Greenfield et al., 1995). Carbohydrate content of current season's growth of *Protea* cv. Sylvia [*P. eximia* (Salisb. ex Knight) Fourc x *P. susannae* Phill.] decreased in early spring, although both starch and sugars were present in low concentrations at all times (Hettasch, 1999). Carbohydrate levels were generally low in both *Leucospermum* R.Br. cv. Red Sunset (Napier, 1985) and *Brunia albiflora* 'Pillans' (Poole, 1999), but starch content increased prior to inflorescence initiation. Shading of 'Red Sunset' during shoot growth reduced flowering, suggesting that current photosynthates were necessary for successful inflorescence initiation. Inflorescence development in 'Carnival' was delayed by defoliation (Gerber, 2000). The delay was most obvious following defoliation before spring budbreak, but was still apparent when defoliation was applied after inflorescence initiation had occurred, but before expansion of leaves on the spring flush was complete. Leaf area was reduced by both defoliation of the shoot and by a reduction in the number of new leaves produced (an effect of defoliation).

Shoots of 'Lady Di' were defoliated at different times, starting prior to spring budbreak, to: 1) determine the dependence of growth of the spring flush on stored carbohydrates in the overwintering shoot; 2) ascertain the importance of leaves in inflorescence initiation and development; and 3) assess the possibility of delaying flowering.

Plant material. Six-year-old *Protea* cv. Lady Di plants were grown in a commercial plantation, spaced 1 m in the row and 4 m between rows, clean cultivated, and were not irrigated or fertilized. The climate in the Stellenbosch district (lat. 33°15'S; long. 19°07'E), is Mediterranean, with an annual rainfall of 600–700 mm, falling mainly in winter. Summers are hot and dry.

'Lady Di' plants were pruned in Sept. 1997 for biennial bearing (Gerber et al., 1995) to improve stem length. Two growth flushes were produced in a growing season: a spring flush produced shortly after pruning in September (Spring 1997), followed by a late summer flush in January/February of the following year (Summer 1998). Vegetative growth ceased during winter and continued from the terminal position in Spring 1998. The spring flush arising from the terminal bud was preformed during winter (Gerber et al., 2001). Inflorescence initiation occurred terminally during elongation of the spring flush, followed immediately by development.

Dry weight and carbohydrate changes during production of the spring flush and inflorescence. From late winter until inflorescence maturity, nondefoliated shoots were harvested at the following phenologically determined intervals: pre-spring budbreak (20 Aug. 1998); at spring budbreak and the start of elongation of the new spring flush (1 Oct. 1998, coinciding with the start of production of involucre bracts) (Gerber et al., 2001); when elongation of the spring flush was about half the final length (29 Oct. 1998); when elongation of the spring flush was complete (19 Nov. 1998); when the inflorescence was ≈30 mm in basal diameter (8 Apr. 1999); and when the inflorescence was ready for commercial harvest (14 July 1999).

Shoots consisted of two mature growth flushes (Spring 1997 and Summer 1998) and, after spring budbreak, a third flush (Spring 1998) and a terminal inflorescence. Three shoots were harvested on each date and separated into leaf and stem portions for each flush and the developing inflorescence. Samples were lyophilized and the dry weights recorded before being milled to a fine powder.

Carbohydrate analysis. A 0.5-g sample of dried tissue was taken for carbohydrate analysis. Samples were extracted by shaking overnight in 100 mL 1% acetic acid and then centrifuged. The supernatant was filtered and brought to volume. Thereafter, the pellet was dissolved in an acetate buffer (pH 4.8) and gelatinized in a boiling steambath for 2 h. After cooling to 60 °C, the starch fraction was hydrolyzed to glucose with amyloglucosidase in an incubator for 18 h.

Reducing sugars and starch were further analyzed on a Sanplus Segmented Flow Analysis System from Skalar, using method number 551-965w/r issue 070798/MH and number 356-001w/r issue 012998/MH97203066 (Skalar, Breda, The Netherlands). Carbohydrate (starch and reducing sugars) in tissues is reported as the total content (mg) in a specific tissue.

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Defoliation treatments. Defoliation entailed cutting leaves off at their point of insertion using scissors, five shoots being defoliated on each date. Shoots consisting of two mature growth flushes (Spring 1997 and Summer 1998) were defoliated starting before spring budbreak (20 Aug. 1998), at spring budbreak (1 Oct. 1998), and when the spring flush (Spring 1998) was half its final length (29 Oct. 1998). All leaves were removed from the two mature flushes.

After completion of spring flush elongation (19 Nov. 1998), shoots were defoliated at 2-week intervals until inflorescences were ≈ 30 mm in diameter (8 Apr. 1999). Defoliation treatments applied to these shoots entailed removal of the leaves on the mature flushes, as well as newly formed leaves of the spring flush, except for the 10–12 uppermost new leaves. These leaves were left on the shoot to enable the mature inflorescence to be harvested and marketed. In commercial practice, the lower two-thirds of the leaves on the protea shoot are removed before being packed for marketing. The response to defoliation was assessed in the field in mid-June 1999.

The diameter of developing inflorescences was measured at 2-week intervals until they reached commercial harvest stage, and the date of harvest recorded. Nondefoliated shoots were used for comparison.

When inflorescences were at the stage of commercial harvest, on 14 July 1999, three shoots that were defoliated in February or March were harvested and separated into leaf and stem portions for each flush and the inflorescences. Samples were lyophilized and the dry weights recorded.

Statistical analysis. Data were tested by analysis of variance using the SAS General Linear Model (SAS Institute, 1990), and mean separation was accomplished by either Duncan's multiple range test or least significant difference (LSD), where applicable.

Results

Dry weight and carbohydrate changes. The dry weight of mature tissues remained constant during growth of both new tissues in spring, and the inflorescence through summer (Fig. 1). The total starch content of mature tissues varied from 300 g to >600 g during this period, but differences were nonsignificant (Fig. 2). The sugar content (total reducing sugars) of mature tissues initially increased in spring (Fig. 2), then decreased, corresponding with the later period of elongation of the spring growth flush (Spring 1998). During growth and development of the inflorescence the sugar content of mature tissues remained unchanged.

The dry weight of new, developing tissues increased gradually during elongation of the spring flush (Fig. 1). By the time elongation was complete the dry weight of new spring flush tissues was 15 g, which was only 30% of the dry weight accumulated by 8 Apr. 1999. The major increase occurred after elongation was complete. Inflorescence development during elongation of the spring flush occurred at the microscopic level (Gerber et al., 2001).

Fig. 1. Changes in dry weight in mature and new tissues during development of new tissues of *Protea* cv. Lady Di in 1998–99. Mature tissues (stem and leaves) are represented by filled triangles, new tissues (stem and leaves) by open circles, and the inflorescence by open triangles. Spring budbreak occurred on 1 Oct. 1998, and spring flush elongation continued until 19 Nov. 1998 (indicated by dotted lines). Mean separation within tissues by Duncan's multiple range test, $P \leq 0.05$.

Fig. 2. Carbohydrate changes in mature tissues during development of new tissues of *Protea* cv. Lady Di in 1998–99. Total reducing sugar content of mature tissues (stem and leaves) is represented by open squares, and total starch content is represented by filled circles. Spring budbreak occurred on 1 Oct. 1998, and spring flush elongation continued until 19 Nov. 1998 (indicated by dotted lines). Mean separation within tissues by Duncan's multiple range test, $P \leq 0.05$.

The major increase in dry weight of the inflorescence occurred only following dry weight accumulation of the developing spring flush. Dry weight of the spring flush did not increase further during the rapid accumulation of dry weight in the inflorescence occurring from 8 Apr. 1999 to anthesis on 14 July 1999.

Defoliation. Overwintering shoots defoliated before or at spring budbreak (29 Oct. 1998) did not produce flowers. These shoots produced either a terminal vegetative flush, or a flower bud which aborted early, or ceased further growth entirely (Table 1). When shoots were defoliated before elongation of the spring flush was complete (before 19 Nov. 1998), flowering was still negatively affected, but some shoots produced inflorescences that developed through to anthesis.

Removal of all mature leaves and 75% of new leaves after elongation of the spring flush was complete (after 19 Nov. 1998) had no effect on flowering. All shoots initiated inflorescences, and development occurred at a similar rate, with all inflorescences reaching com-

mercial harvest stage within a 6-week period (Fig. 3).

On shoots defoliated after completion of the spring flush, only the top 20% to 25% of the leaves on the spring flush remained [10–12 out of the normal complement of ≈ 47 leaves (Gerber et al., 2001)]. The leaves that remained following defoliation in February and March supported normal accumulation of dry weight by the inflorescence (data not shown). The dry weights of neither mature nor new stem tissues were significantly affected by defoliation, and levels were similar to those in nondefoliated shoots.

Discussion

Nonstructural carbohydrates contribute a small proportion to the dry weight of flowering shoots of 'Lady Di'. New spring growth was supported by new photosynthates produced by mature leaves on the overwintering shoot. The increase in dry weight of the new spring flush (≈ 50 g) resulted predominantly

from current photosynthesis, since the starch content of mature tissues was low and remained unchanged, and the small change in reducing sugars (from a high of 3 g on 29 Oct. 1998 to 1 g on 19 Nov. 1998) could not account for the increase. Greenfield et al. (1995) reported low levels of carbohydrate in the 2-year-old structural branches of 'Carnival', so new growth was probably not supported by reserves stored in the permanent parts of the plant, as in deciduous trees.

Inflorescence initiation did not occur in 'Lady Di' shoots that were defoliated before or at spring budbreak. In 'Carnival', flowering was prevented only when defoliation was done earlier than 6–7 weeks before spring budbreak (Gerber, 2000). The presence of mature, overwintering leaves was considered essential for inflorescence initiation in 'Carnival'. It was concluded that environmental factors probably play an inductive role and that the carbohydrate status of the shoot, while making a small contribution to tissue growth, does not play a definitive role in inflorescence initiation. Mature leaves that had overwintered appeared to be essential for inflorescence initiation in 'Lady Di', as seen by the failure to initiate on the late summer flush, and the inductive factors may be the same as in 'Carnival'. Unlike 'Carnival', however, where inflorescence initiation (once induced) can occur in the absence of mature leaves on a shoot, successful inflorescence initiation in 'Lady Di' requires the continuous presence of leaves. Defoliation of 'Lady Di' no longer affected flowering when applied after the leaves on the spring flush were expanded.

Unlike 'Carnival', defoliation of 'Lady Di' shoots caused some flower abortion. Defoliation before or at spring budbreak either prevented inflorescence initiation or caused reversion of the meristem to the vegetative state. Such reversion can occur in *P. cynaroides* L., even after the production of numerous involucre bracts (personal observation). Defoliation of 'Lady Di' after inflorescence initiation, but before differentiation of the involucre bracts was complete (29 Oct. 1998), caused flower bud abortion at an early stage of development in some shoots, indicating that leaves on the overwintering shoot are necessary for sustained differentiation of the inflorescence.

Bud abortion also occurred when shoots were totally defoliated after completion of elongation of the spring flush (unpublished results). All leaves on the shoot, including the newly formed spring flush leaves, were removed, starting 4 weeks after completion of spring flush elongation in 1996 and continuing until the inflorescence was macroscopically visible. All shoots had initiated inflorescences and undergone a degree of differentiation before defoliation, and all aborted. Therefore, current photosynthates are essential for inflorescence development.

Inflorescence development in 'Lady Di' continued unimpeded until anthesis, when shoots were partially defoliated after spring flush growth, leaving only the uppermost 10–12 leaves of the newly formed spring flush. An increase in photosynthesis in remaining leaves

Table 1. Effects of defoliation on vegetative growth and flowering of *Protea* cv. Lady Di. Spring budbreak occurred on 1 Oct. 1998, and elongation of the spring flush was complete on 19 Nov. 1998. All mature leaves were removed from shoots defoliated before completion of the spring flush. Shoots defoliated later had all mature leaves removed, plus the lower three-quarters of new leaves on the spring flush (+ indicates leaf removal; – indicates leaves remaining). Response to defoliation was assessed in the field in mid-June 1999.

Date of defoliation (1998–99)	Leaves removed		No. shoots observed ^z	Vegetative meristems		Reproductive meristems	
	Mature	New		Quiescent	Active	Aborted	Anthesis
20 Aug.	+	–	3	3			
1 Oct.	+	–	4	2	2		
29 Oct.	+	–	4	2		1	1
19 Nov.	+	+	4				4
3 Dec.	+	+	3				3
17 Dec.	+	+	4				4
31 Dec.	+	+	5				5
13 Jan.	+	+	5				5
27 Jan.	+	+	4				4
11 Feb.	+	+	5				5
25 Feb.	+	+	5				5
11 Mar.	+	+	5				5
25 Mar.	+	+	5				5
8 Apr.	+	+	5				5
Nondefoliated	–	–	5				5

^zFive shoots were defoliated on each date. Where less than five shoots are reported, shoots were mechanically damaged during normal cultural practices.

Fig. 3. Basal diameter of *Protea* cv. Lady Di inflorescences during development in 1998–99 (mean of 50 shoots for each date, including defoliated and nondefoliated shoots). Inflorescences reached anthesis within the 6-week period marked by vertical lines.

has been described as a compensatory mechanism to overcome reduced leaf area (Bhatt and Srinivasa Rao, 1993; Meyer, 1998). The rate of inflorescence development of 'Lady Di' was not affected by partial defoliation, suggesting that the 10–12 new leaves remaining were capable of sustaining normal inflorescence development.

In conclusion, the presence of mature, overwintering leaves is essential for inflorescence initiation in 'Lady Di', and also for the early stages of inflorescence differentiation. Low reserve carbohydrate content of mature tissues indicates that new spring growth is supported by current photosynthesis. Partial defoliation does not delay flowering in 'Lady Di', as the remaining leaves can support normal growth, presumably by an increase in photosynthetic efficiency.

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