

DECLARATION

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

SUMMARY

The high establishment cost of growing proteas under intensive cultivation has necessitated young plant management to attain an economically viable crop as soon as possible after planting. Rooted cuttings of *Protea* cv. Sylvia (*P. eximia* x *P. susannae*) were planted and shoots were pinched and topped at various growth stages to increase the vegetative complexity of the plants during the first growing season. During the second growing season, one-year-old plants of *Protea* cv. Sylvia were pruned in early spring, and the resultant regrowth thinned to between three and six shoots per bearer or left unthinned. Plants which were not pinched or topped during the first growing season, grew a single stem; pinching after the spring flush resulted in an average of 4.34 shoots forming while not pinching the spring flush, but topping after the summer flush resulted in an average of 6.82 shoots forming by late summer. Pinching both the spring and summer flushes resulted in an average of 8.84 shoots per plant. One-year-old Sylvania plants, pruned in early spring, produced an average of 5.5 shoots per bearer. Where all the shoots were left, some became dominant and the remainder became weak, spindly, non-flowering shoots. Thinning the sprouts on the bearers in the spring to 3, 4 or 5 shoots per bearer, did not result in longer shoots or a higher percentage of flowering shoots than leaving 6 shoots per bearer.

Whole shoots of *Protea* cv. Sylvania (*P. eximia* x *P. susannae*) and *Protea* cv. Cardinal (*P. eximia* x *P. susannae*) plants were sampled at the conclusion of each successive growth flush over a period of one growing season. The dry mass of leaves and stems of each flush was measured separately and a sample analysed for total sugars and starch concentrations. Total dry mass of both stems and leaves of 'Sylvia' and 'Cardinal' remained similar during growth of the spring and first summer flushes and then increased significantly during the formation of the second summer flush and the autumn flush. The concentration of carbohydrates (total sugars and starch combined) in the leaves and stems of the whole shoot, increased significantly from one flush to the next, up to and including the second summer flush. The concentration of carbohydrates in the leaves of the whole

shoot was significantly reduced after the winter, while in the stems the concentration remained similar. Total sugars and starch concentrations in the leaves of both 'Sylvia' and 'Cardinal' were significantly higher than in the stems on all flushes.

The dry mass, concentration of starch and concentration of total sugars of different plant parts of *Protea* cv. Sylvia were measured at two sampling dates. Plants were grown for two years in 10l bags in a nursery. During July and September, whole plants were sampled, dissected into leaves, shoots, stem (trunk) and roots and laboratory analysed for dry mass, starch and sugars. The two sampling dates corresponded to the end of active vegetative shoot growth in early winter and its resumption in spring. In all plant parts monitored, dry mass did not increase significantly from one sampling date to the next. Tissue content of both total sugars and starch in the leaves represented half the amount of the total plant at both sampling dates. Significant increases in the concentration and tissue content of total sugars were noted in the leaves, shoots and roots between the first and second sampling dates. In the case of starch a significant increase in concentration and tissue content was noted in the leaves and a decrease in the stem.

STUDIES VAN DIE VEGETATIEWE ONTWIKELING VAN *PROTEA* CV. 'SYLVIA' EN 'CARDINAL'.

OPSOMMING

Die hoë vestigings kostes betrokke by die intensiewe verbouing van proteas noodsaak jong plant bestuur om 'n ekonomies betekenisvolle oes te behaal so gou moontlik na plant. Bewortelde steggies van *Protea* cv. *Sylvia* (*P. eximia* x *P. susannae*) is geplant en die lote se groeipunte is op verskillende groei stadia getop of terug gesny om vegetatiewe kompleksiteit van die plante in die eerste groeiseisoen te verhoog. Gedurende die tweede groeiseisoen is een-jaar-oue plante van *Protea* cv. *Sylvia* in die vroeë lente gesnoei en die nuwe groei is dan uitgedun na tussen drie en ses lote per draer, of dit is ongedun gelos. Plante wat nie getop of terug gesny was in die eerste groei seisoen nie, het 'n enkele loot geproduseer; lote wat na die lente groeistuwing getop is het 'n gemiddeld van 4.34 lote geproduseer terwyl lote waar die lente groeistuwing nie getop is nie, maar wat wel terug gesny is na die somer groeistuwing het gemiddeld 6.82 lote geproduseer teen laat somer. Waar beide die lente en somer groeistuwings getop is, het die plante gemiddeld 8.84 lote geproduseer. Een-jaar-oue *Sylvia* plante wat in die vroeë lente gesnoei is het gemiddeld 5.5 lote per draer geproduseer. Waar al dié lote behou is, het sommige lote dominant geraak en ander was swak, nie blommende lote. Uitdun van die knoppe in die vroeë lente na 3, 4 of 5 per draer, het nie gelei tot langer lote of 'n hoër persentasie blommende lote as waar 6 lote gelos is nie.

Intakte lote van *Protea* cv. *Sylvia* (*P. eximia* x *P. susannae*) en *Protea* cv. *Cardinal* (*P. eximia* x *P. susannae*) plante is versamel aan die einde van elke opeenvolgende groeistuwing oor 'n periode van een groei seisoen. Die droë massa van die blare en stele van elke groeistuwing is apart gemeet en 'n monster is geanaliseer vir totale suiker en stysel konsentrasie. Totale droë massa van beide stele en blare van 'Sylvia' en 'Cardinal' het eeners gebly gedurende die lente en eerste somer groeistuwings en het dan betekenisvol toegeneem met die ontwikkeling van die tweede somer groeistuwing en die herfs groeistuwing. Die konsentrasie koolhidrate (totale suikers en stysel gekombineer)

in die blare en stele van intakte lote het betekenisvol toeneem van een groeistuwning na die volgende, tot en met die tweede somer groeistuwning. Die konsentrasie van koolhidrate in die blare van die hele lote was betekenisvol minder in die winter, terwyl die konsentrasie in die stele dieselfde gebly het. Totale suikers en stysel in die blare van beide 'Sylvia' en 'Cardinal' was by al die flushes betekenisvol hoër as in die stele.

Die droeë massa, konsentrasie stysel en konsentrasie totale suiker van die verskillende plant dele van *Protea* cv. Sylvia is bepaal op twee datums. Plante is twee jaar lank gekweek in 10l sakke in 'n kwekery. Gedurende Julie en September, is intakte plante versamel, opgedeel in blare, stele, stam en wortels en geanaliseer vir droeë massa, stysel en suikers. Die twee datums het ooreengestem met die einde van aktiewe vegetatiewe lootgroeï in die vroeë winter en die begin van lootgroeï in die lente. In al die plant dele wat gemonitor is het droeë massa nie betekenisvol toeneem tussen die twee datums nie. Die inhoud van beide totale suikers en stysel in die blare het die helfde van die totale hoeveelheid in die plant verteenwoordig by albei datums. Betekenisvolle toenames is getoon in die konsentrasie en totale inhoud van totale suikers in die blare, stele en wortels tussen die eerste en die tweede datums. In die geval van stysel is 'n betekenisvolle toename in die konsentrasie en totale inhoud van blare opgemerk, asook 'n afname in die stam.

**To my parents,
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2. DEVELOPING VEGETATIVE COMPLEXITY, DRY MASS ACCUMULATION AND CARBOHYDRATE ALLOCATION IN WOODY PERENNIALS WITH SPECIAL REFERENCE TO *PROTEA*

2.1 INTRODUCTION

Very little has been written on the topics under discussion for Proteaceae in general, not to mention *Protea* cv. *Sylvia* in particular. Although some general guidelines do exist for the pruning of young and adult Proteaceae plants (Malan, 1995), none address the issue of young plant complexity building directly. More work has been done on carbohydrate levels in Proteaceae plants, but usually involving the reproductive phase, with reference to post-harvest leaf blackening (Newman *et al.*, 1990; Paull & Daie, 1990; McConchie & Lang, 1991; Bieleski *et al.*, 1992; McConchie & Lang, 1993a & b; Dai & Paull, 1995). Greenfield *et al.* (1995) report on reserve carbohydrate levels in bark and wood of two-year-old shoots of *Protea* cv. *Carnival* over a 12 month period.

The economic importance of Proteaceae as cutflowers for export is increasing, especially for flowers produced in high intensity cultivation, due to the superior quality of these products compared to those picked from the wild. High intensity cultivation is however extremely capital intensive and early returns are essential for this approach to be economically viable. Thus, cultural practices which contribute to the viability of growing *Protea* under intensive cultivation are of great importance. Knowledge of physiological activities in the plant such as sink-source relationships, dry mass accumulation and carbohydrate allocation can contribute significantly to understanding responses to manipulations and determining the best possible cultural procedures.

2.2 ECONOMIC IMPORTANCE OF *PROTEA* CULTIVAR SYLVIA

Fresh cutflower exports of Proteaceae and related families (Fynbos) has increased steadily over the last five years, as can be seen in Table 1.

Table 1. Fresh cutflower exports of Fynbos 1993 - 1998

YEAR	KG EXPORTED	%INCREASE
1993	2 427 156	
1994	2 476 363	+ 2.02%
1995	2 861 212	+15.50%
1996	3 300 266	+15.35%
1997	3 570 838	+ 8.20%
1998	3 666 349	+ 2.67%

Source: SAPPEX (Unpublished)

The fresh cutflower exports of Fynbos produced a net foreign currency income of R 60 million for the industry in 1998 (SAPPEX, Unpublished).

With the general move away from pure specie production from seedling plants to the use of hybrids and clonal cutting plants, *Protea* cv. Sylvia has become the single most important *Protea* hybrid being produced in South Africa (SAPPEX, Unpublished), making up almost 25% of the total *Protea* hybrid flower production. Both *Protea* cv. Sylvia and *Protea* cv. Cardinal are hybrids between *P. eximia* (Salisb. ex Knight) Fourcade and *P. susannae* (E.P. Phillips). Table 2 shows how 'Sylvia' and 'Cardinal' have grown from "Not specified" in the 1993/4 survey to 40% of all *Protea* hybrids produced in the 1997/8 season.

Table 2. Relative importance of some important Proteas in the South African protea cutflower industry (1993/4 vs. 1997/8).

PRODUCT	NUMBER OF STEMS EXPORTED	
	1993/4	1997/8
Total Protea Hybrids	471 239	450 379
Protea cv 'Sylvia'	Not specified	107 737
Protea cv 'Cardinal'	Not specified	73 902

Source: Hettasch *et al.* (unpublished) and SAPPEX (unpublished)

One of the main reasons for the popularity of 'Sylvia' as a *Protea* variety for cultivation is the fact that its flowering time can be manipulated (Gerber, 1999), making it one of the few *Protea* varieties which flower during the desired marketing time from September to January. This variety has also gained popularity in other protea growing countries, for example Zimbabwe (Archer, pers. comm.).

2.3 DEVELOPING VEGETATIVE COMPLEXITY

A balance between high production and quality (shoot length) can be achieved through correct complexity management. Brits *et al.* (1986) reported that in *Protea*, thinning and heading must be balanced to produce the correct number of stems. Too much thinning could result in under-production and too much heading in the formation of too many, too short, lateral shoots. Malan (1995) mentions that in young *Protea* cutting plants "growth should be headed back by removing only the tip of the growing section". Later work was done by Allemand *et al.* (1995), Allemand *et al.* (1997) and Montarone *et al.* (1997), on the architectural structure of *Protea eximia* and *Protea cynaroides* in soilless culture.

2.4 DRY MATTER PARTITIONING IN WOODY PERENNIALS

Detailed studies of dry matter production and partitioning have been done in many crops, including apple (Heim *et al.*, 1979; Young & Warner, 1984), citrus (Koch *et al.*, 1986) and avocado (Scholefield *et al.*, 1985). Brouwer (1962) discusses the physiology of growth correlations between various plant organs. Gerber *et al.* (1995) made use of pruning techniques to shift dry matter partitioning and thereby optimise economic biomass production of *Protea* cv. Carnival.

2.5 CARBOHYDRATE ALLOCATION IN WOODY PERENNIALS

2.5.1 Importance of carbohydrates in woody plants

Carbohydrates, the direct products of photosynthesis, are the primary energy storage compounds in plants and are the building blocks for other organic compounds synthesised in the plants. Uses of carbohydrates found in plants include: forming part of cell walls; used in synthesis of fats and proteins; are the energy source for respiration and serve as storage of reserves. Altogether, carbohydrates can make up as much as 75% of the dry mass of woody plants (Kramer & Kozlowski, 1979; Oliviera & Priestly, 1988).

2.5.2 Kinds of carbohydrates

Principally carbohydrates consist of carbon, hydrogen and oxygen, but may also contain phosphorus or nitrogen. Depending on the complexity of the molecules, they are categorised as monosaccharides, oligosaccharides and polysaccharides. Monosaccharides are the simple sugars and their derivatives e.g. glucose and fructose, oligosaccharides are complexes of two or more monosaccharides, joined by glycosidic linkages, e.g. sucrose and polysaccharides are long chains of monosaccharides e.g. starch and cellulose.

Starch and sucrose represent the majority of reserve carbohydrates in most plants. Sucrose is considered the most important disaccharide in most plants, followed by

maltose. Sucrose occurs in high concentration in the cells and in most plants makes up 95% of the translocated material in the sieve tubes of the phloem (Kramer & Kozlowski, 1979; Oliviera & Priestly, 1988). Work done by Boeyens *et al.* (1983) showed that in certain *Protea* species the dominant sugar moiety is D-glucose.

Starch serves as the major reserve for plants and starch grains in cells cannot move from cell to cell, but must be degraded enzymatically to glucose for use in metabolic processes for transfer to other parts of the plant. In woody plants starch grains are found in the living parenchyma cells of sapwood, in the phloem cells of the bark and in the leaves.

2.5.3 Uses of carbohydrates

Carbohydrates produced by the process of photosynthesis are used in growth, food storage, and respiration. Different parts of the plant serve as carbohydrate sinks or sources over time. Carbohydrates are generally mobilised from parts of the plant closest to the sink organ first. The rate of carbohydrate production and transfer to other parts varies continuously depending on sink-source relations which are related to growth rates of the various plant organs and hormonal levels. Carbohydrates are produced primarily in the actively photosynthetic leaves, although chlorophyll containing stems and other organs also contribute limited amounts of photosynthates (Kozlowski, 1992; Daie, 1985; Gifford & Evans, 1981). Sink strength is a combination of net carbon gain vs. respiratory carbon loss (Kozlowski, 1992). Partitioning of carbohydrates in leaves is controlled hormonally by auxins, gibberellins and cytokinins which can increase sink activity and abscisic acid, which may increase or decrease sink activity (Brenner *et al.*, 1989; Kozlowski, 1992).

The accumulation of carbohydrates is especially important in perennial plants because the carbohydrates accumulated in the growing season are used for respiration during the winter and resumption of growth in the spring. In deciduous trees carbohydrates are stored in the leaves during spring and summer, but are transferred to the permanent structure before leaf drop (Oliviera & Priestly, 1988). Evergreens store the majority of their carbohydrates in their leaves, from where they are transferred for growth and

metabolism throughout the plant (Kozłowski, 1992). In *Leucospermum*, Napier (1985) found that levels of reducing sugars in the leaves were significantly higher than in the shoots throughout the year. Greenfield *et al.* (1995) recorded relatively low levels of reducing sugars and starch in the wood and bark of two-year-old shoots of *Protea* cv. Carnival.

Starch was found by Kozłowski (1992) to be the predominant form of carbohydrate in roots in deciduous trees, which accumulate there during autumn and winter and are mobilised in the spring. Borrás *et al.* (1984) reported higher levels of sugars than starch in the roots of Washington Navel throughout the year, although leaf levels of both sugars and starch were relatively much higher.

When studying carbohydrate accumulation in plants it is important to consider both the concentration (percentage of dry mass) and the total amount of carbohydrates in different plant parts. The concentration of carbohydrates in certain parts may be high, but due to the relatively small dry mass component, they do not constitute an important source.

Although storage of carbohydrates is more critical in deciduous plants, reserve carbohydrates also play an important role in evergreens. Some early root growth may depend largely on reserve carbohydrates and early season growth may depend on late season storage (Kramer & Kozłowski, 1979; Kozłowski, 1992; Oliviera & Priestly, 1988).

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PAPER 1: DEVELOPING VEGETATIVE COMPLEXITY IN YOUNG *PROTEA* CV. SYLVIA PLANTS DURING THE ESTABLISHMENT YEARS

INTRODUCTION

Growing proteas using intensive cultivation practices, results in high establishment costs and young plant management is essential to attain economically viable returns as soon as possible after planting. Young protea plants are usually planted from late autumn through to early spring from rooted cuttings (Malan & De Kock, 1995). When terminal cutting are used, a single shoot arises from the terminal bud in spring (Malan, 1995). If plants are left unmanipulated, these single shoots continue to grow terminally and may branch naturally, especially later in the growing season (Fig. 1).

At the end of the first growing season, these single shoots must then be cut back to allow multiple side shoots to develop, forming the production framework of the plant. Often stems of such unmanipulated plants are thick and woody after a season of growth and the severe pruning required to form the plant can be fatal. Plants with long single shoots, sometimes bearing a flower or multiple side shoots high up on the stem are extremely susceptible to wind damage as depicted in Fig. 2.

The desired plant shape for extended economic life of the plant is shown in Fig. 3, where vegetative complexity is attained early and the framework of the plant is low to the ground. This reduces the chances of wind damage to the plant and by starting the first harvesting tier low, the potential number of harvests before the plant becomes unmanageable is increased.

By planting sub-terminal cuttings and pinching or cutting back successive growth flushes, during the first season of growth, a high degree of vegetative complexity can be achieved. A number of shoots can then be chosen as bearers to carry the shoots of the first crop (Fig. 4). These bearers are not as thick and woody as in the case of unmanipulated plants and the probability of pruning mortality is greatly reduced.

In late winter or early spring, one year after planting, shoots which were produced during the first growing season are topped to become bearers. Numerous shoots develop from these bearers during the spring of the second growing season. In *Leucospermum*, it is generally accepted that the more shoots produced on a bearer, the shorter the shoots will be, but all shoots will flower (Brits *et al.*, 1986). In this trial with *Protea* cv. *Sylvia*, different numbers of shoots were left per bearer and the resultant growth measured and the percentage of shoots forming flowers noted.

MATERIALS AND METHODS

Plant material

Young, rooted cutting plants of *Protea* cv. *Sylvia*, a hybrid between *P. eximia* (Salisb. ex Knight) Fourcade and *P. susanna* (E.P. Phillips) were planted in mid-August 1996 in a commercial plantation in Elgin, Cape Province, South Africa (34°09' S, altitude 340 m). The area has a Mediterranean climate with a mean annual rainfall of 960 mm. Plants were spaced 1 m in the row and 4 m between rows and irrigated, fertilised and sprayed according to commercial standards.

Establishment year complexity

Sub-terminal rooted cuttings of similar quality were selected from the nursery and planted in August 1996. Four treatments with six replications per treatment were used in a randomised complete block design. The treatments consisted of pinching the spring flush (mid-November) which developed from the rooted cuttings after establishment, cutting back to the intercalation between the spring flush and the summer flush after

cessation of the summer flush (mid-January), pinching both the spring flush and the resultant summer flush (mid-January) and no treatment, in the case of the control.

The number of shoots formed by the end of the first growing season were counted. In most cases only one axillary bud developed from the cutting in spring, however, some of the cuttings produced two or even three shoots during the initial spring flush. The number of shoots resulting directly from the rooted cutting plant in the spring was used as a covariate in the statistical analysis.

Complexity management in the second growing season

Shoots of one-year-old plants (planted in August 1995) were pruned by topping to form bearers in August of 1996. Between four and six strong shoots per plant were chosen to become bearers, while the other shoots were removed by thinning cuts. Five treatments with six replications per treatment were used in a randomised complete block design. Four of the treatments consisted of thinning the resultant sprouting from the bearers to the three, four, five or six most terminal sprouts on the bearers, shortly after sprouting. The fifth treatment was the control treatments, in which all the sprouts on the bearers were retained.

The number of shoots were reduced on all the bearers on the plants to the number required by the treatment, to eliminate intra-plant interactions. The final shoot length, the percentage of shoots with only one or two flushes and the percentage of non-flowering shoots, of all the shoots on all the bearers on the plants were measured and an average per replication calculated.

Statistical analysis

The General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) was used to analyse the data (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

Establishment year complexity

The average number of shoots formed by rooted cutting plants during the first growing season after planting with no complexity manipulation was 1.16 (Table 1). Pinching once, pinching twice or cutting back after the summer flush all produced significantly higher numbers of shoots per plant than the control during the same time. Cutting back to the intercalation between the spring flush and the summer flush after cessation of the summer flush, did not produce a statistically significant higher number of shoots than pinching both the spring flush and the resulting summer flush (Table 1). Thus, either of these treatments can be used to attain maximum vegetative complexity during the first growing season of 'Sylvia' plants.

Complexity management in the second growing season

The average shoot length between treatments did not differ significantly, although the contrast between shoot lengths of the control and the treatments differed at a significance level of 11% (Table 2). Thus, thinning the resultant sprouts on the bearers to fewer than six shoots per bearer did not result in improved average shoot length. In the control treatments, all having in excess of six shoots per bearer, and as many as nine shoots per bearer, 38.2% of all shoots produced only one or two growth flushes. This contrasts significantly with all the treatments in which the number of shoots per bearer were thinned, in which case only between 9.7% and 15.0% of shoots produced only one or two flushes (Table 3). The percentage of shoots which did not initiate flowers after one full growing season is shown in Table 3. The control treatments, where the sprouts on the bearers were not thinned, resulted in a significantly higher percentage of non-flowering shoots. The different thinning treatments did not differ significantly in their percentage of non-flowering shoots. Thus, the treatment in which the shoots were thinned to six per bearer was the most productive in terms of flowering stems of marketable quality. Having a high percentage of flowering shoots also leads to greater uniformity in the plant and a synchronisation of the vegetative and reproductive cycles. The synchronisation of

Protea cv. Carnival and the resultant higher percentage of flowering shoots is well documented both by Gerber *et al.* (1995) and by Hettasch *et al.* (1997).

Planting sub-terminal cuttings and pinching both the spring and summer flushes, or cutting back to the spring flush after completion of the summer flush, produced a plant, after one growing season, from which the desired number of bearers could be chosen, giving a framework of bearers close to the ground. Thinning the resultant sprouting on the bearers at the beginning of the second growing season to six shoots per bearer gave the highest number of flowering stems without compromising the stem length.

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Table 1. Number of shoots produced by *Protea* cv. *Sylvia* rooted cuttings during the establishment year, using different strategies for development of vegetative complexity.

Treatment	Number of Shoots
Control	1.16
Pinch Spring Flush	4.34
Cut back to Spring Flush	6.82
Pinch Spring and Summer Flush	8.84

Contrasts	DF	Significance
Between Treatments	3	0.001
Control vs. Treatments	1	0.001
Cut Back vs. Double Pinch	1	0.1118

Table 2. The effect of number of shoots per bearer on the average shoot length (cm) of *Protea* cv. *Sylvia*

No. Shoots per Bearer	Average Length (cm)
3	48.17
4	52.83
5	49.33
6	54.17
Control	44.83

Contrasts	DF	Significance
Between Treatments	4	0.3335
Control vs. Treatments	1	0.1107

Table 3. The effect of the number of sprouts per bearer on percentage of shoots with only 1 or 2 flushes and percentage non-flowering shoots.

No. Shoots per Bearer	% Shoots with only 1 or 2 flushes	% Non-flowering shoots
3	14.8 ^b	7.8 ^b
4	12.2 ^b	12.2 ^b
5	15.0 ^b	18.8 ^b
6	9.7 ^b	14.0 ^b
Control (>6 shoots)	38.2 ^a	36.2 ^a
L.S.D. (0.05)	18.826	16.805

Contrasts	DF	Significance	Significance
Between treatments	4	0.0306	0.0182
Treatment vs. Control	1	0.0018	0.0015



Figure 1. 18-month-old, unmanipulated, *Protea* cv. *Sylvia* plant, showing thick woody stem and natural branching high on the stem.

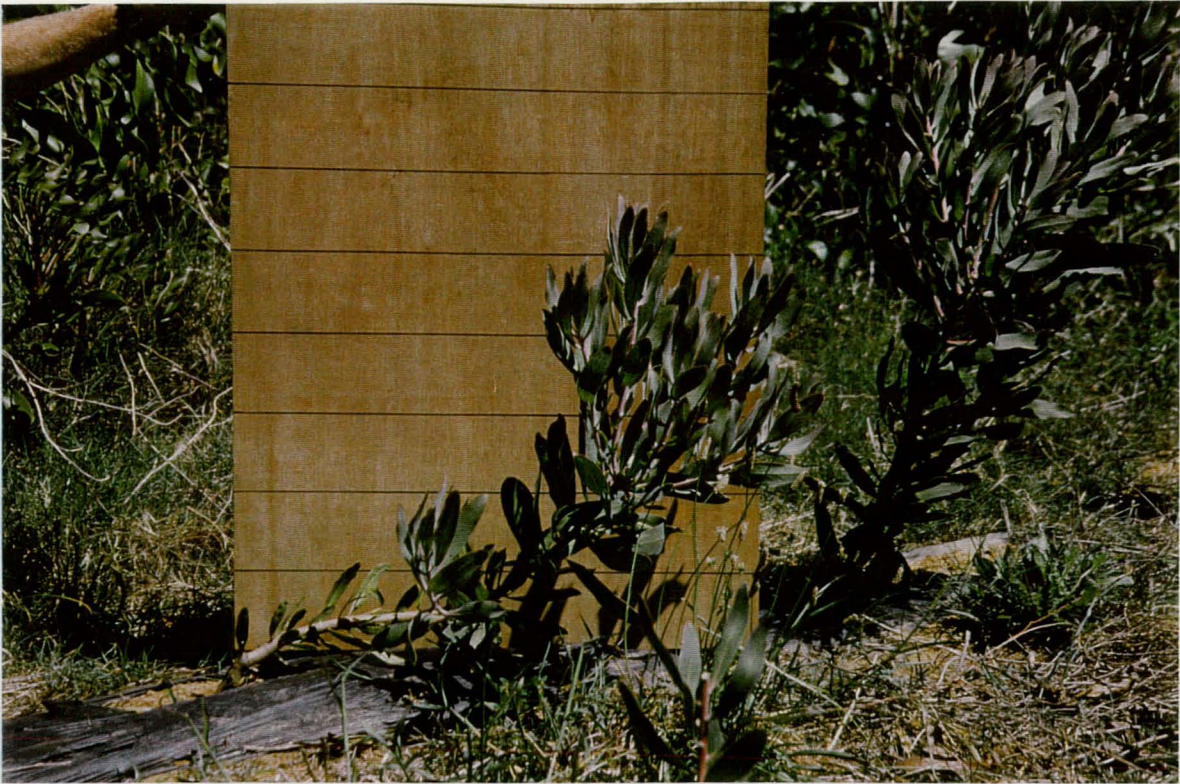


Figure 2. 18-month-old, unmanipulated, *Protea* cv. *Sylvia* plant, showing wind damage.



Figure 3. Framework of bearers near the ground level on a 12-month-old, complexity manipulated, *Protea* cv. *Sylvia* plant.



Figure 4. Complexity manipulated *Protea* cv. *Sylvia* plant 18 months after planting.

PAPER 2: DRY MASS ACCUMULATION AND CARBOHYDRATE ALLOCATION IN SUCCESSIVE GROWTH FLUSHES OF *PROTEA* CV. SYLVIA AND *PROTEA* CV. CARDINAL SHOOTS

INTRODUCTION

Protea cv. Sylvia (*P. eximia* x *P. susannae*) and *Protea* cv. Cardinal (*P. eximia* x *P. susannae*) plants produce three to four vegetative flushes during a growing season under normal climatic and growing conditions in the Western Cape. These flushes are referred to as the spring flush, first summer flush, second summer flush and autumn flush.

The aim of this paper is to strengthen our understanding of the vegetative growth dynamics over a season in terms of dry mass accumulation and carbohydrate allocation. We report on the quantitative increase in dry mass of each vegetative shoot flush, measured just prior to the start of the following flush. The levels of total reducing sugars and starch of each of the vegetative shoot flushes was measured at the same time to determine the carbohydrate allocation between the successive flushes.

The patterns of dry mass accumulation and carbohydrate allocation of 'Sylvia' and 'Cardinal' were very similar, although the vigour of the 'Cardinal' plants was slightly less than that of the 'Sylvia' plants, leading to lower total values. This paper will focus on the 'Sylvia' data and refer to the 'Cardinal' data only where significant differences occurred.

MATERIALS AND METHODS

Plant material

Experiments were done on one-year-old *Protea* cv. *Sylvia* and *Protea* cv. *Cardinal*, both hybrids between *P. eximia* (Salisb. ex Knight) and *P. susannae* (E.P. Phillips), plants in a commercial plantation in Elgin, Cape Province, South Africa (34°09' S, altitude 340 m). The area has a Mediterranean climate with a mean annual rainfall of 960 mm. Plants were spaced 1 m in the row and 4 m between rows and irrigated, fertilized and sprayed according to commercial standards. The shoots on the plants were pruned to between 4 and 6 bearers during August of 1996 and the subsequent shoot growth from these bearers, during the 1996/97 growing season, was analysed.

Treatments and experimental design

Four sampling dates (treatments) with five replications per sampling date were laid out in a randomised complete block design for each variety. Each treatment and replication was on a separate plant so as to eliminate intra-plant interactions due to shoot removal. Whole shoots were removed with a thinning cut just prior to the start of the first summer flush (10 December 1996), the second summer flush (12 February 1997), the autumn flush (3 April 1997) or spring bud break (19 September 1997).

Shoots were divided into their separate flushes at the intercalation and leaves removed from the stems.

Dry mass and carbohydrate analysis

Stems and leaves were placed separately in paper bags, lyophilised and milled to a fine powder to determine the total dry mass of each treatment. A sample was then taken for laboratory analysis of total sugars and starch. Each sample was extracted overnight in 1% acetic acid by shaking and thereafter centrifuged. The supernatant was filtered and brought to volume. Thereafter the pellet was taken up in an acetate buffer (pH 4.8) and gelatinised in a boiling steam bath for two hours. After cooling to 60°C, the enzyme amyloglucosidase was added and incubated for 18 hours facilitating the hydrolysis of starch to glucose. Further analysis for reducing sugars and starch (glucose) were done on a Sanplus Segmented Flow Analysis System from Skalar using Method No. 551-

965w/r issue 070798/MH and No. 365-001w/r issue 012998/MH/97203066.

For the automated determination of reducing sugars the sample was mixed and dialysed against a sodium carbonate solution. After addition of a copper neocuprion reagent, the steam was heated to 97°C. The copper neocuprion chelate was reduced by the present sugars, forming a yellow cupro-neocuprion complex which was measured colorimetrically at 460 nm. To include non-reducing sugars the sample extract was hydrolysed with β -fructosidase prior to the above procedure.

The automated determination of glucose is based on an enzymatic reaction whereby the glucose in the sample is oxidised to gluconic acid by glucose oxidase. Hydrogen peroxide, which is formed simultaneously reacts with an indicator reagent (peroxidase hydroxybensoate-4-amino-phenazone) to form a stable red quinone compound which is then measured colorimetrically at 520 nm. The intensity of the absorption is directly proportional to the concentration of d-glucose in the sample.

Statistical analysis

The General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) was used to analyse the data (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

Dry mass

Fig. 1a and 1b show the dry mass accumulation of leaves and shoots of 'Sylvia' and 'Cardinal' over time. During the formation of the first two flushes very little dry matter is accumulated and of this, the majority is incorporated in the leaves. Thereafter, with the formation of the second summer flush, the total dry mass of both the leaves and the stems increased dramatically with a further large increase in accumulation during the formation of the autumn flush and during winter until the beginning of the new spring flush. By the last sampling date in September, the contribution to the total shoot dry mass by the leaves and by the stems was almost 50:50. When the buds sprout from the pruned bearers in spring, there is minimal support of growth in terms of carbohydrate supply from the bearer (Greenfield *et al.*, 1995). Only once the new shoot has

developed enough leaf area, can significant accumulation of dry matter commence.

Leaves: The leaves of the spring flush alone consisted of 10 g dry mass, in the case of 'Sylvia', which did not change significantly after the first summer flush had been completed (at the February sampling date) as shown in Fig. 2a. This first summer flush leaves consisted of 12 g dry mass at the February sampling date (Fig. 2b). During the production of the second summer flush, the subtending spring and first summer flushes' leaves increased significantly in dry mass to 17 g and 26 g respectively, while an additional 24 g dry mass was accumulated by the leaves with the second summer flush (Fig. 2c). The shoot at this stage was capable of supporting sufficient dry mass accumulation produce a substantial second summer flush and contribute to significant dry mass accumulation of the leaves in the subtending spring and first summer flushes. The dry mass of the leaves of the first three flushes did not change significantly from 3 April to 16 September (Fig. 2a-c). An additional 29 g of dry mass was added with the formation of the autumn flush (Fig. 2d). The pattern of dry mass accumulation of leaves of *Protea* cv. *Cardinal* followed a similar pattern as can be seen from Fig. 2e-h.

Stems: The dry mass accumulation in the stems of successive flushes of 'Sylvia' followed a very similar pattern to that of the leaves (Fig. 2a-d) except that all the shoot flushes accumulated dry mass through secondary thickening during the winter period which was not the case with the leaves, except in the case of the autumn flush. De Swardt (1989) found that secondary thickening of shoots of *Protea* cv. *Ivy* continued throughout the year. The stem of the spring flush alone consisted of 2.9 g dry mass, which did not change significantly after the first summer flush had been completed (Fig. 2a). The first summer flush initially consisted of 2.5 g dry mass (12 February) as seen in Fig. 2b. During the production of the second summer flush, the subtending spring and first summer flushes increased significantly in dry mass to 19 g and 13 g respectively (Fig. 2a-b), while an additional 12 g dry mass was accumulated with the second summer flush (Fig. 2c). The shoot at this stage had gained sufficient photosynthetic capacity to produce a substantial second summer flush and contribute to the significant secondary thickening of the subtending spring and first summer flushes. The dry mass of the stems of the first three flushes increased significantly from 3 April

to 16 September (Fig. 2a-d) presumably as a result of further secondary thickening. An additional 16 g dry mass was added with the formation of the autumn flush (Fig. 2d). The pattern of dry mass accumulation of stems of *Protea* cv. Cardinal followed a similar pattern as can be seen from Fig. 2e-h.

By the final sampling date (16 September), the leaves of the spring flush of both cultivars only made up approximately one third of the total dry mass of the spring flush (Fig. 2a and 2e). In the case of the first summer flush, the leaves and stems of the shoots contributed equally to the total shoot dry mass (Fig. 2b and 2f). The leaves of the second summer flush contributed a greater proportion to the total dry mass of the shoot than the stem (Fig. 2c and 2g) and in the autumn flush, measured in spring, the stem only contributed half the amount of dry mass that the leaves contributed (Fig. 2d and 2h). Thus, there is a pronounced shift in the contribution, from stems to leaves, more distally on the mature shoot. Secondary thickening of the stem in the older, spring flush causes the stem to be more important in terms of dry mass, while in the younger, autumn flush where the stem has not thickened much, but the leaves are fully developed, the leaves are contributing most in terms of dry mass.

The time period during which the dry mass accumulation takes place drastically shortens from one flush to the following. In the case of 'Sylvia' the spring flush took six and a half months, from 15 September (bud break) to 3 April (third sampling date), to accumulate 37 g of dry mass (Fig. 2a). The first summer flush accumulated 39 g of dry mass in three and a half months (10 December to 3 April), as can be seen in Fig. 2b, while the second summer flush only needed one and a half months to accumulate 36 grams of dry mass (Fig. 2c). As the shoot develops sufficient photosynthetic capacity to support its own development, the rate of dry mass accumulation increases. While the absolute values of dry mass accumulation in 'Cardinal' are lower than those of 'Sylvia', the pattern of accumulation and the proportional importance of leaves and stem is very similar as can be see in Fig. 2e-h.

Total carbohydrate accumulation

As can be seen from Fig. 3a and 3b, total carbohydrate accumulation by the leaves of 'Sylvia' and 'Cardinal' is very gradual during the formation of the first two flushes,

while accumulation by the stems is negligible. With the formation of the second summer flush, carbohydrate accumulation by both leaves and stems increased dramatically, although the amount of carbohydrates in the leaves continued to outweigh that in the stems at a ratio of approximately 5:1. Over the winter period up to the final sampling date, there is no significant change in the accumulation of total carbohydrates in the leaves or stems of either 'Sylvia' or 'Cardinal'.

In Fig. 4a-e for 'Sylvia' and Fig. 4f-h for 'Cardinal' it can be seen that the time period for accumulation of carbohydrates becomes shorter with the progression of the successive growth flushes, even though the total amount of carbohydrates in various flushes is similar at the last two sampling dates. It is also clear from these figures that leaves are by far the most important point of accumulation of carbohydrates in the shoot.

Tissue content of starch

Accumulation of starch takes place mainly in the leaves and not in the stems of both 'Sylvia' and 'Cardinal' shoots (Fig. 5a and 5b). Initial accumulation of starch was slow, but a rapid accumulation took place with the formation of the second summer flush. While a large reduction in leaf starch took place over the winter period, stem starch levels continued to increase, but at very low levels. Thus the total shoot starch levels mirror those of the leaves.

Leaves: Starch accumulation by the leaves of 'Sylvia' was slow during the spring and first summer flushes and then increased dramatically after the second summer flush (Fig. 6a-c). Sanz *et al.* (1987) report an accumulation of non-structural carbohydrates in citrus shoots during the period from bud sprouting to flowering, when photosynthesis exceeds carbohydrate consumption. After winter, total starch in the leaves had decreased to 1.17 g in the four flush shoot with a contribution of 0.18 g from the autumn flush (Fig. 6d). Leaves are a major source of carbohydrate reserves for evergreen plants and leaf starch is converted to sugars and then transported to sink organs in the plant.

Stems: Starch accumulation by the stems of 'Sylvia' were much lower than in the leaves, starting at 0.019 g after the spring flush (Fig. 6a) and increasing to 0.65 g by the last sampling date in September. Starch accumulation by all the stems of all the flushes increased over winter and were not depleted as in the case of the leaves (Fig. 6a-c). The increase in dry mass of the stems over the winter period, however, was also significantly higher than for the leaves (Fig. 1a).

Fig. 6e-h show similar patterns of total tissue starch content in leaves and stems of 'Cardinal'.

Tissue content of total sugars

The overall accumulation of total sugars in the leaves and stems of 'Sylvia' and 'Cardinal' shoots is depicted in Fig. 7a and 7b. Leaves are far more important sources of total sugars than stems. During the formation of the first two flushes, total sugars in the stems of the shoots is almost zero. Only with the formation of the second summer flush, did a significant increase in accumulated total sugars take place by the leaves and the stems, whereafter the levels remained constant through the winter, even with the formation of the additional autumn flush.

Leaves: After completion of the spring flush, the leaves of 'Sylvia' contained 0.69 g of total sugars (Fig. 8a). The tissue content of total sugars in the spring flush increased to 1.2 g by the time the second summer flush had been completed. Koch *et al.* (1986) reported that a new flush in citrus is a substantial sink throughout its expansion period, whereafter it becomes the predominant source for the next flush. Although an additional 1.53 g of total sugars was accumulated in the autumn flush (Fig. 8d), the total in the whole shoot only increased to 5.36 g due to a reduction in the total sugars stored in the subtending three flushes, when measured in the spring (16 September), as can be seen in Fig. 5a-c. The reduction of the total sugars stored in the leaves over the winter period indicates that the sugars were used in the leaves to support them while photosynthesis is low or that the leaves acted as a source of sugars for export to other parts of the plant, possibly the roots and the autumn flush.

Stems: Amounts of total sugars in the stem of 'Sylvia' were much lower than in the leaves (Fig. 8a-d). After completion of the initial spring flush, the leaves contained a total of 0.12 g of total sugars compared to 0.69 g in the leaves of this same flush (Fig. 8a). The total sugars stored in the stems was substantially higher after the completion of the second summer flush, at 1.43 g in all three flushes together. As in the leaves, the autumn flush contributed additional sugar reserves to the stem (Fig. 8d), but the sugar content of the subtending flushes remained constant (Fig. 8a-c). The dry mass of the spring, first summer and second summer flushes increased over the winter period (Fig. 1a). The concentration of total sugars thus decreased during this period.

Fig. 8e-h show similar patterns of total sugars tissue content in leaves and stems of 'Cardinal'.

Concentration total sugars

Leaves: The concentration of total sugars in the leaves of the spring flush of 'Sylvia' shoots did not vary significantly between the first three sampling dates, but was lower at the last sampling date after winter (Fig. 9a). The concentration of total sugars in the leaves of the first summer and second summer flushes was similar to that of the spring flush for the first three sampling dates. On the fourth sampling date (16 September) the concentration of total sugars in all the flushes was significantly reduced, but remained similar between flushes (Fig. 9a). Borrás *et al.* (1984) reported that in citrus, leaf sugar levels remained fairly constant throughout the year, but were reduced during the flowering period when carbohydrate demand is at its highest. The leaves of Sylvia shoots maintain relatively stable reserves of total reducing sugars, except over the winter period, when the rate of use through respiration is higher than the rate of production through photosynthesis or when the required level of total reducing sugars in the leaves for respiration is lower. The average concentration of total reducing sugars in the leaves of 6.5% over the four sampling dates, compares well with the findings of Napier (1985) of 6.4% total reducing sugars in the leaves of *Leucospermum* cv. Red Sunset over a period from 1 February to 29 August. Napier (1985) also reported a reduction in leaf sugar levels in *Leucospermum* plants grown under shade.

In the 'Cardinal' plants the pattern was the same and the average concentration of total sugars for the first three flushes was only slightly lower than in the case of 'Sylvia', 6.6% versus 7.3% respectively (Fig. 9e).

Physiological disorders like leaf tip burn found on a number of *Protea* species and hybrids, could possibly be related to the low levels of sugars in the leaves during the winter period. Sieckmann & Boe (1978) relate that reducing and non-reducing sugars are probably associated with cold hardiness of leaf tissue of evergreen species such as English boxwood and American Cranberry.

Stems: Concentration of total sugars in the stems of 'Sylvia' shoots was 4.3% in the initial spring flush, reduced to 3.0% and 2.8% respectively, after the first and second summer flushes had been completed and reduced further to 1.8% by 16 September (Figure 9c). The concentration of total sugars in the first summer flush was unchanged between 12 February and 3 April, but significantly reduced by 16 September. The second summer flush started with 3.7% total sugars, which depleted to 2.4% after winter. The autumn flush contained 3.1% total reducing sugars at the sampling date, which was significantly higher than any of the subtending flushes at that time. According to Wardlaw (1968) the reserves of the more terminal parts of shoots will be fed to the terminal growth point of the shoot while reserves from the basal part of the shoot will be fed to the basally situated organs like stems and roots. The average concentration of total sugars in the stems of 3.4% over the four sampling dates, compares well with the findings of Napier (1985) of 3.0% total reducing sugars in the stems of *Leucospermum* cv. Red Sunset over a period from 1 February to 29 August. Napier (1985) also reported a reduction in shoot sugar in *Leucospermum* plants grown under shade. Comparatively, Scholefield *et al.* (1985) reported between 2 and 4% sugars in the two-year-old wood of avocado.

'Cardinal' showed a similar pattern of total sugar concentration in the stems (Figure 9g).

Concentration starch

Leaves: The concentration of starch in the leaves of the initial spring flush of 'Sylvia' was 6.0% (Fig. 9b). With the formation of the first summer flush, the concentration of starch in the spring flush was reduced to 4.0%, the other 2% probably being mobilised for the production of the first summer flush. By the time that the second summer flush was completed (3 April) the level of starch in the subtending spring flush had increased to 5.7%, almost its starting value. From 12 February to 3 April, the concentration of starch in the first summer flush increased from 1.9% to 3.8%. Thus, during this period both the spring flush and the first summer flush were producing carbohydrates in excess of the plant's immediate needs and were able to build up reserves in the form of starch. Koch *et al.* (1986) noted on citrus that a new flush is a substantial sink throughout its expansion period, whereafter it takes over as the predominant source of photosynthates for the next flush.

With the formation of the second summer flush (completed 3 April) the starch levels in this flush were 4.6%, significantly higher than that of the first summer flush immediately after completion. During its formation the second summer flush was being supported by two strong subtending flushes and could thus accumulate carbohydrates sooner than the first summer flush. After winter, starch levels in all four flushes were significantly reduced, by an average of 1.2%. Reserve starch in the leaves is used during winter, for maintenance of the plant and possibly for mobilisation to the roots during root growth. Borrás *et al.* (1984) showed that in citrus leaves, starch accumulated during the spring and early summer, then decreased during flowering and fruiting.

'Cardinal' leaves contained similar levels of starch per flush to 'Sylvia' leaves (Fig. 9f).

Stems: The average concentration of starch stored in the stems of shoots of 'Sylvia' was significantly lower than in the leaves, 0.9% and 3.1% respectively (Fig. 9d). It is characteristic of evergreen plants to store carbohydrates in the leaves rather than in the stems. Borrás *et al.* (1984) found leaf sugars in Washington Navel ranging between 13.9% and 19.2% of dry mass, while leaf starch was between 3.3% and 8.4%. The

starch content of the spring flush of 'Sylvia' increased from 0.7% to 2.2% between the first two sampling dates. During this time the dry mass of this flush increased from 2.8g to 4.3g. At the latter two sampling dates the starch concentration in the spring flush was reduced to 0.6% and 0.8% respectively. The concentration of starch in the first summer, second summer and autumn flushes at the different sampling times ranged from 0.6% to 1.0%. The shoots do not serve as an important organ of starch storage.

'Cardinal' stems showed a similar pattern of starch concentration in the stems (Fig. 9h).

Initially dry mass accumulation in leaves and stems is slow, as the shoot utilises the available carbohydrates to support the grow of the next flush. After the second flush has been completed the shoot has enough photosynthetic capacity to produce another flush and accumulate dry mass in the subtending flushes. Dry mass of the leaves per flush is the same before and after winter, while stem dry mass of the three oldest flushes increases from before winter to after winter, probably as a result of secondary thickening. Tissue content of starch in the leaves increased significantly through the summer period, but was drastically reduced after winter. The leaves serve as the main source of storage carbohydrates. Tissue content of starch in the stems increased continuously, but represented only a small part of the reserve base. Tissue content of total sugars in the leaves and stems increased dramatically from the sampling of the two-flush shoot to the sampling of the three-flush shoot. The level of total sugars in the leaves was more than double that of the stems, although the pattern of accumulation was similar. Patterns of dry mass accumulation and carbohydrate allocation of *Protea* cv. Sylvia and *Protea* cv. Cardinal were similar in all respects.

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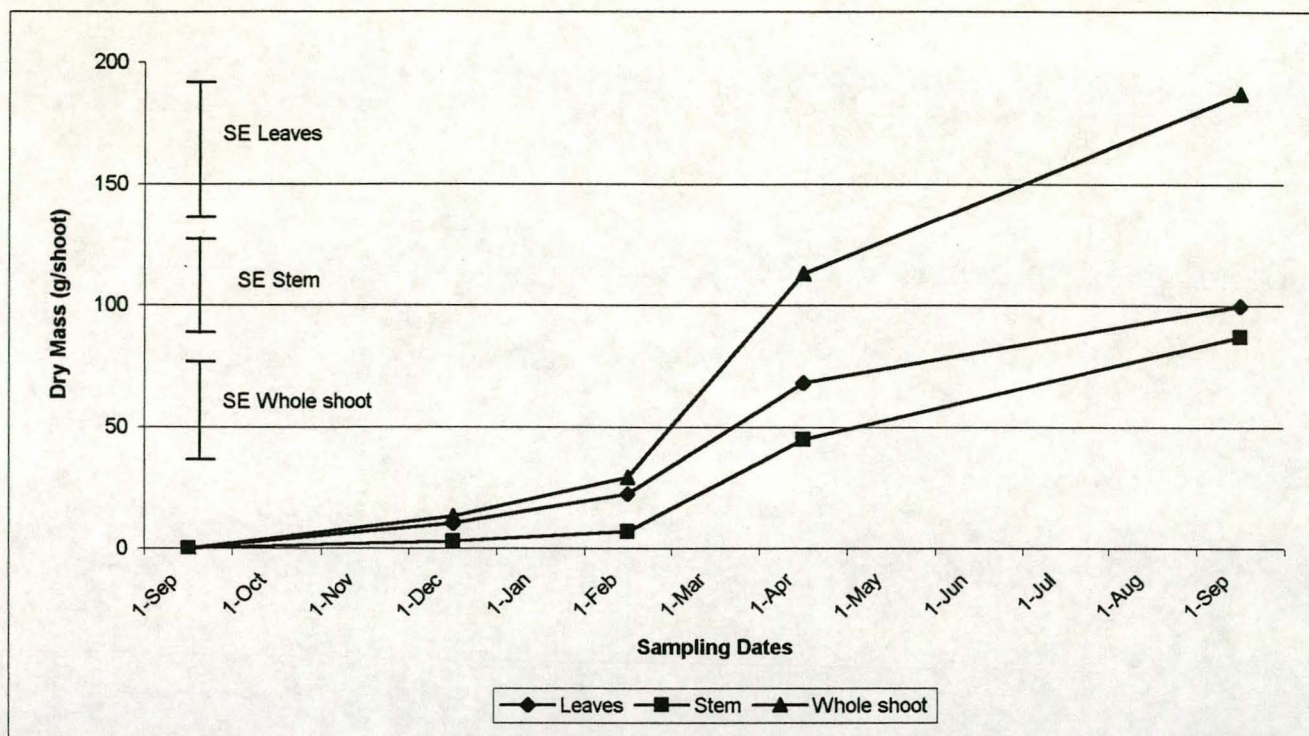


Figure 1a. Total dry mass accumulation of the leaves and stem of 'Sylvia' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

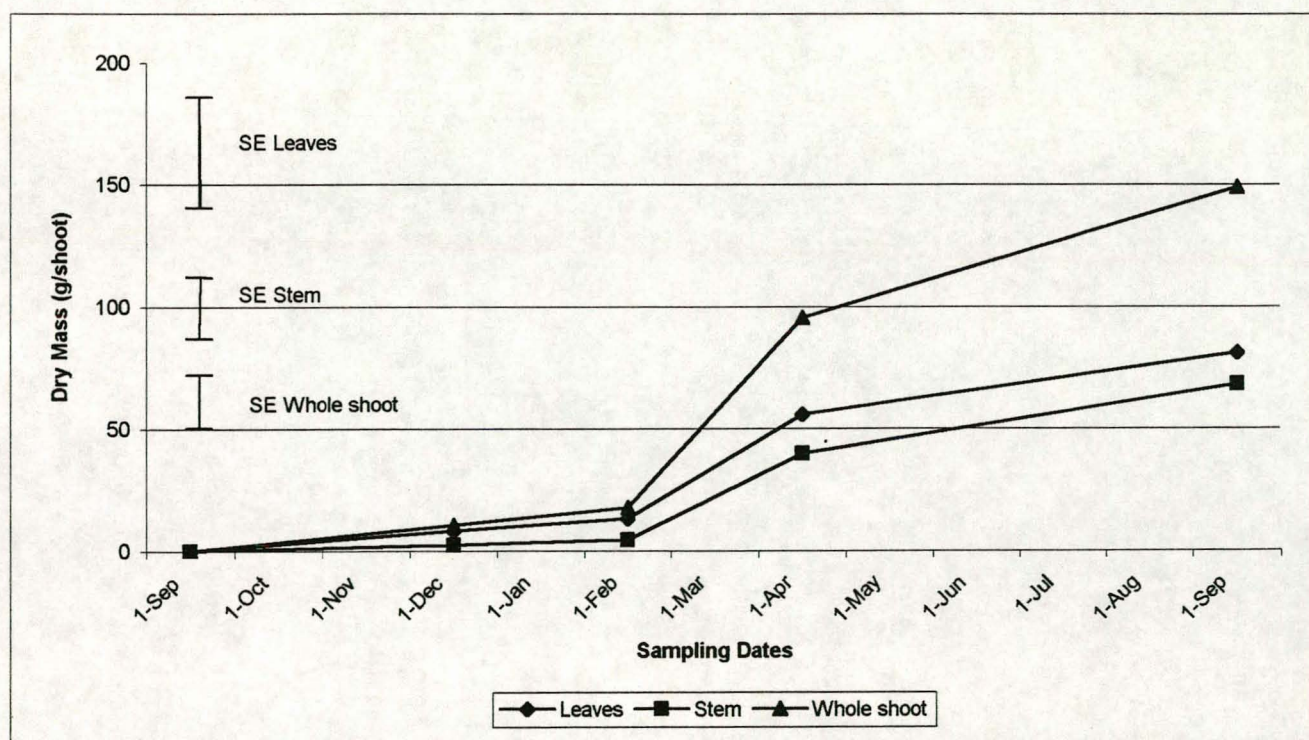


Figure 1b. Total dry mass accumulation of the leaves and stem of 'Cardinal' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

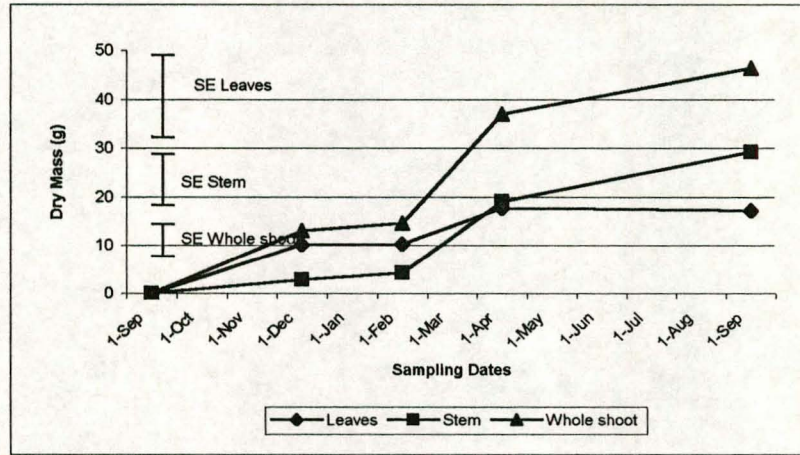


Figure 2a. Dry mass accumulation of the leaves and stem of the spring flush of 'Sylvia' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

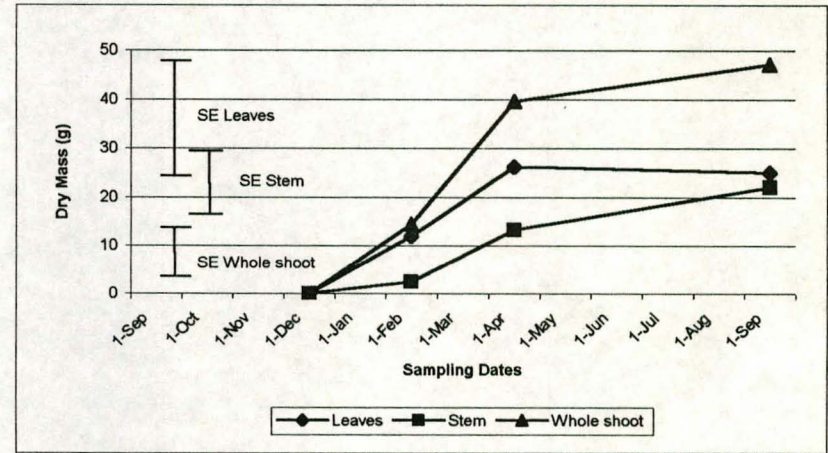


Figure 2b. Dry mass accumulation of the leaves and stem of the 1st summer flush of 'Sylvia' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

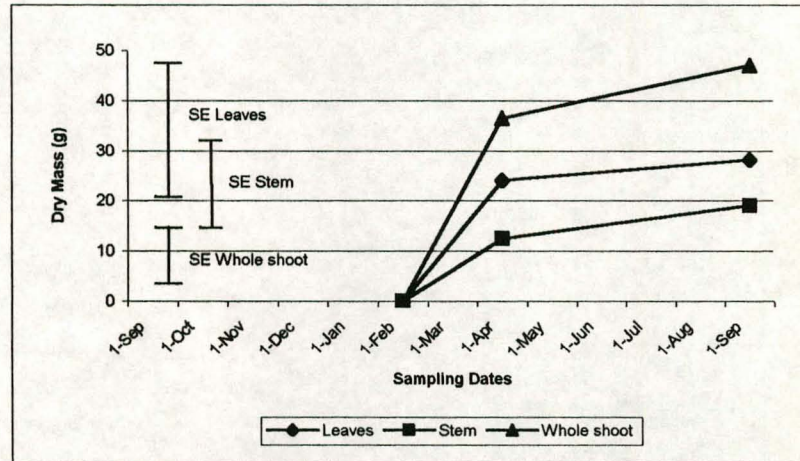


Figure 2c. Dry mass accumulation of the leaves and stem of the 2nd summer flush of 'Sylvia' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

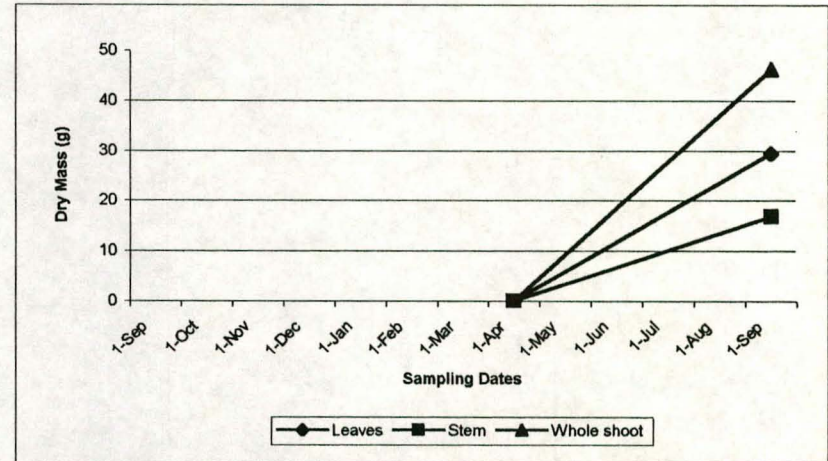


Figure 2d. Dry mass accumulation of the leaves and stem of the autumn flush of 'Sylvia' shoots over time. Spring bud break on 16 September.

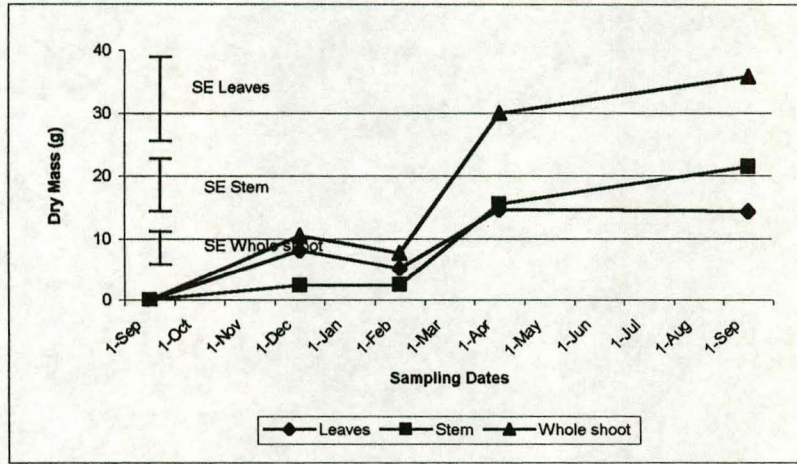


Figure 2e. Dry mass accumulation of the leaves and stem of the spring flush of 'Cardinal' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

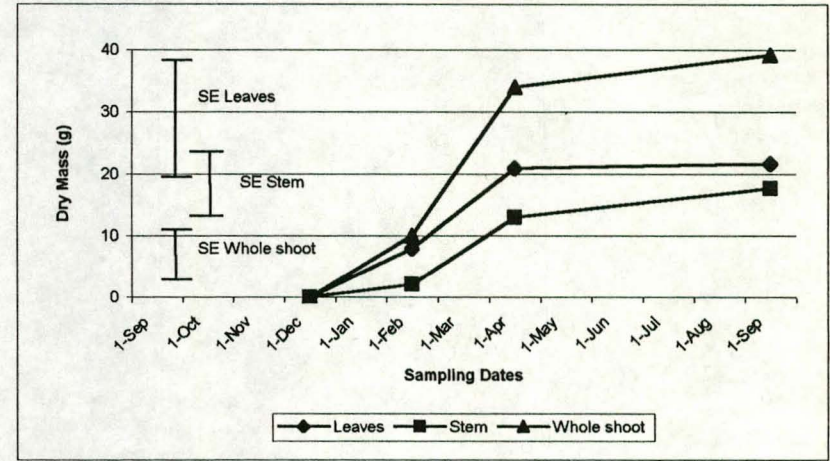


Figure 2f. Dry mass accumulation of the leaves and stem of the 1st summer flush of 'Cardinal' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

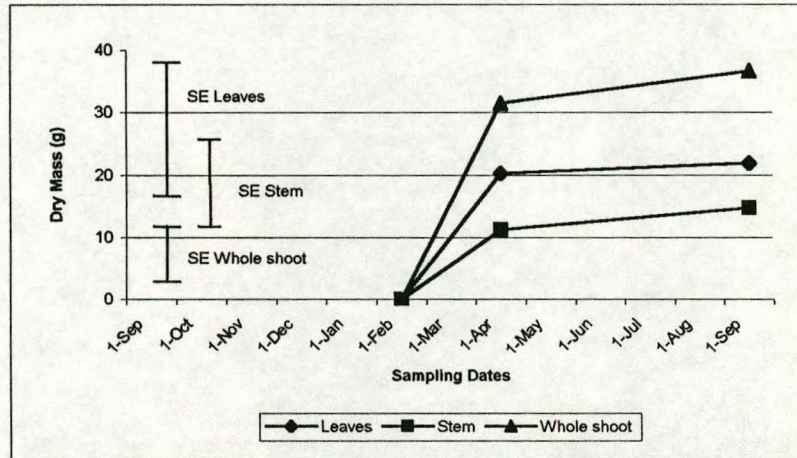


Figure 2g. Dry mass accumulation of the leaves and stem of the 2nd summer flush of 'Cardinal' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

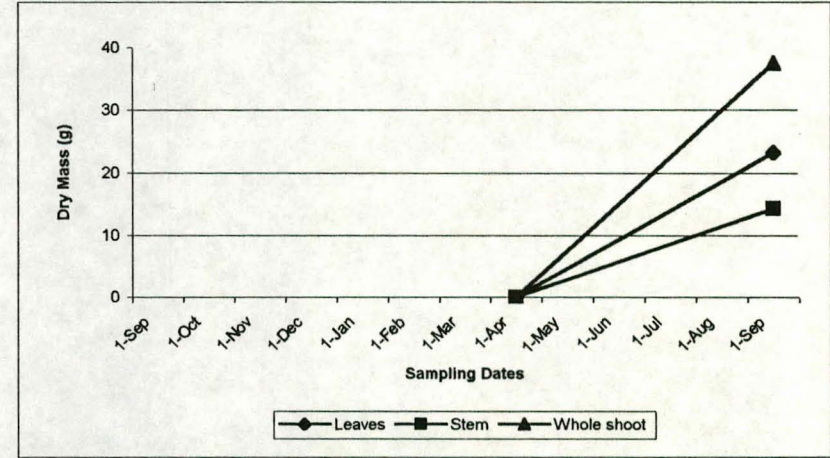


Figure 2h. Dry mass accumulation of the leaves and stem of the autumn flush of 'Cardinal' shoots over time. Spring bud break on 16 September.

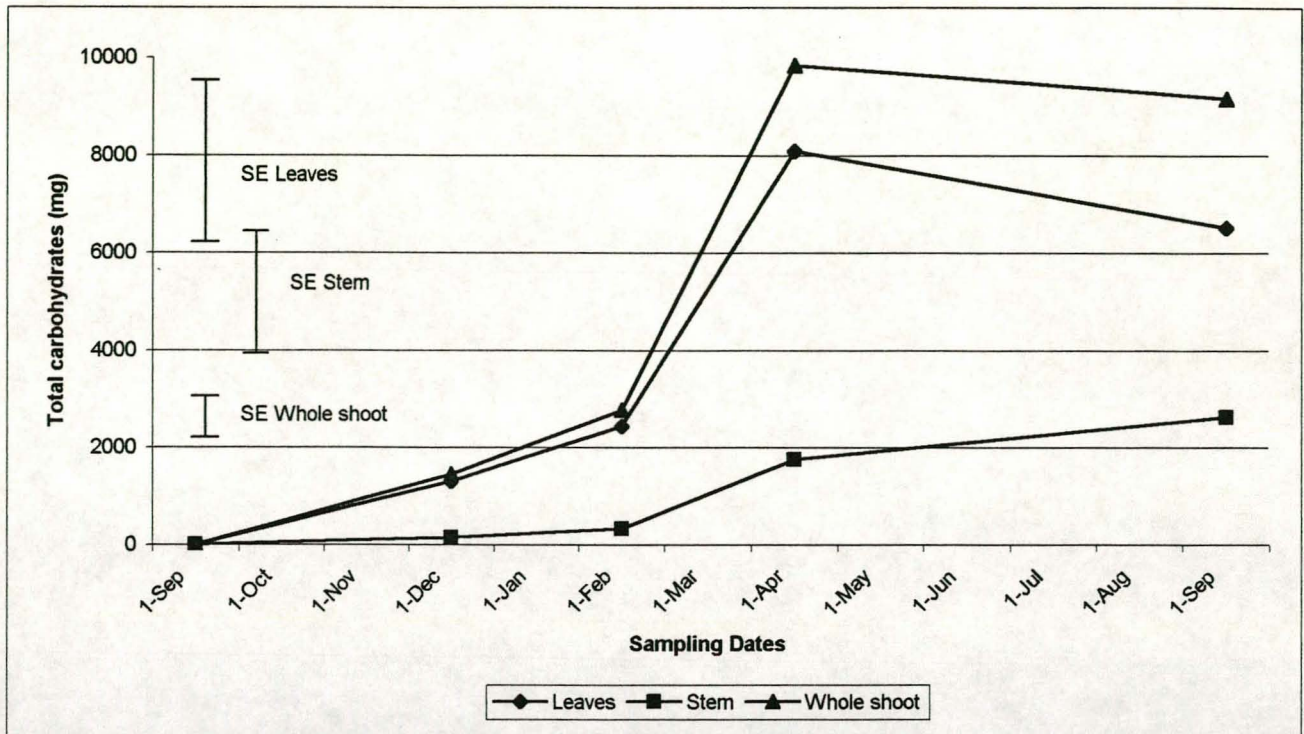


Figure 3a. Total carbohydrate accumulation in the leaves and stems of 'Sylvia' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

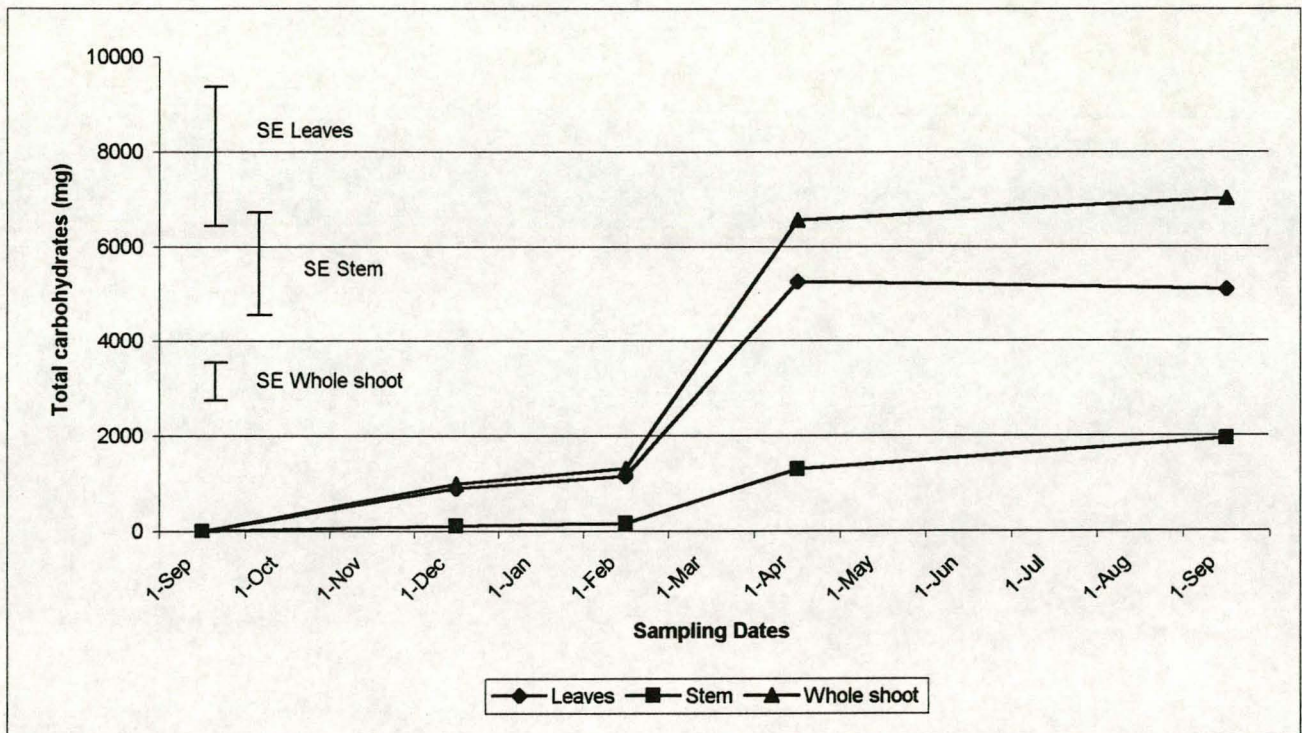


Figure 3b. Total carbohydrate accumulation in the leaves and stems of 'Cardinal' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

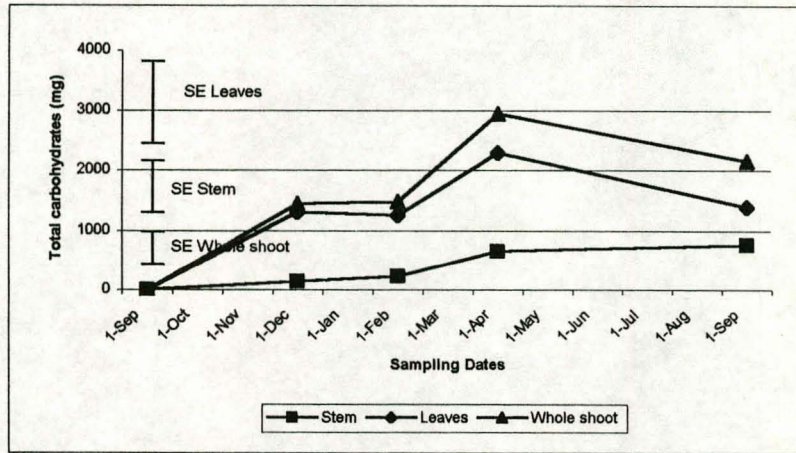


Figure 4a. Carbohydrate accumulation in the leaves and stem of the spring flush of 'Sylvia' over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

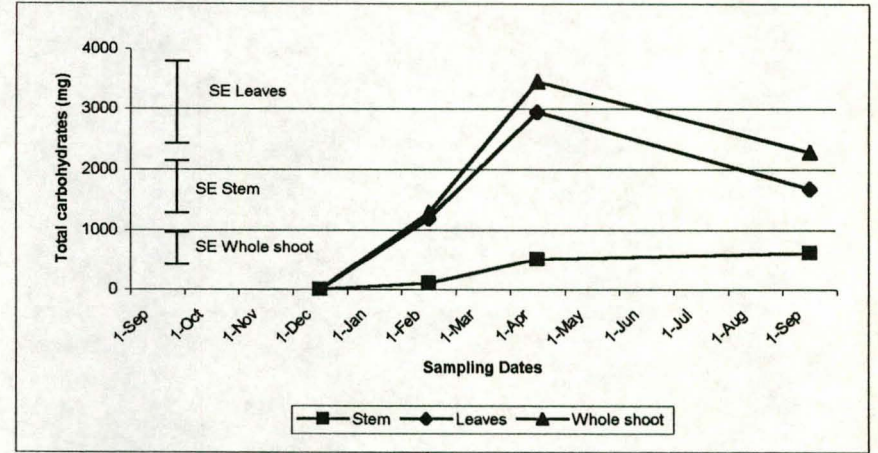


Figure 4b. Carbohydrate accumulation in the leaves and stem of the 1st summer flush of 'Sylvia' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

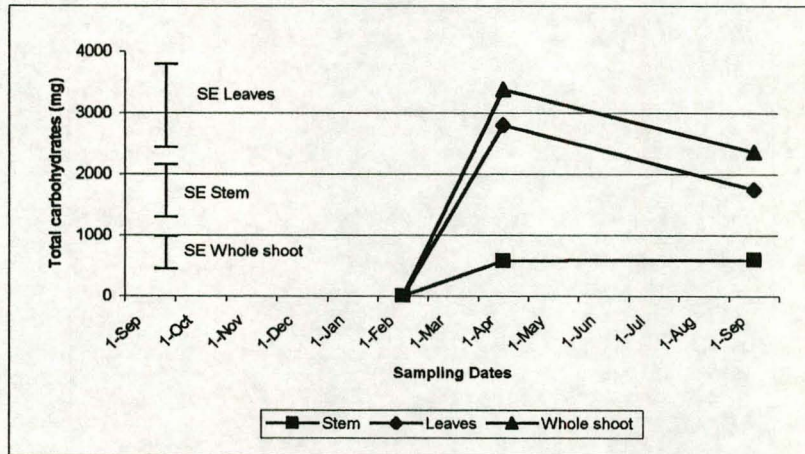


Figure 4c. Carbohydrate accumulation in the leaves and stem of the 2nd summer flush of 'Sylvia' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

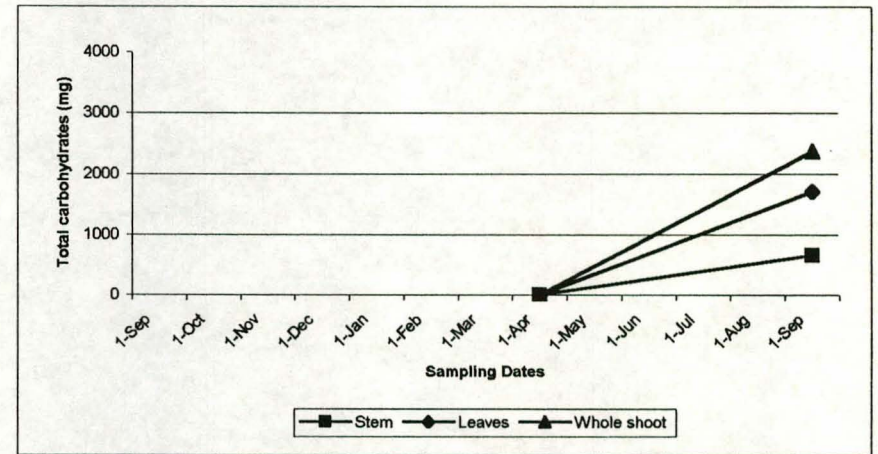


Figure 4d. Carbohydrate accumulation in the leaves and stem of the autumn flush of 'Sylvia' shoots over time. Spring bud break on 16 September.

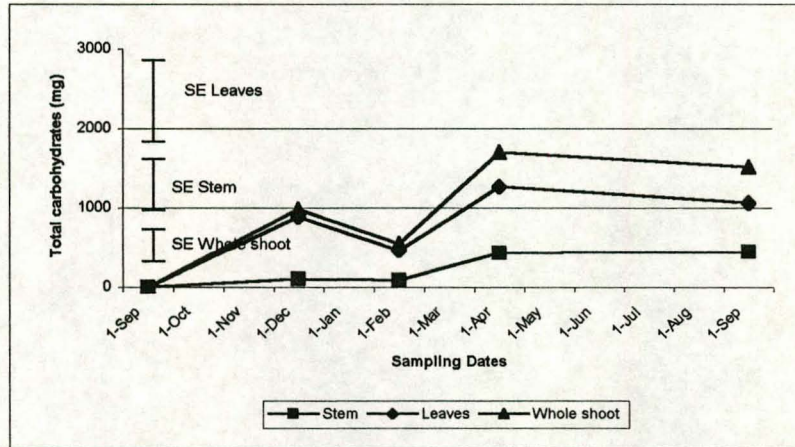


Figure 4e. Carbohydrate accumulation in the leaves and stem of the spring flush of 'Cardinal' over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

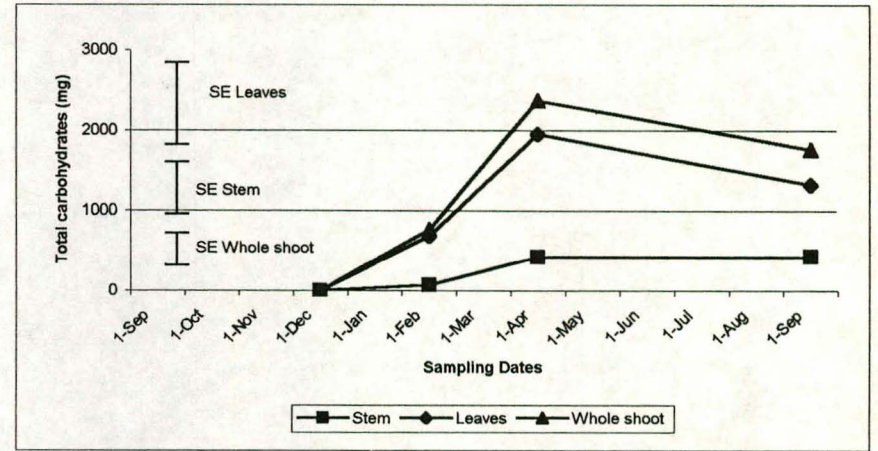


Figure 4f. Carbohydrate accumulation in the leaves and stem of the 1st summer flush of 'Cardinal' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

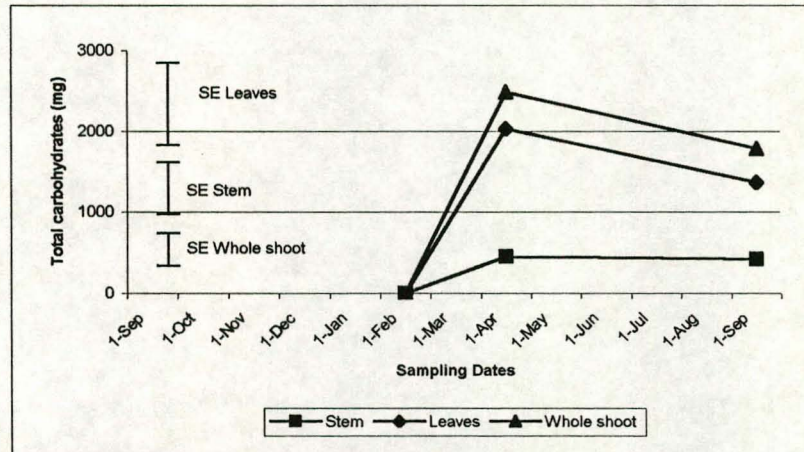


Figure 4g. Carbohydrate accumulation in the leaves and stem of the 2nd summer flush of 'Cardinal' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

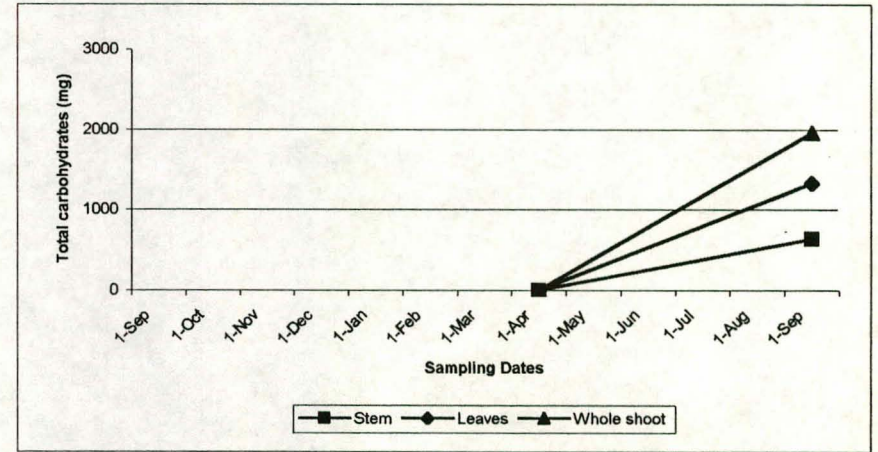


Figure 4h. Carbohydrate accumulation in the leaves and stem of the autumn flush of 'Cardinal' shoots over time. Spring bud break on 16 September.

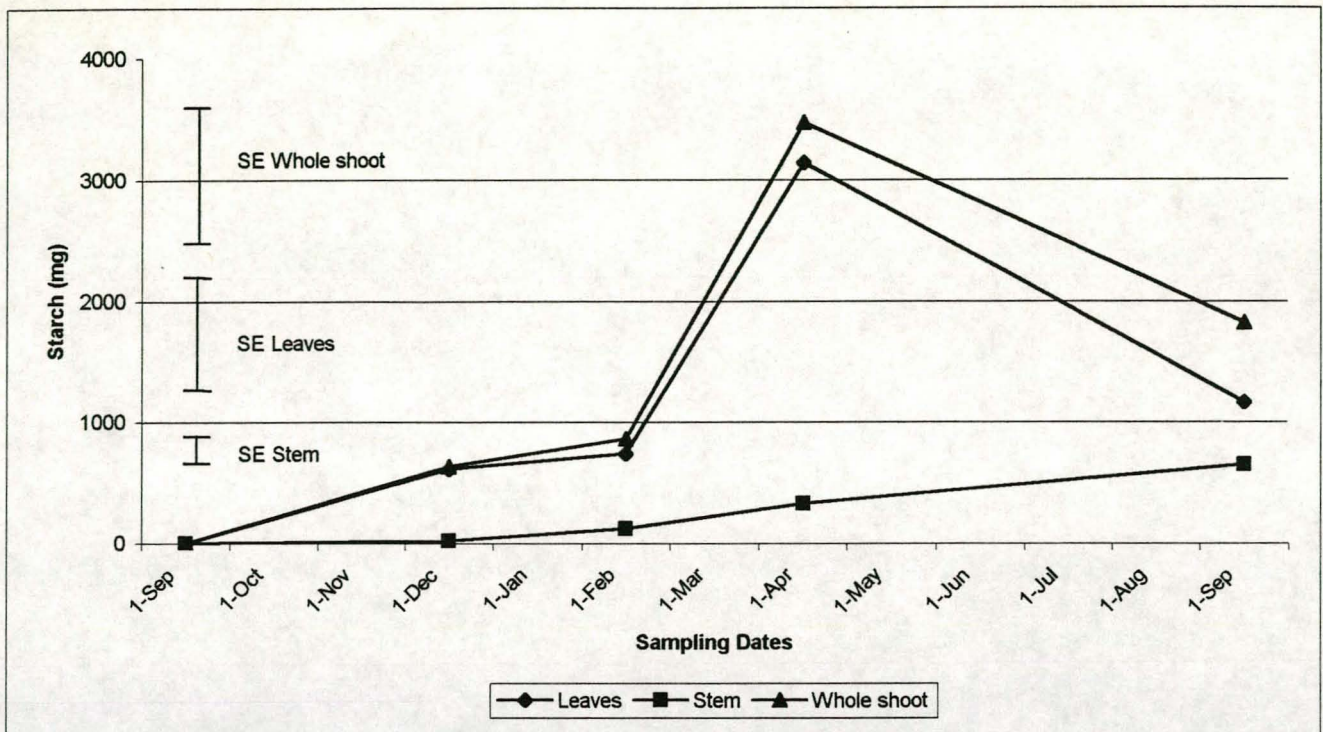


Figure 5a. Total starch accumulation in the leaves and stems of 'Sylvia' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

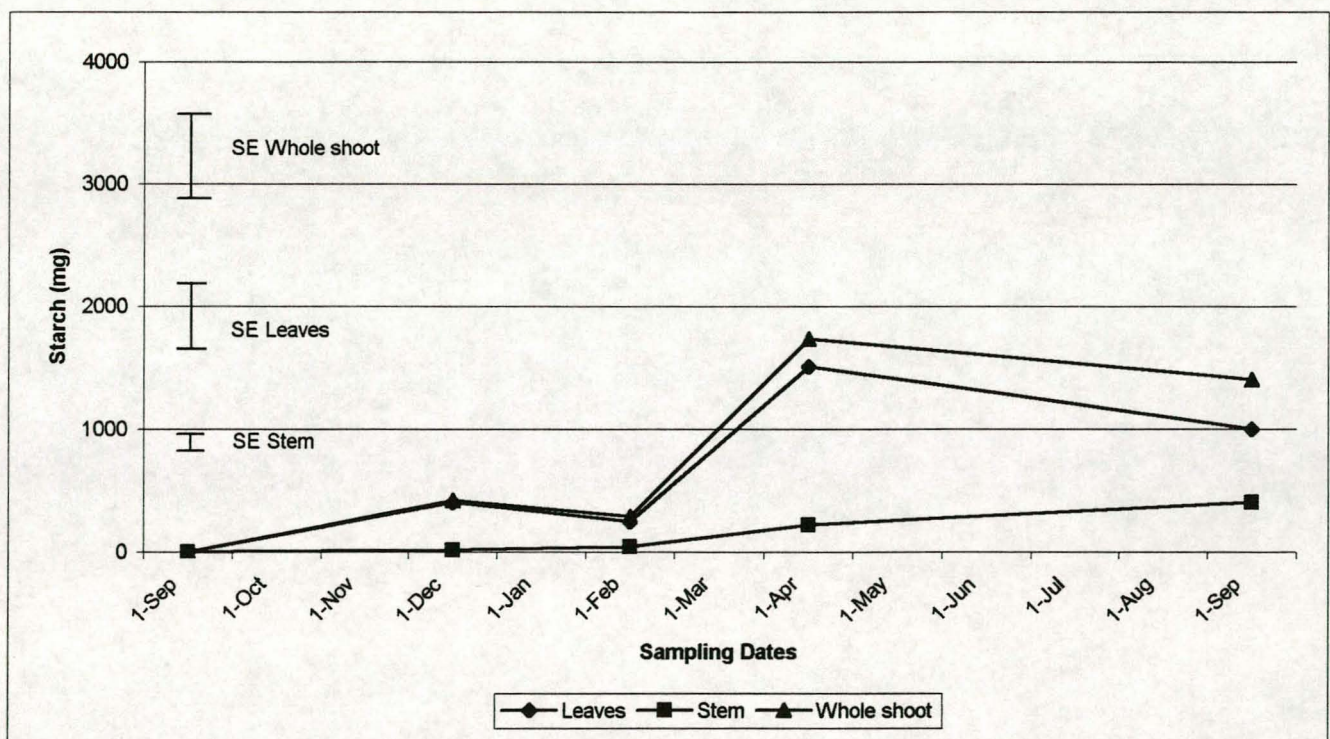


Figure 5b. Total starch accumulation in the leaves and stems of Cardinal shoots over time.

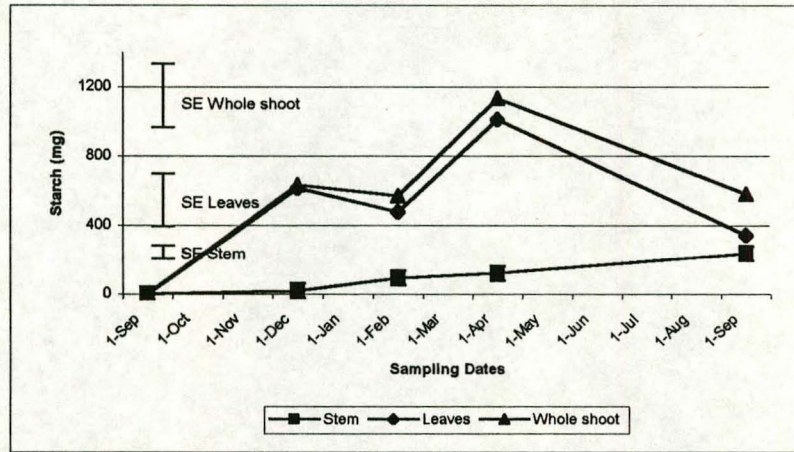


Figure 6a. Starch accumulation in the leaves and stem of the spring flush of 'Sylvia' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

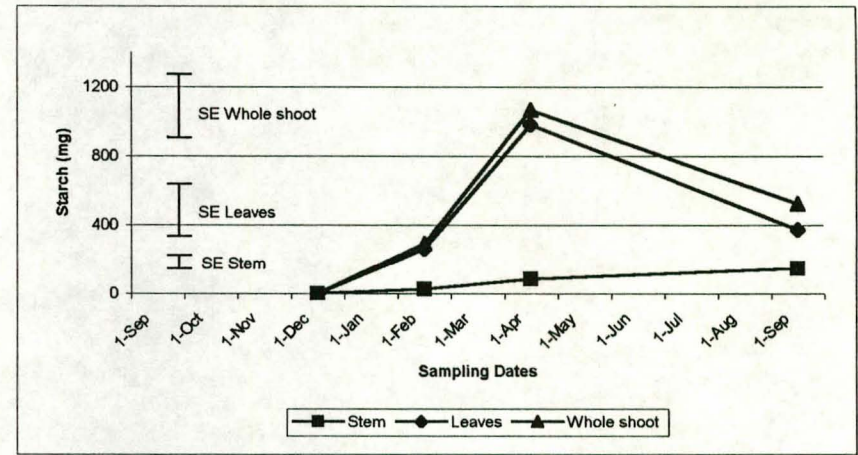


Figure 6b. Starch accumulation in the leaves and stem of the 1st summer flush of 'Sylvia' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

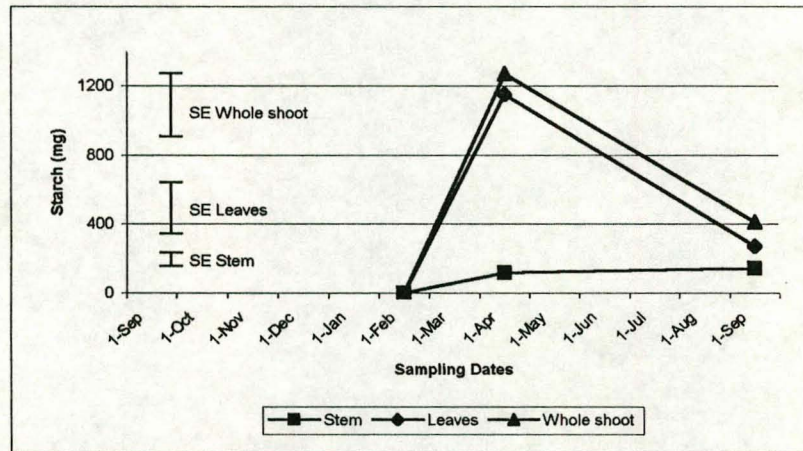


Figure 6c. Starch accumulation in the leaves and stem of the 2nd summer flush of 'Sylvia' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

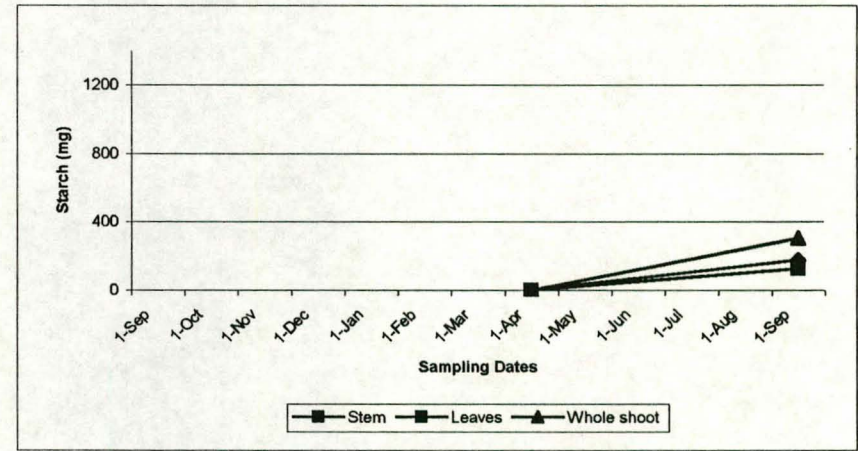


Figure 6d. Starch accumulation in the leaves and stem of the autumn flush of 'Sylvia' shoots over time. Spring bud break on 16 September.

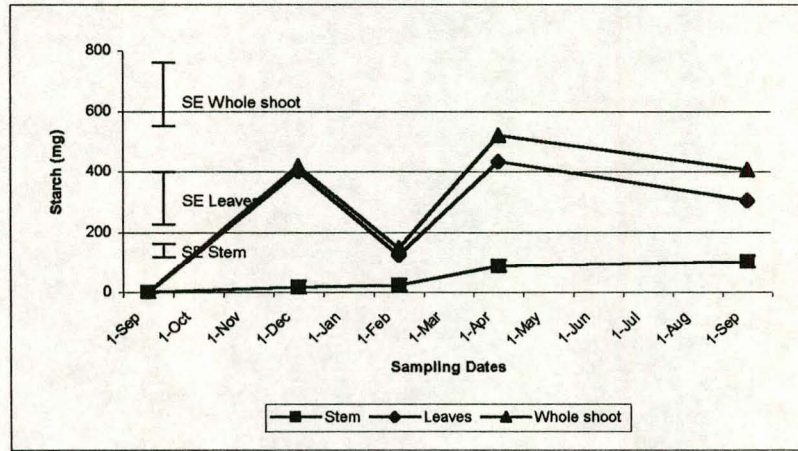


Figure 6e. Starch accumulation in the leaves and stem of the spring flush of 'Cardinal' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

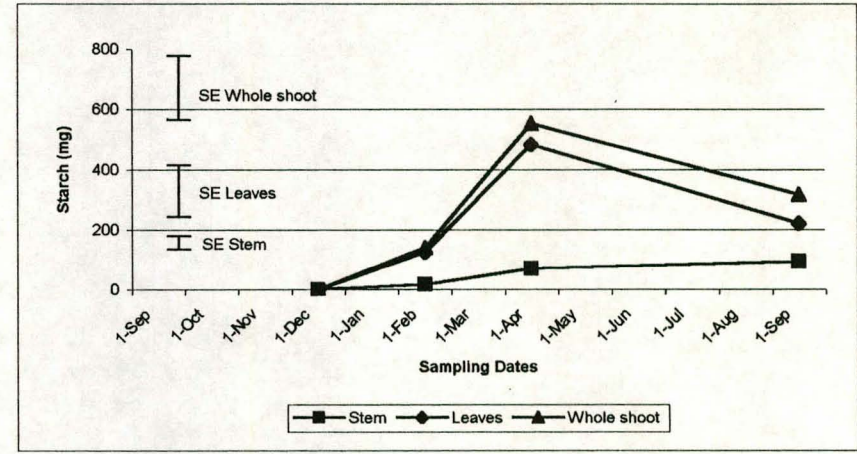


Figure 6f. Starch accumulation in the leaves and stem of the 1st summer flush of 'Cardinal' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

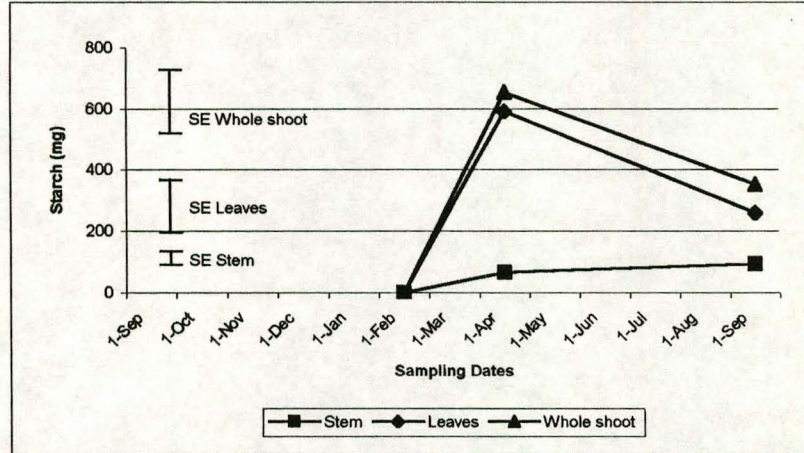


Figure 6g. Starch accumulation in the leaves and stem of the 2nd summer flush of 'Cardinal' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

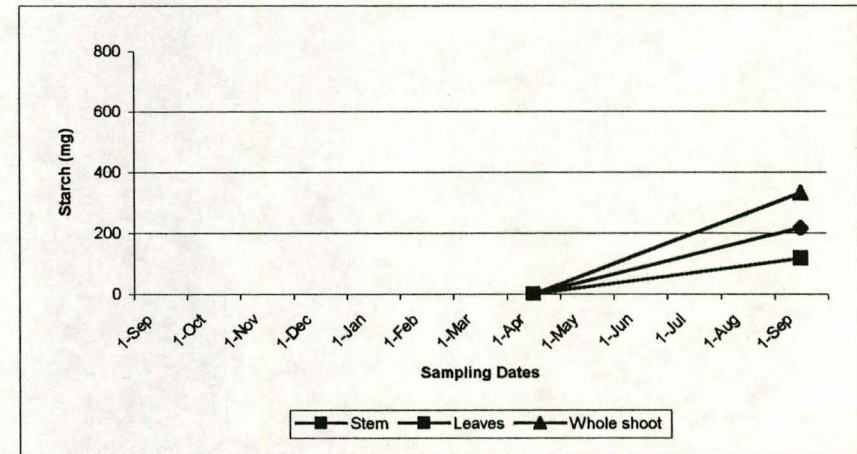


Figure 6h. Starch accumulation in the leaves and stem of the autumn flush of 'Cardinal' shoots over time. Spring bud break on 16 September.

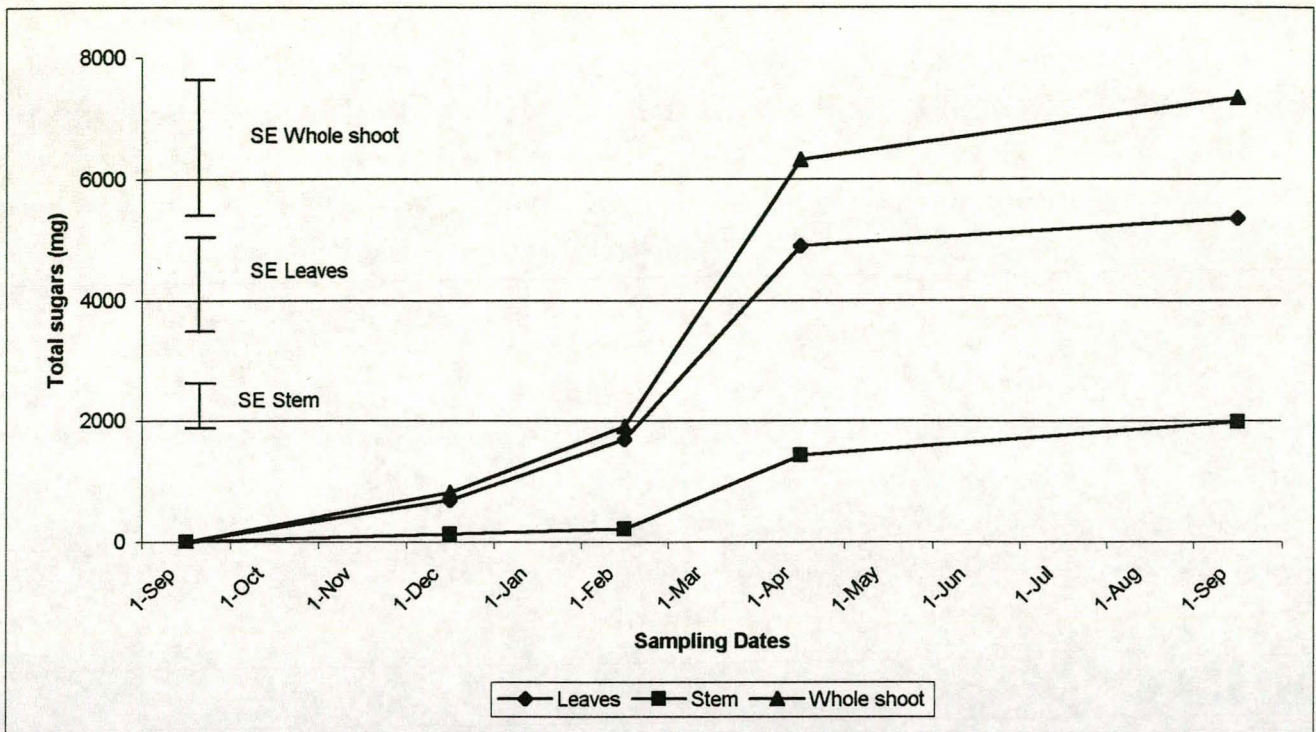


Figure 7a. Total sugars accumulation of the leaves and stem of 'Sylvia' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

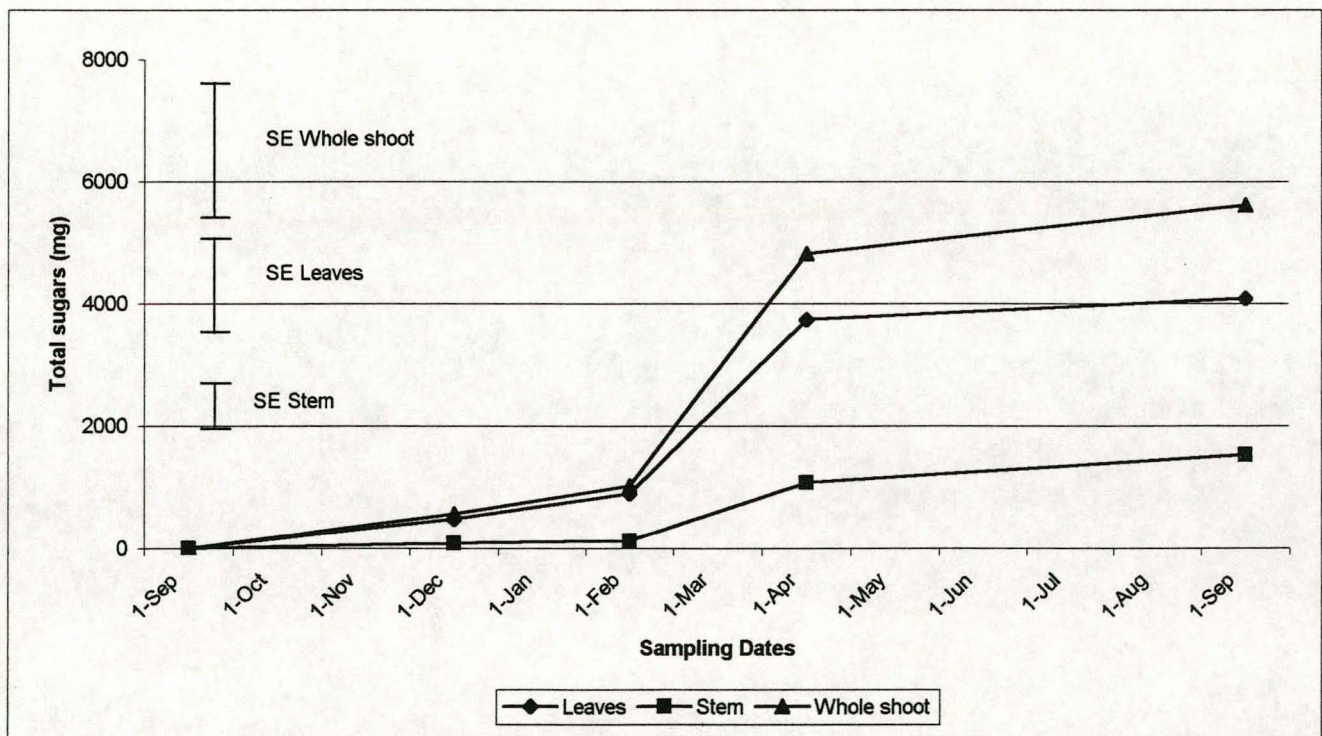


Figure 7b. Total sugars accumulation of the leaves and stem of 'Cardinal' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

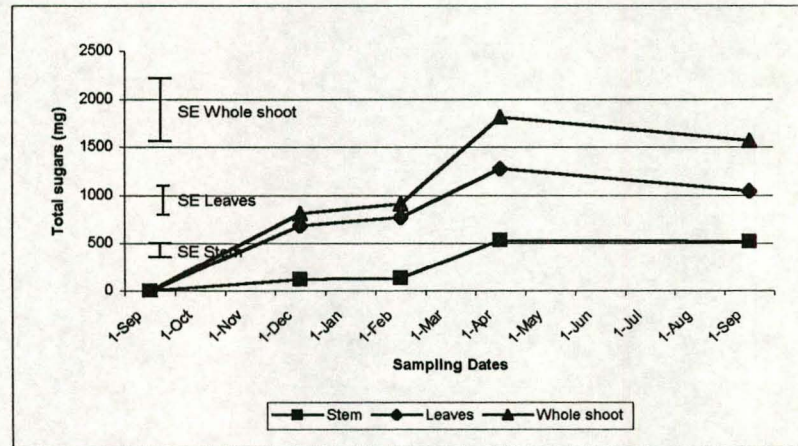


Figure 8a. Total sugars accumulation in the leaves and stem of the spring flush of 'Sylvia' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

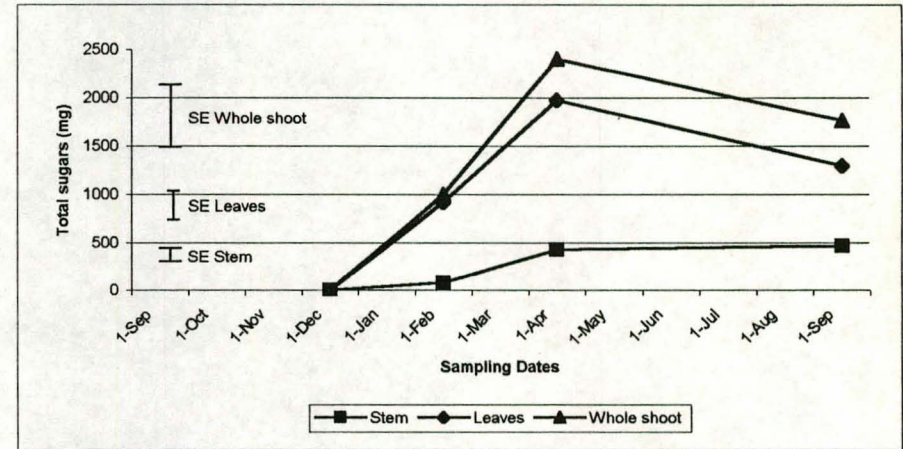


Figure 8b. Total sugars accumulation in the leaves and stem of the 1st summer flush of 'Sylvia' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

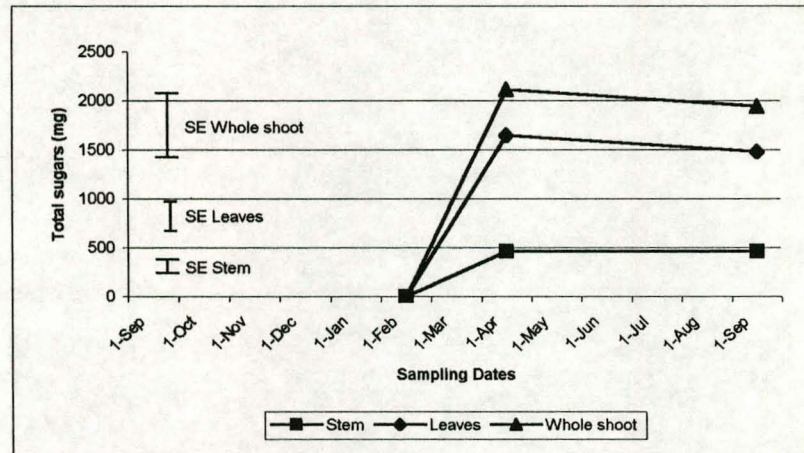


Figure 8c. Total sugars accumulation in the leaves and stem of the 2nd summer flush of 'Sylvia' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

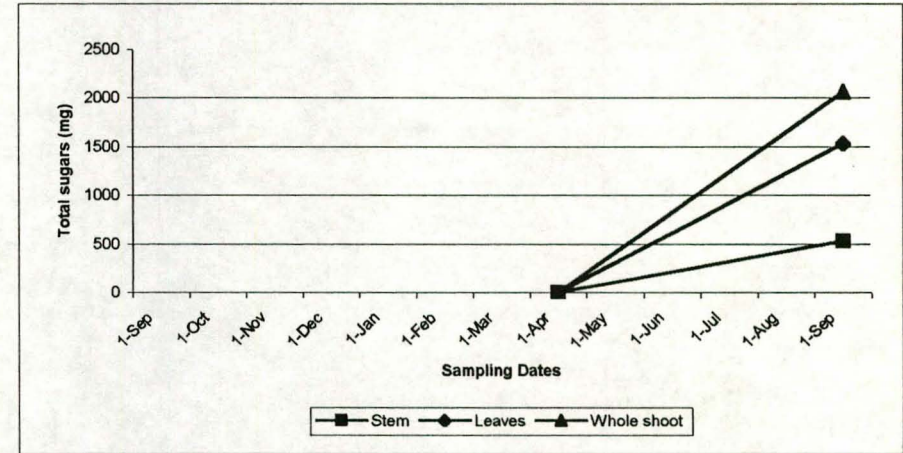


Figure 8d. Total sugars accumulation in the leaves and stem of the autumn flush of 'Sylvia' shoots over time. Spring bud break on 16 September.

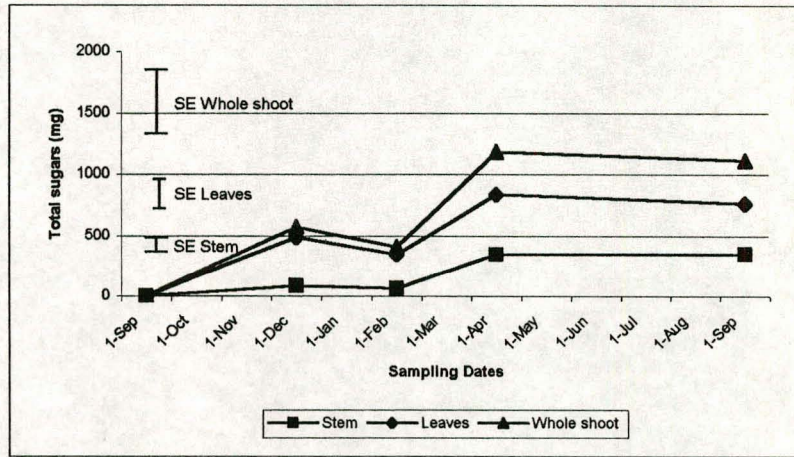


Figure 8e. Total sugars accumulation in the leaves and stem of the spring flush of 'Cardinal' shoots over time. Start of first summer flush on 10 December, second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

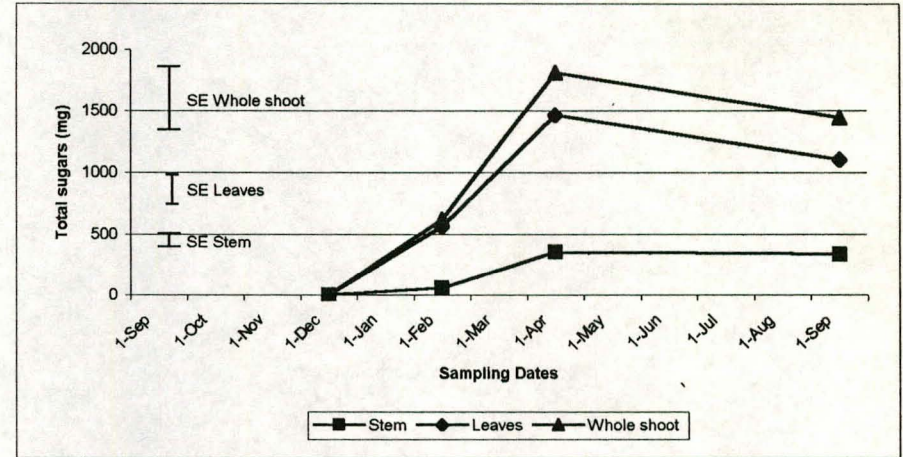


Figure 8f. Total sugars accumulation in the leaves and stem of the 1st summer flush of 'Cardinal' shoots over time. Start of second summer flush on 12 February, autumn flush on 3 April and spring bud break on 16 September.

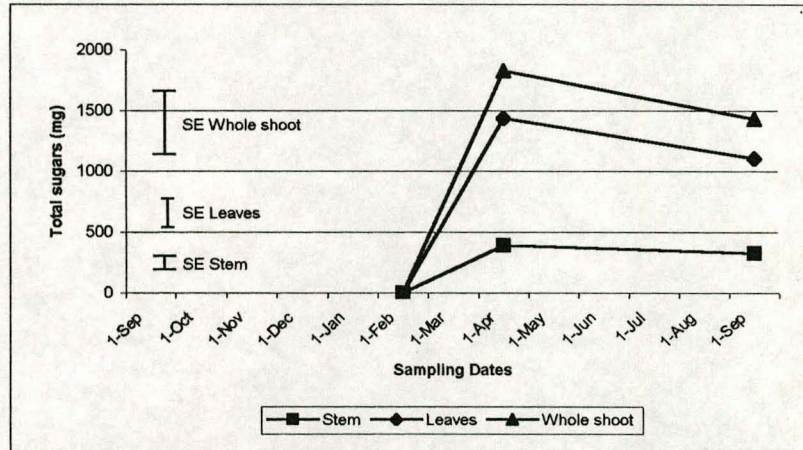


Figure 8g. Total sugars accumulation in the leaves and stem of the 2nd summer flush of 'Cardinal' shoots over time. Start of autumn flush on 3 April and spring bud break on 16 September.

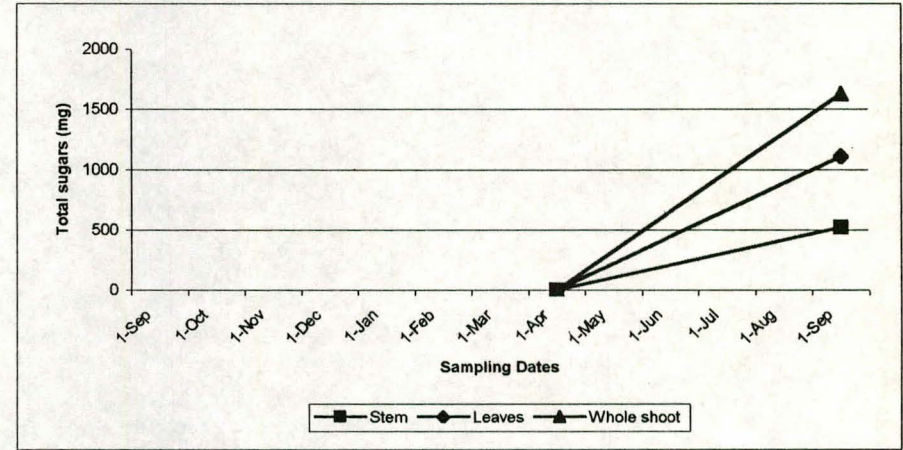


Figure 8h. Total sugars accumulation in the leaves and stem of the autumn flush of 'Cardinal' shoots over time. Spring bud break on 16 September.

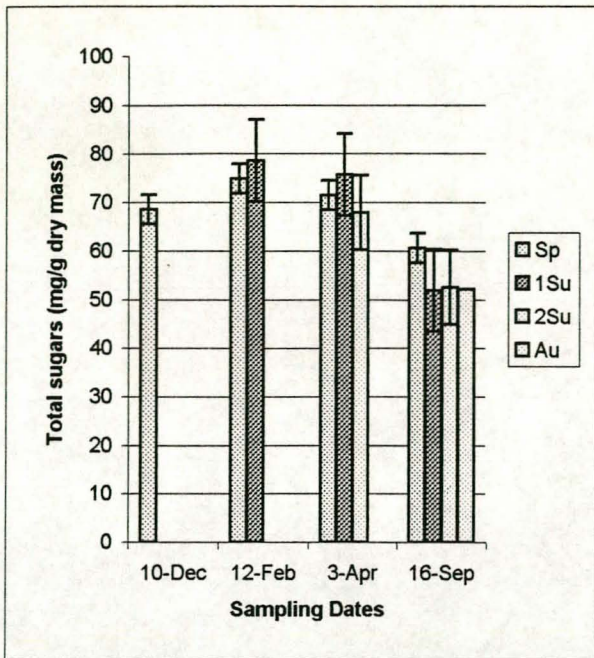


Figure 9a. Concentration of total sugars in 'Sylvia' leaves per flush.

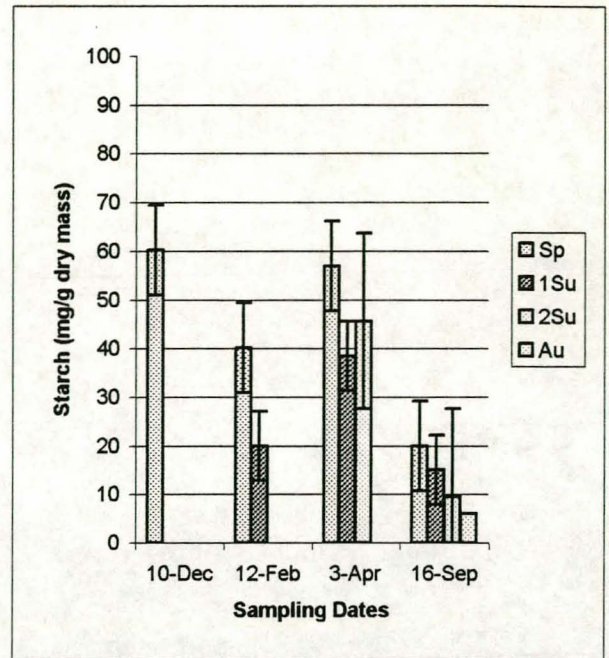


Figure 9b. Concentration of starch in 'Sylvia' leaves per flush.

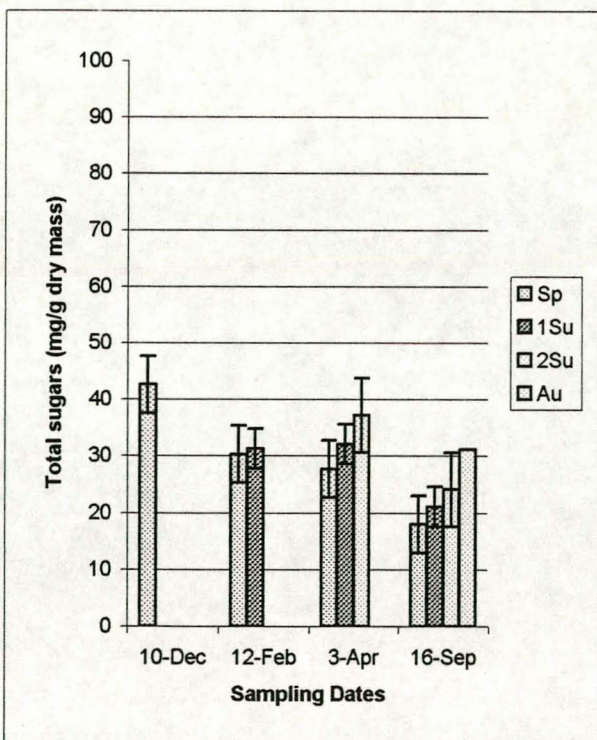


Figure 9c. Concentration of total sugars in 'Sylvia' stems per flush.

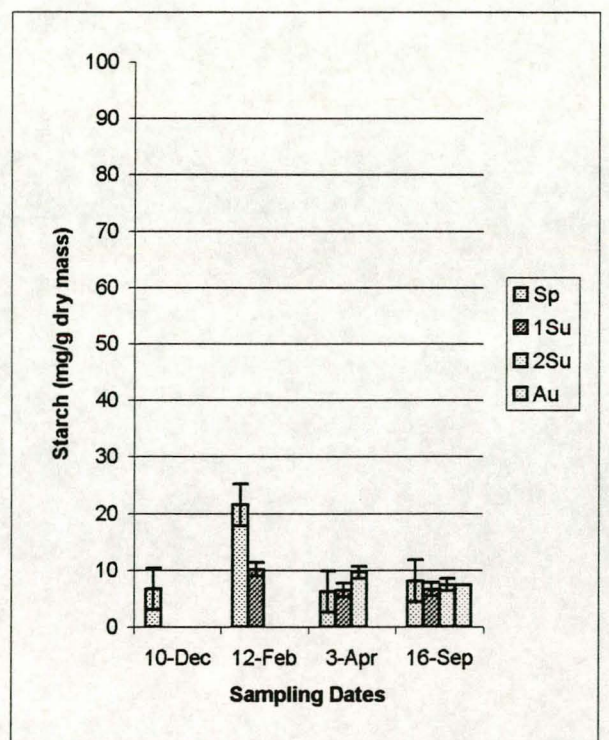


Figure 9d. Concentration of starch in 'Sylvia' stems per flush.

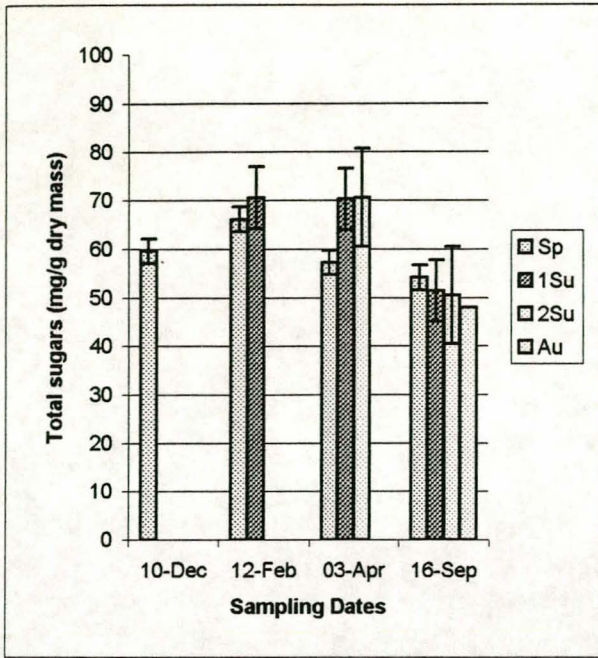


Figure 9e. Concentration of total sugars in 'Cardinal' leaves per flush.

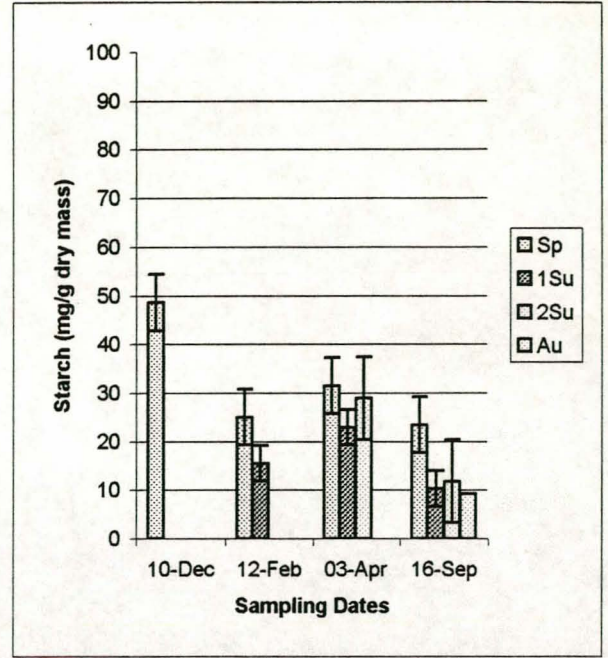


Figure 9f. Concentration of starch in 'Cardinal' leaves per flush.

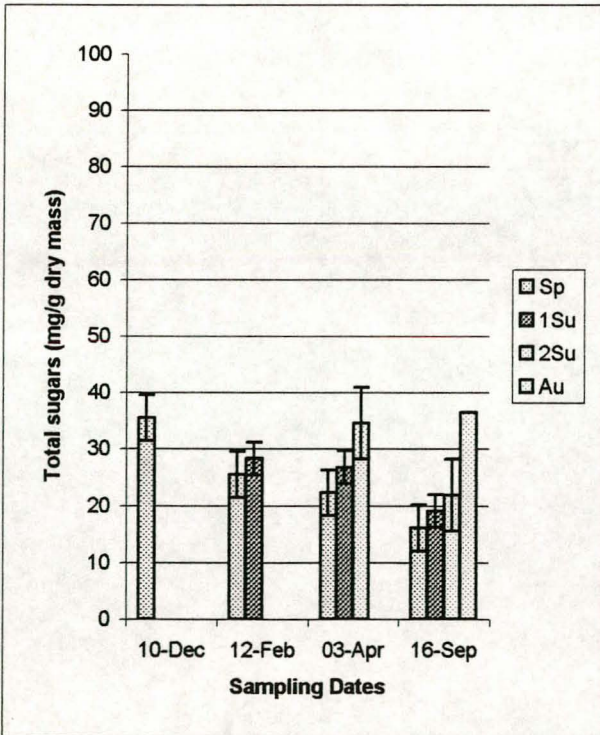


Figure 9g. Concentration of total sugars in 'Cardinal' stems per flush.

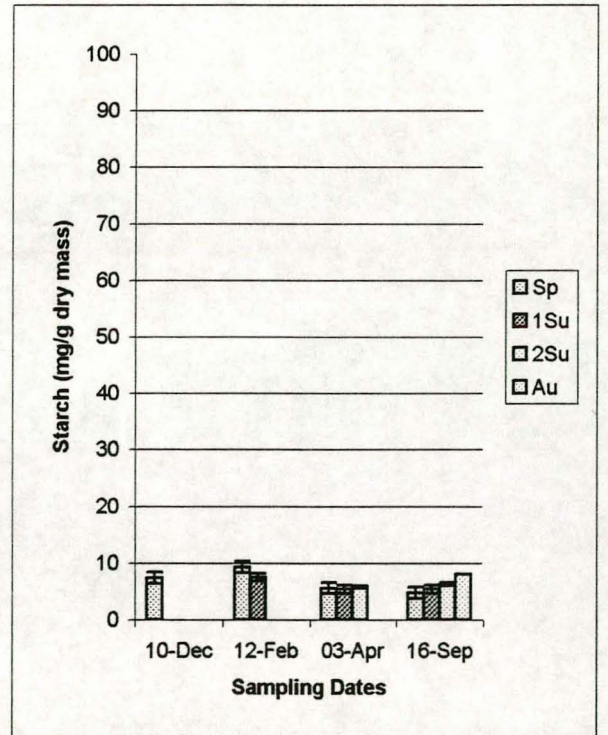


Figure 9h. Concentration of starch in 'Cardinal' stems per flush.

PAPER 3: DRY MATTER PARTITIONING AND CARBOHYDRATE ALLOCATION IN WHOLE PLANTS OF *PROTEA* CV. SYLVIA

INTRODUCTION

Successful manipulation of a plant's vegetative and reproductive cycles depends largely on an understanding of the growth dynamics of the plant. In this paper the changes in dry matter partitioning and the allocation of storage carbohydrates in the plant are examined over the winter period when no vegetative shoot growth took place. Paper II deals with the dry matter accumulation and storage carbohydrate allocation of successive vegetative shoot flushes. Due to the difficulties in extracting roots from a soil medium, plants were grown in nursery bags in an artificial medium, which could be easily washed off the roots. Under normal field growing conditions in the Western Cape three to four vegetative flushes are formed during the year, one in spring, one or two in summer and one in autumn. Secondary thickening of the stem or trunk, and root growth takes place in winter (De Swardt, 1989). The plants in the bags in this experiment all formed shoots with three or four flushes during the second growing season, before the sampling took place. The aim of this trial was to establish the extent of secondary thickening of the shoots and stem (trunk) and the development of the roots. In addition, the accumulation and allocation of storage carbohydrates was studied to determine changes in source-sink relationships over time.

MATERIALS AND METHODS

Plant material

Two-year-old *Protea* cv. *Sylvia* (*Protea eximia* x *Protea susannae*) plants grown in 10l pots in a nursery were used. The pots were placed in an unshaded area of a commercial

protea nursery in Elgin, Cape Province, South Africa (34°09' S, altitude 340 m). The area has a Mediterranean climate with a mean annual rainfall of 960 mm. Overhead irrigation was applied twice a day during summer and once a day during spring, autumn and winter. The potting mix consisted of one part coco-peat, one part polystyrene granules and two parts coarse sand. A commercial slow release fertiliser, Osmocote Plus (17:3:10 + 1 MgO + trace elements), was applied to the potting mix at the recommended rate of 1g/l.

Rooted cuttings were planted in the pots in June 1996 and pinched and pruned to attain complexity (as described in Paper I). In August 1997 the shoots were cut back to bearers and subsequent shoot growth thinned to a maximum of six shoots per bearer, and left to develop through the 1997/98 growing season.

Treatments and experimental design

Two treatments with 10 replications per treatment were laid out in a randomised complete block design. The two treatments were two sampling dates, 14 July 1998 and 15 September 1998. These two dates corresponded with the completion and hardening-off of the autumn vegetative flush and the beginning of spring bud break or flower bud development, respectively. Each replication was represented by a whole plant. On each of the two treatment dates, 10 pots were randomly selected and the leaves, stems (shoots after removal of the leaves), trunk and roots of each plant separated for laboratory analysis of dry mass, starch and sugar concentrations.

Dry mass and carbohydrate analysis

Leaves, stems, trunk and roots were placed in paper bags, lyophilised and milled to a fine powder to determine the total dry mass of each treatment. A sample was then taken for laboratory analysis of total sugars and starch. Each sample was extracted overnight in 1% acetic acid by shaking and thereafter centrifuged. The supernatant was filtered and brought to volume. Thereafter the pellet was taken up in an acetate buffer (pH 4.8) and gelatinised in a boiling steam bath for two hours. After cooling to 60°C the enzyme amyloglucosidase was added and incubated for 18 hours facilitating the hydrolysis of

starch to glucose. Further analysis for reducing sugars and starch (glucose) were done on a Sanplus Segmented Flow Analysis System from Skalar using Method No. 551-965w/r issue 070798/MH and No. 365-001w/r issue 012998/MH/97203066.

For the automated determination of reducing sugars the sample was mixed and dialysed against a sodium carbonate solution. After addition of a copper neocuprion reagent, the steam was heated to 97 °C. The copper neocuprion chelate was reduced by the present sugars, forming a yellow cupro-neocuprion complex which was measured colorimetrically at 460 nm. To include non-reducing sugars the sample extract was hydrolyzed with β -fructosidase prior to the above procedure.

The automated determination of glucose is based on an enzymatic reaction whereby the glucose in the sample is oxidised to gluconic acid by glucose oxidase. Hydrogen peroxide, which is formed simultaneously, reacts with an indicator reagent (peroxidase hydroxybensoate-4-amino-phenazone) to form a stable red quinone compound which is then measured colorimetrically at 520 nm. The intensity of the absorption is directly proportional to the concentration of d-glucose in the sample.

Statistical analysis

The General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) was used to analyse the data (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

Dry matter partitioning

None of the plant parts analysed for dry mass: leaves, stems, trunk or roots showed any significant change in total dry mass over the period between the two treatments (Table 1). Although an increase in dry mass would not be expected in the case of leaves, secondary thickening of the stems and the trunk, as well as root development would be expected. In the case of this experiment, the time period of two months between the treatments may

have been too short to result in significant differences. A larger sample (more repetitions) may also have given significant differences as can be deduced from the large L.S.D. values of the data presented in Table 1.

Carbohydrate allocation

Leaves: In the leaves, both starch and total sugars concentrations increased significantly from the first sampling date to the second (data not shown). Starch increased from 9 mg.g⁻¹ dry mass to 63 mg.g⁻¹ dry mass and total sugars from 46 mg.g⁻¹ dry mass to 83 mg.g⁻¹ dry mass. The increase in starch concentration over this period indicates that energy, in excess of that needed for normal respiration, was being produced, and partitioned to storage starch in the leaves. The higher concentration of sugars in the leaves at the second sampling date indicates a higher metabolic rate of the plants at this time i.e. spring compared to winter.

The total tissue content of both total sugars and starch was also significantly higher in September than in July (Fig. 1 & 2). Borrás *et al.* (1984) noted higher nutrient flow to the vegetative buds at times of higher cytokinin levels in the buds. The September sampling date in this trial coincided with the onset of spring bud break, at which time the hormonal activity is high. Sanz *et al.* (1987) noted that changes in carbohydrate levels are brought on by a combination of sink strength and hormonal activity. At the second sampling date, active shoot growth had not commenced, thus the sink was probably already being strengthened by hormonal activity.

The two sampling dates correspond to winter and spring. The much higher total sugars concentration at spring sampling can also be attributed to warmer air and soil temperatures, especially as the plants were being grown in bags. At both sampling dates, starch and total sugars in the leaves represented 50% or more of the starch and total sugars in the whole plant. In the case of starch, the major reserve component of carbohydrates in the plant, the leaves contained 91% of the total starch in the plant at the second sampling date. Greenfield *et al.* (1995) also reported reserve carbohydrates in the wood and bark of *Protea* cv. Carnival to be low throughout the year.

Stems: As shown in Paper II, the stems were not as important a source of starch accumulation as the leaves. Concentration of starch in the stems of whole plants in this experiment were 6.8 mg.g^{-1} dry mass in mid-June and 5.8 mg.g^{-1} dry mass in mid-September (data not shown). Higher concentrations of total sugars at the second sampling date again point to higher metabolic activity at this time.

Although the tissue content of total sugars in the stems increase significantly from 1.2 g in mid-June to 2.4 g in mid-September (Fig. 1), tissue content of starch remained unchanged at 0.2 g at both sampling times (Fig. 2). The higher levels of total reducing sugars in the stems at the second sampling date reflects the increase in the tissue content of the leaves at this time. Due to the high production of sugars in the leaves, translocation was increased. However, the stems were comparatively unimportant for the storage of carbohydrates in the form of starch. Greenfield *et al.* (1995) also reported reserve carbohydrates in the wood and bark of *Protea* cv. Carnival to be low throughout the year.

Trunk: Greenfield *et al.* (1995) showed a significant increase in starch concentration between 2 July and 1 October in the bark of two-year-old branches of *Protea* cv. Carnival (*Protea compacta* x *Protea neriifolia*), although no significant difference was shown in the starch concentration of the wood over the same time. The percentage starch found in the bark of two-year-old stems of *Protea* cv. Carnival by Greenfield *et al.* (1995), varied between 0.16% and 0.36%, measured at monthly intervals over a period of one year. At the two sampling dates in this experiment, percentage starch in the two-year-old stems (trunks) of *Protea* cv. Sylvia was 0.45% and 0.35% respectively, although this did not represent a significant difference (data not shown). The plants in this experiment were younger and grown in favourable nursery conditions, with irrigation and fertilisation, compared with those used by Greenfield *et al.* (1995) which were six-years-old and grown in a commercial plantation without irrigation or fertilisation. The tissue content of starch between the two sampling dates in this experiment showed a significant decrease from 0.10 g to 0.06 g (Fig. 2), indicating that reserve carbohydrates were depleted over

this period. The amount is, however, negligibly small in comparison to leaf starch content.

Concentration of total sugars in the trunk increased from 29 mg.g⁻¹ dry mass at the first sampling date to 41 mg.g⁻¹ dry mass at the second sampling date (data not shown). Again this points to increased metabolic activity in the plants during September compared to July. However the tissue content of total sugars showed no significant increase between the two sampling dates (Fig. 1). The percentage reducing sugars found in the bark and wood of *Protea* cv. Carnival by Greenfield *et al.* (1995) varied between 2% and 4% in the bark and between 0.5% and 1.1% in the wood, measured monthly over the period of one year, but did not vary significantly in the period between July and September.

Roots: Roots of protea grow actively during autumn and spring, therefore becoming strong sinks for metabolites, including carbohydrates, during this time. The concentration of total sugars in the roots of *Protea* cv. Sylvia plants in mid-September was 44 mg.g⁻¹ dry mass, compared to 12 mg.g⁻¹ dry mass in mid-July (data not shown). Tissue content of total sugars also increased from 0.25 g to 0.83 g (Fig. 1). Starch tissue content did not change and remained at very low levels, 0.05 g and 0.07 g, respectively (Fig. 2).

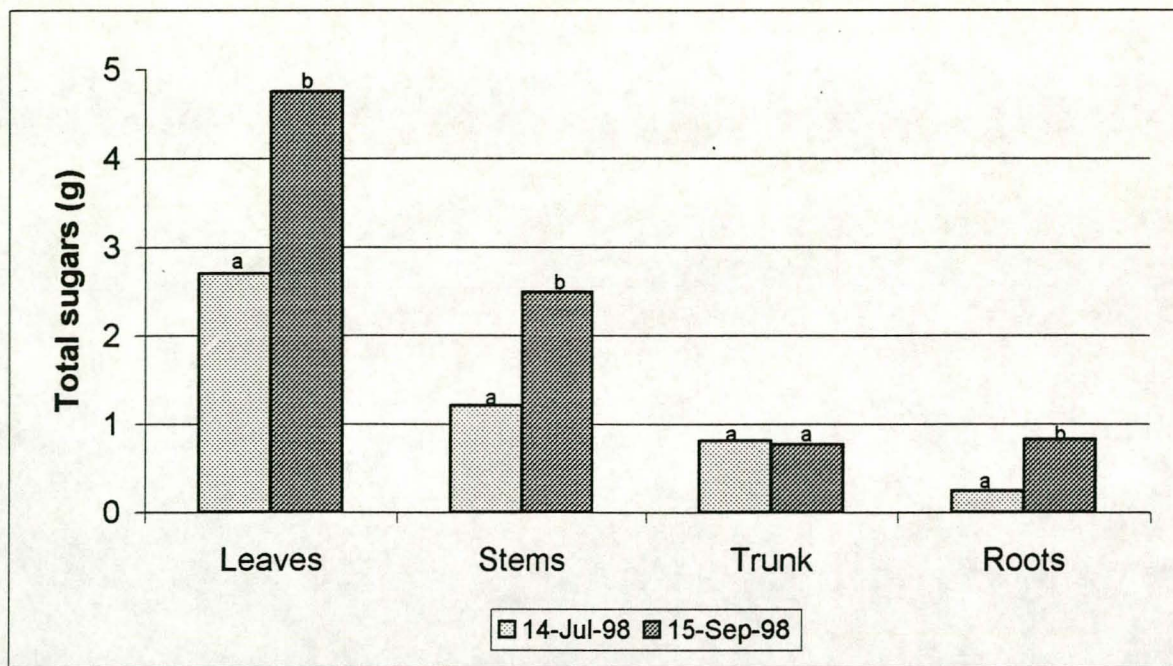
No significant dry mass accumulation was found in the leaves, stems, trunk or roots of *Protea* cv. Sylvia plants grown in bags over a two month period during the winter. The majority of reserve carbohydrates (total sugars and starch) in terms of tissue content was found in the leaves at both sampling dates, showing that newly produced carbohydrates outweigh storage carbohydrates in importance. Although the amount of total sugars was significantly higher in both stems and roots at the second sampling date in spring, no increase in the amount of starch was recorded. Total sugars content of the trunk remained constant and starch content decreased over the winter period, although its relevance in terms of total plant starch was minimal.

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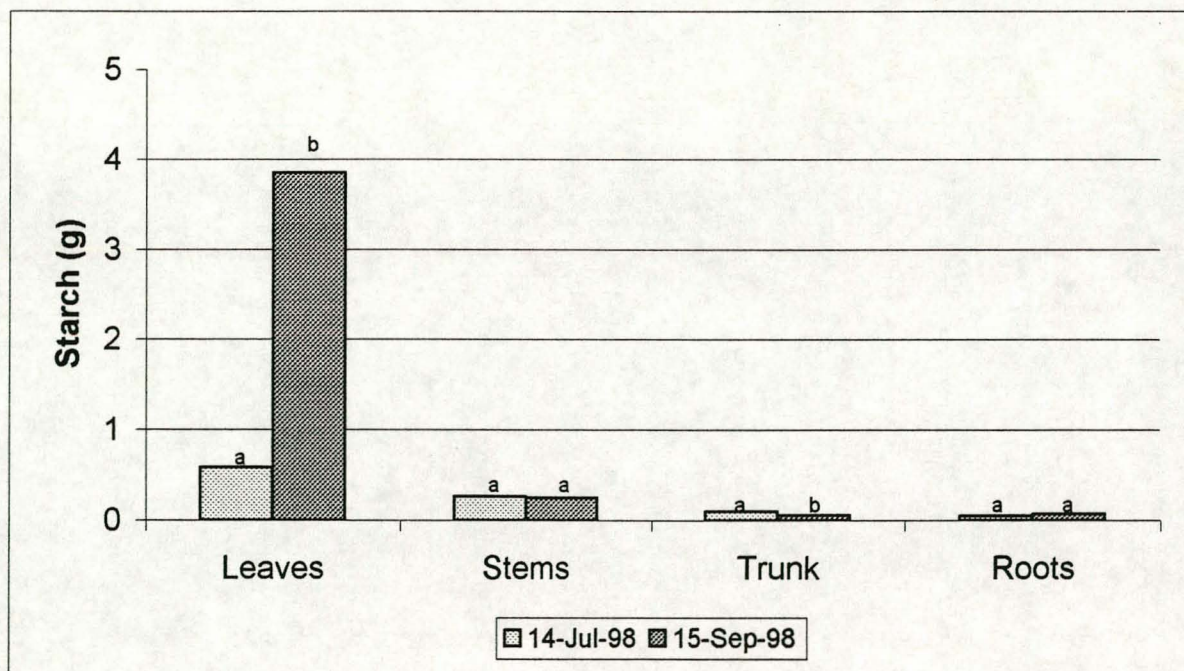
Table 1. Dry mass of different plant organs over time of *Protea* cv. *Sylvia*.

		Leaves	Stems	Trunk	Roots
14 July 1998		55.61 ^a	36.02 ^a	22.01 ^a	19.43 ^a
15 Sept. 1998		57.01 ^a	41.39 ^a	18.30 ^a	19.74 ^a
L.S.D. (0.05)		18.715	14.767	5.4645	6.4054
Contrasts	DF	Significance	Significance	Significance	Significance
Between dates	1	0.8764	0.4547	0.1713	0.9183



L.S.D. (0.05): Leaves=1.3893; Stems=0.8086; Trunk=0.4380; Roots=0.1775

Figure 1. Tissue content of total sugars over time in different parts of 'Sylvia' plants.



L.S.D. (0.05): Leaves=1.3723; Stems=0.1351; Trunk=0.0264; Roots=0.0277

Figure 2. Tissue content of starch over time in different parts of 'Sylvia' plants.

GENERAL DISCUSSION AND CONCLUSIONS

With the advent of high input cultivation of proteas, many questions of technical and practical nature have arisen. Although some questions can be answered purely in a practical manner, others firstly require a knowledge of the relevant physiology. This study has aimed to answer some practical questions as well as to lay the basis of theoretical knowledge of aspects of physiology which are essential for the understanding of how the plant functions.

To optimise return on investment in intensive protea plantings, vegetative development of young plants must be manipulated to produce an economically viable crop as soon as possible after planting. Planting sub-terminal cuttings of *Protea* cv. Sylvia and pinching or cutting back successive growth flushes during the first year of growth, improves the plant complexity and minimises wind damage and pruning mortality. Instead of having a single thick shoot after one year of growth, six to eight shoots are formed from which bearers can be chosen. By thinning the resultant growth from the bearers to six shoots per bearer, shoots which would not develop into economically viable units were minimised. This work lays the basis for young *Protea* plant manipulation. As potential total bio-mass production increases, the limit to increasing vegetative complexity without compromising the quality of the economically valuable units (flowers), will be adapted. While this work was carried out only on *Protea* cv. Sylvia, it provides a model for the vegetative development of all non-lignotuberous *Protea* varieties grown from cuttings.

Knowledge of the carbohydrate dynamics of a plant is a prerequisite for any judicious plant manipulations of bio-mass production and allocation. The dry mass accumulation of new shoots developing on bearers of *Protea* cv. 'Sylvia' and *Protea* cv. 'Cardinal' was studied together with the carbohydrate allocation in the successive vegetative shoot flushes. These studies showed that dry mass and carbohydrate accumulation were extremely low initially, with more significant accumulation only after completion of the second summer flush. The time taken for a flush to reach a set amount of dry mass or carbohydrate content decreased for every successive shoot flush. By the end of the

growing season, dry mass in the spring flush (most proximal) was partitioned more in favour of the stem while in the autumn flush (most distal) dry mass was partitioned more in favour of the leaves. Carbohydrate accumulation in the leaves was much higher than in the stems of shoots. Total sugars made up more than two thirds of the total carbohydrate content of both leaves and stems. While total sugar levels in the leaves and stems of the whole shoot remained stable over the winter period, starch levels decreased in the leaves and increased slightly in the stems. Compared to fruit trees, whether citrus or apple, the actual values of reserve carbohydrates found in both *Protea* cv. 'Sylvia' and *Protea* cv. 'Cardinal' in this study were extremely low. It is clear that these plants do not store large amounts of carbohydrates as in apple and are primarily dependant on carbohydrates being produced through photosynthesis on a day to day basis.

Although the studies of the dry matter partitioning and carbohydrate allocation of the different plant parts of whole *Protea* cv. 'Sylvia' plants over time did not produce the expected changes, this study confirmed that the leaves are the predominant point of accumulation of carbohydrates in the plant and that storage of carbohydrates in all the plant parts (including the leaves) was extremely low.

Further work should be done to quantify the dry matter and carbohydrate dynamics of *Protea* in the reproductive phase.

7. STATISTICAL ANALYSIS OF DATA

The statistical analysis of the experimental data presented in this thesis can be found on a diskette stored on the inside back cover of the thesis. Data files are in Exel and the results of the statistical analysis are saved as SAS files.

Paper One:

ESTCOMP.exl

ONEYSAS.exl

PERCENTAGE NON FLOWERING SHOOTS.exl

PERCENTAGE SHOOTS WITH ONLY 1 OR 2 FLUSHES.exl

% NON FLOWERING SHOOTS.sas

% SHOOTS WITH 1 OR 2 FLUSHES.sas

AVERAGE SHOOT LENGTH.sas

SHOOTS AT END OF YEAR ONE.sas

Paper Two:

Dry Mass Sylvia Leaves.exl

Dry Mass Cardinal Leaves 1S.sas

Dry Mass Cardinal Leaves 2S.sas

Dry Mass Cardinal Leaves Sp.sas

Dry Mass Cardinal Stems 1S.sas

Dry Mass Cardinal Stems 2S.sas

Dry Mass Cardinal Stems Sp.sas

Dry Mass Sylvia Leaves 1S.sas

Dry Mass Sylvia Leaves 2S.sas

Dry Mass Sylvia Leaves Sp.sas

Dry Mass Sylvania Stems 1S.sas

Dry Mass Sylvania Stems 2S.sas

Dry Mass Sylvania Stems Sp.sas

Paper Three:

Whole plant dry mass LEAVES.exl

Whole plant dry mass ROOTS.exl

Whole plant starch and sugar LEAVES.exl

Whole plant starch and sugar ROOTS.exl

Whole plant starch and sugar SHOOTS.exl

Whole plant starch and sugar STEM.exl

Whole plant dry mass.sas

Whole plant starch LEAVES.sas

Whole plant starch ROOTS.sas

Whole plant starch SHOOTS.sas

Whole plant starch STEM.sas

Whole plant sugars LEAVES.sas

Whole plant sugars ROOTS.sas

Whole plant sugars SHOOTS.sas

Whole plant sugars STEM.sas