EFFECTS OF DEFOLIATION ON REGROWTH AND CARBON BUDGETS OF THREE SEMI-ARID KAROO SHRUBS

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ABSTRACT

Plant regrowth, nonstructural carbohydrate utilization patterns, photosynthesis and the partitioning of photosynthetic products in response to foliage removal were studied for the following karoo shrubs: *Osteospermum sinuatum*, a dwarf deciduous shrub with fleshy leaves, *Pteronia pallens*, a dwarf evergreen shrub with sclerophyllous leaves and *Ruschia spinosa*, a dwarf evergreen shrub with succulent leaves. Defoliation adversely affected both vegetative growth and reproductive output for periods up to 26 weeks following foliage removal. A gradient of increasing regrowth capacity with decreasing defoliation intensity and frequency was observed in all species. In terms of biomass production, defoliation was the least detrimental to the deciduous shrub, *O. sinuatum*, and the evergreen shrub, *P. pallens*, and the most injurious to the succulent shrub, *R. spinosa*. All species regrew better during spring and autumn, and no regrowth was recorded in the moderate (40%) or intensely (80%) defoliated plants during summer and winter over the 6-week monitoring periods. Spatial patterns of carbohydrate accumulation were the same for all species, with most of the total nonstructural carbohydrates (TNC) being stored in the twigs and stems. Karoo shrubs can be divided into two distinct groups based on the primary nonstructural polysaccharides accumulated in their plant parts. The Asteraceous plants, *O. sinuatum* and *P. pallens*, accumulate predominantly fructans. In contrast, the succulent species, *R. spinosa*, accumulate starch and fructans in equal proportions. Differences among species in terms of seasonal changes in TNC levels of undefoliated plants reflect the extent to which different species are dependent on stored carbohydrates or photosynthesis for normal vegetative growth processes. Repeated defoliations at a moderate frequency (26-week interval) resulted in the elevation of TNC concentrations of *O. sinuatum* and *P. pallens*. In contrast, defoliations at heavy or at leniant frequencies caused decreases in TNC concentrations in all plant parts of *Ruschia spinosa*. Restoration of plant storage TNC levels in excess of undefoliated plant TNC levels occurred prior to complete vegetative regrowth in the two Asteraceous shrubs which suggests that some factor(s) other than the carbon resource was limiting vegetative regrowth in karoo shrubs. Analyses of short-term changes (2-weekly) in TNC levels in response to defoliation demonstrated the elevation in TNC concentrations of *Pteronia pallens* plant parts only during the periods when no regrowth was recorded. This phenomenon

illustrates that on a short-terms basis, regrowth and over-replenishment of reserves represent two alternate responses to defoliation. However, during periods when regrowth was recorded for *P. pallens* (autumn and spring), and during all seasons of the year for *O. sinuatum* and *R. spinosa*, depressions in TNC concentrations were observed in most plant parts up to six weeks following defoliation. This illustrates the large dependence these shrubs have on stored carbohydrates following defoliation. Defoliation had no effect on the photosynthetic rates of karoo shrubs for at least 11 days following defoliation. Foliage removal resulted in the redistribution of photoassimilates in all plant species. These changes in the allocation of newly produced photosynthates appear to be associated largely with the replenishment of carbohydrate reserves following the initial TNC utilization caused by foliage removal. Comparison of TNC utilization patterns, following defoliation of *O. sinuatum* in the dark (no photosynthesis) and in the light resolved the question of the relative importance of reserve carbohydrates and photosynthates following defoliation. Reserve carbohydrates were used only for the first 2 weeks following defoliation for respiratory functions while photoassimilates were used for the production of new foliage. The magnitude of nonstructural carbohydrate utilization in the absence of photosynthesis emphasized the importance of continuing photosynthesis to the survival of defoliated karoo shrubs. The differences among species in terms of the timing and the extent of changes (elevations or decreases) in TNC levels in response to defoliation are interpreted as being the result of alterations in plant chemistry which in turn are governed by species specific physiolgical adaptations to environmental constraints. Rangeland management guidelines are recommended within the framework of the observed short-term and long-term defoliation effects on karoo shrub plant production.

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CONTENTS

CHAPTER 1
INTRODUCTION

1.1 ARID ECOSYSTEMS IN PERIL

Arid ecosystems are characterized as regions with a mean annual rainfall of 60 to 500 mm and where biological processes are controlled primarily by the infrequent, discrete and largely unpredictable nature of rainfall events (Noy-Meir, 1973). A number of plant growth forms are found in arid zones which seldom occur in other ecosystems. These unique patterns of plant morphological diversification which have occurred in arid regions are probably the result of the evolution of a broad range of adaptations by plants to utilize efficiently the limited and infrequent moisture supply (Cody, 1989). Historical and scientific evidence indicate that agricultural practices in arid zones of the world are primarily reponsible for the extinction of arid zone plant species, changes in species composition, decreases in primary productivity and the concomitant lowering of economic potential (Schlesinger *et al.*, 1990), putting both biological diversity as well as human welfare in arid regions at risk.

The soils of arid zones often have inherently low nutrient levels and this is further exacerbated by slow microbial nutrient return processes which are limited by the lack of water (Noy-Meir, 1974; Hadley & Szarek, 1981). Low nutrient levels and lack of water appear to be the primary factors limiting plant growth and development in these regions. Other factors which may limit plant growth in arid zones are strong and dry winds, extremes of temperatures, saline soils and stress associated with high light intensities (Berry *et al.*, 1983). Native invertebrate and vertebrate herbivores do not appear to have a detrimental effect on total primary production of arid zones (Noy-Meir, 1974; Hadley & Szarek, 1981). The high degree of seed consumption by small mammals, however, can have a significant effect on future plant populations (Chew & Chew, 1970).

The greatest stress factor affecting productivity of arid ecosystems, however, is the grazing impact of large domestic animals which may consume up to 75 % of the total primary productivity (Noy-Meir, 1974). Overgrazing by livestock is exceptionally severe in arid

ecosystems because of the suite of environmental stress factors which limit vegetative as well as reproductive growth. Moreover, during prolonged droughts, livestock are forced to feed on plants which have not fully recovered from prior grazing, which often leads to irreversible damage (Noy-Meir, 1974). Over-utilization of the arid ecosystem vegetation by livestock leads towards the creation of desert-like conditions. Desertification has become a world wide threat and is likely to become even worse with global climate warming phenomena (Schlesinger *et al.*, 1990). Addressing the problem of desertification, which has too often been associated with livestock production, ultimately necessitates a knowledge of how much, how often and when grazing can be undertaken. This in turn relates to an understanding of fundamental plant physiological processes. Comparatively little is known of arid zone plant physiology or how it relates to plant form. Moreover, the physiological understanding of the growth responses following defoliation of arid zone plants is lacking, although it is essential for the effective grazing management of arid ecosystems.

1.1.1 Karoo Ecosystems

The semi-arid to arid zone of South Africa incorporates the karoo biome (Venter *et al.*, 1986) which covers 31.4% of southern Africa south of 22^{0}S parallel (Rutherford & Westfall, 1986). The vegetation of the Karoo consists of dwarf shrublands and arid grasslands which in former times have been utilized by a wide range of migratory ungulates (Roux & Theron, 1987). Replacement of these migratory grazers by enclosed domestic stock (goats and sheep) marked the change in plant utilization patterns (Roux & Theron, 1987) which have resulted in the change in plant composition and phytomass. Overstocking and the ensuing overgrazing is considered as the most common cause for the desertification processes of the Karoo (Acocks, 1955; Roux & Voster, 1983). Karoo rangeland deterioration, which is a result of ecologically inopportune grazing practices, has been acknowledged for over a century (Cowling *et al.*, 1986).

There is a distinct lack of scientific evidence to indicate to what extent karoo plants can be utilized (defoliated), hence the management of karoo rangelands to maintain them as a viable natural resource is problematical. Resolving the dilemma between continued livestock production and the maintenance of ecological processes in the Karoo requires grazing practices based on a thorough understanding of the plant physiological processes which

determine vegetative and reproductive development following defoliation.

The investigation reported on in this thesis was carried out as part of the Karoo Biome Project which aimed to develop a fundamental, and predictive understanding of the semi-arid karoo ecosystems. The Karoo Biome Project was co-ordinated and funded by the Foundation for Research Development of the Council for Scientific and Industrial Research, South Africa (Cowling, 1986). With the initiation of the Karoo Biome project, investigations of plant adaptive physiology in relation to climatic and defoliation regimes were considered as a research field where studies were urgently required in order to effectively deal with the ecological problems of the karoo biome (Cowling, 1986). This thesis was undertaken to address this deficiency in the understanding of karoo plant function in relation to foliage removal.

1.2 DEFOLIATION STUDIES

Attempts to resolve the question of ecologically prudent grazing can be divided into two principal experimental approaches. The first approach dominates the literature and comprizes of extensive grazing trials which aim to establish a threshold level of grazing above which the status of some species in the community is impaired (Coetzee, 1975; Brown, 1985; Sala *et al.*, 1986; Heitschmidt *et al.*, 1987; Gibb *et al.*, 1989; Weigel *et al.*, 1989). The disadvantages of grazing trials are the long duration of these studies and the difficulty of generalizing the results. The second approach involves the quantification of plant growth and development of individual species and their responses to defoliation. Study species should be selected in such a way that different guilds are represented which would enable the extrapolation of the observed defoliation responses to other plant communities of the ecosystem. These studies entail the measurement of biomass yields and allocations to various organs for plants defoliated at different intensities, frequencies, seasons, or combinations of these (Tainton *et al.*, 1970; Wright, 1970; Cook & Child, 1971; Hobson & Sykes, 1980; McLean & Wikeem, 1985). Investigations of this nature have been augmented by studies involving fundamental plant physiological responses in order to define more accurately the factor(s) which determine the vegetative and reproductive growth responses following

defoliation. These mechanistic physiological studies have been largely concerned with the effects of leaf removal upon plant nutrient relations and plant carbon economy. Most studies of the effects of defoliation on plant nutrient relations have demonstrated alterations in nutrient allocation patterns following photosynthetic tissue removal. For example, increased nutrient investment has been demonstrated in new leaves (Chapin, 1980) and in remaining leaves (Ericsson *et al.*, 1985) following defoliation. Reports of increases in nutrient uptake rates (Chapin & Slack, 1979; Ruess *et al.*, 1983; Ruess, 1988; McNaughton & Chapin, 1985) support observations of elevated plant nutrient levels following defoliation.

Studies of plant responses to defoliation have most often been concerned with aspects of plant carbon balance because leaf removal effectively removes the photosynthetic organs of the plant thereby reducing the potential for carbon acquisition. Since the effects of defoliation on components of plant carbon economy comprise a major part of this research project, studies related to these aspects will be considered in some detail in section 1.3.

1.3 EFFECTS OF DEFOLIATION ON PLANT CARBON BUDGET

Many defoliation studies have quantified levels of storage carbohydrates (reserves) because this group of carbon based compounds are thought to be closely associated with plant vigour. Carbohydrates are stored when carbohydrate production exceeds immediate demands such as respiration, growth and production (Crawley, 1983). This classical definition of storage in plants has been recently questioned by Chapin *et al.*(1990). The alternative definition of storage by these authors includes the allocation to defence, growth and accumulation. The traditional definition of carbohydrate reserves, often used by ecologists, rangeland scientists and whole plant physiologists, includes a range of carbon based compounds such as mono- and disaccharides, oligosaccharides, fructans and starch (White, 1973).

Many studies have observed a decrease in the amount or concentration of nonstructural carbohydrates after defoliation and have therefore implied a causal role for these compounds in initiating regrowth (Kinsinger & Hopkins, 1961; Steinke & Booysen, 1968; Bartholomew & Booysen, 1969; Trlica & Cook, 1971; Buwai & Trlica, 1977). Differences in carbohydrate concentrations between plant species or between defoliation treatments of the

same species have therefore often been used to explain recovery rates following grazing. The evidence provided by earlier studies such as those by Weinmann (1948 & 1961) and Sullivan & Sprague (1943) led to the widely used assumption that sufficient carbohydrate reserves are required for regrowth following defoliation. Such generalizations are implicit in many defoliation studies where changes in biomass are recorded. Another point of concern with many of the earlier studies is the manner in which carbohydrate levels have been expressed. Concentrations of carbohydrates have been used almost exclusively in the published literature to quantify carbohydrate reserves. However, carbohydrate concentrations may not be indicative of carbohydrate content (pool size) of plant tissues, the latter being the product of "concentration" and the "size of organ". It has been illustrated that an improved appreciation of the importance of carbohydrates for foliage regrowth can be obtained when pools rather than concentrations are considered (Caldwell *et al.*, 1981; Richards, 1986).

Although it has been emphasized by many that carbohydrate reserves are an overriding determinant of foliage regrowth following defoliation, there has been some opposition to this tradional concept (May, 1960; Ryle & Powell, 1974; Richards & Caldwell, 1985; Richards, 1986). Experimental evidence for the school of thought which disputes the paramount significance of carbohydrate reserves for regrowth has taken the form of quantification of the relative contribution of both stored carbohydrates and current photosynthates (produced by remaining leaf biomass) to regrowth (Ryle & Powell, 1975; Richards and Caldwell, 1985; Danckwerts & Gordon, 1987). Radioactive tracer experimentation to determine the exact role of current photosynthates as a resource for regrowth (Ryle & Powell, 1975; Danckwerts & Gordon, 1987) or etiolated regrowth techniques to examine the relationship between soluble carbohydrates and foliage regrowth (Richards & Caldwell, 1985) have been used in these studies. It was concluded that most of the vegetative growth following defoliation can be attributed to photosynthesis and very little to reserve carbohydrates. It is also evident from these studies that (i) the length of time that the defoliated plant is reliant on the stored carbohydrates and (ii) the quantitative contribution of photosynthates and reserve carbohydrates to regrowth differs among species.

Another aspect of plant carbon balance that has been used to evaluate differences in regrowth capacity among species is the rate of photosynthesis. Increases in photosyntetic rates of the

remaining leaves or the new leaves (following defoliation) have been reported for a number of species (Hodgkinson, 1974; Alderfer & Eagles, 1976; Detling *et al.*, 1979; Painter & Detling, 1981; Wallace *et al.*, 1984). It has been hypothesized that the stimulated productivity following defoliation of many species, specifically grasses, can be attributed to elevated photosynthetic rates (Wallace *et al.*, 1984).

1.4 OBJECTIVES OF THE THESIS

In order to obtain an appreciation of optimal plant utilization (grazing regimes) of the karoo flora, the factors which determine plant responses to defoliation need to be clearly understood. Because leaf removal decreases total plant photosynthetic carbon gain, this thesis considered how plant carbon balance relates to plant growth responses of karoo shrubs following defoliation. The published literature on defoliation studies is dominated by reports of defoliation responses of grasses and economically important crops and trees. The carbon economy of shrubs in relation to defoliation has been studied to a far lesser extent. The responses of arid zone plants (Senock *et al.*, 1988), in particular, have not been well documented. This thesis endeavours to alleviate this deficiency in the literature. The primary aim of this thesis is to determine the effects of experimental defoliation on the carbon economy of selected karoo shrubs (from various plant guilds) and to relate this to measured plant growth responses following defoliation. The experimental component of this thesis consists of 6 parts which have been designed and executed as separate studies and which correspond with Chapters 2 to 7. Each chapter, therefore, has its own introduction, methodology, results and discussion sections. One of the first objectives was to determine the effects of intensity, frequency and season of defoliation on the regrowth potential of three karoo shrubs representing different leaf consistency classes, palatabilities and rooting depths (Chapter 2). The second objective involved the quantification of individual nonstructural carbohydrate components, characterization of reserve carbohydrate groups and the determination of spatial patterns and sizes of carbohydrate pools in these woody and succulent karoo shrubs (Chapter 3). The effects of different frequencies (Chapter 4), seasons and intensities (Chapter 5) of experimental defoliation on the spatial variation in carbohydrate stores were also investigated. Defoliation effects on photosynthesis and the allocation of

recently assimilated carbon (using radiocarbon techniques) are considered in Chapter 6. A subsidiary aim of this study (Chapter 6) was to quantify the roles of reserve carbohydrates and current photosynthate in initiating regrowth after defoliation. Finally, using an etiolated regrowth technique, an exact relationship between regrowth and reserve carbohydrates was determined to provide a measure of the ability of the study species to mobilize stored carbohydrates and to produce new above-ground tissue without the confounding effects of current photosynthesis (Chapter 7). Since chemical defenses and a high regrowth capacity are often considered as two alternative strategies against herbivores, and since both these strategies represent carbon allocation routes in the plant, the effects of defoliation on tannins and polyphenolic compounds were also investigated. This project was an ancillary one to the thesis and was undertaken in co-operation with colleagues at the University of Cape Town. I undertook 33% of this work and include it as an Appendix to the thesis.

1.5 STUDY SITE AND SPECIES

Field studies were carried out at the Tierberg Karoo Research Centre (33°10'S, 22°17'E) near the town of Prince Albert on the southern boundary of the Great Karoo, South Africa. Average annual rainfall for the site is 167 $\pm$ 7mm (n = 92 years). Heavy rains often occur between February and May and dry periods are most likely between September and January (Milton *et al.*, in press). Total monthly rainfall and temperature minima and maxima for the period June 1988 to December 1989 are illustrated in Figure 1.1. Details of the study site have been described by Milton *et al.*(in press). Yeaton & Esler (1990) described the temporal and spatial patterns of seedling emergence and the relative abundance and distribution of plant species occurring at the Tierberg study site. Livestock had been excluded from the study site for two years prior to commencement of field studies.

Three study species were selected on the basis of their dominance in the study area and to represent various growth forms, leaf consistency classes and degrees of palatability. *Osteospermum sinuatum* [(DC.) T.Norl.], is a dwarf (< 0.25m) Asteraceous deciduous shrub with orthophyllous and fleshy leaves, and is one of the most palatable shrubs in the Karoo. *Pteronia pallens* (L.f), a dwarf (< 0.25m) Asteraceous evergreen shrub with ericoid

sclerophyllous leaves, is recognized as a poisonous plant causing livestock losses (Vahrmeijer 1981). *Ruschia spinosa* [(L.) H.E.K Hartm. & Stuber] (family: Mesembryanthemaceae) is a (< 0.25m) dwarf evergreen shrub with succulent leaves and spinescent stems. Although palatable, it is not a preferred species and is grazed less extensively than *O. sinuatum*. The three study species possess different rooting systems (personal observation). The tap root of *O. sinuatum* penetrates to a depth of 40 cm and is supported by lateral roots. *P.pallens* has a massive ramified system of roots extending to a depth of 40 cm. *R. spinosa* has a branching lateral root system which is confined to the upper 8 cm of the soil.

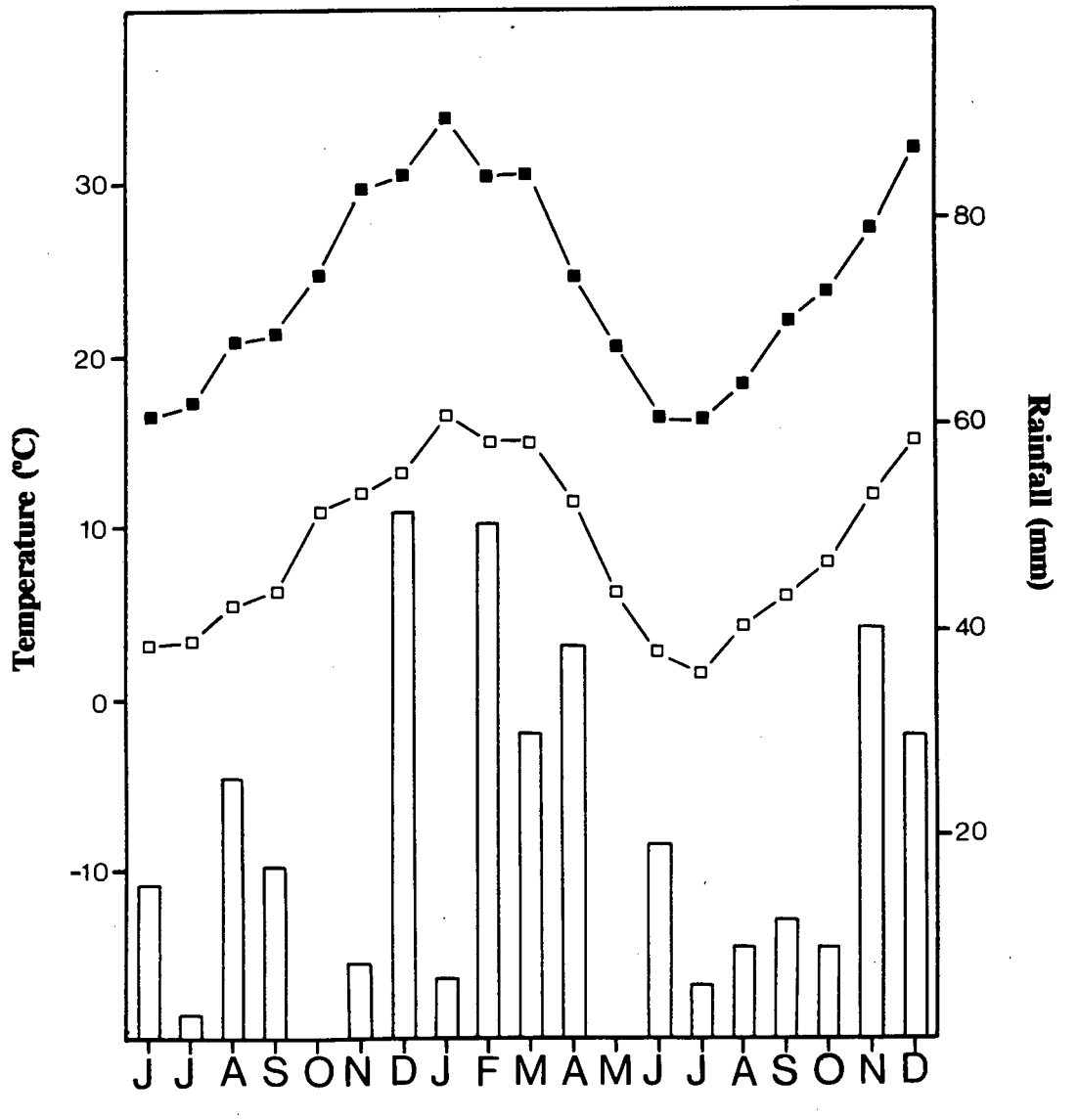

Figure 1.1: Monthly rainfall (histograms) and monthly maximum (closed squares) and minimum temperatures (open squares) recorded at Tierberg Karoo Research Centre, South Africa from June 1988 to December 1989. (adapted from Milton *et al.* in press)

CHAPTER 2

EFFECTS OF EXPERIMENTAL DEFOLIATION ON THE GROWTH OF THREE SEMI-ARID KAROO SHRUBS

2.1 INTRODUCTION

Problems associated with the over-utilization of rangelands for animal production have been universally acknowledged and studies have accordingly been undertaken in an attempt to provide management guidelines for sustainable production. Investigations have traditionally taken the form of extensive grazing trials aimed at establishing grazing threshold levels for different plant communities (Brown, 1985; Olson *et al.*, 1985; Sala *et al.*, 1986; Heitscmidt *et al.*, 1987; Welsch *et al.*, 1987). Such gross ecosystem type approaches have been augmented by intensive studies of the growth responses of individual species to various defoliation intensities and frequencies. The latter approach provides a valuable predictive basis for understanding species responses but it requires a considerable time investment since measurements of biomass yields and allocation patterns of a specific plant species defoliated at various intensities, frequencies, seasons or combinations of these (Garrison, 1953; Wright *et al.*, 1970; Mclean & Wikeem, 1985; Undersander & Naylor, 1987) all need to be carried out. In addition, defoliation effects in relation to factors such as watering frequency (Coughenour *et al.*, 1985b) and nutrient supply (Brown, 1986; McNaughton & Chapin, 1985; Simms, 1985) have also been studied. Such studies have focussed almost exclusively on the responses of temperate crops, pasture species and rangeland grasses with the effects of defoliation on growth of shrubs, especially shrubs from arid and semi-arid regions, being poorly studied.

Studies of the effects of leaf removal or tissue damage on plant growth and development have demonstrated that plants of different growth form differ widely in their responses to

defoliation. The effects of defoliation on the regrowth of grasses have been well documented and increases in plant production have often been reported. The occurrence of stimulated regrowth following defoliation of grasses is associated with increased photosynthetic rates (Detling *et al.*, 1979; Caldwell *et al.*, 1981; Painter & Detling, 1981; Wallace *et al.*, 1984) and increases in water and nutrient availability (McNaughton *et al.*, 1983; Ruess *et al.*, 1983). There is also substantial evidence that dicotyledonous crop species overcompensate after damage by herbivores (Banks & Macaulay, 1967; Taylor & Bardner, 1968; Hirst *et al.*, 1973). For non-crop dicotyledons, defoliation has been found to increase reproductive output (Inouye, 1982; Islam & Crawley, 1983) as well as biomass production (Davidson & Donald, 1958; Mattson & Addy, 1975; Heichel & Turner, 1984). The reponses of woody plant species to defoliation are thought to be primarily determined by resource availability (Bryant *et al.*, 1988) with plants from low resource environments being characterized by slow regrowth rates and the presence of chemical defence compounds. In contrast, woody plants from high resource environments, such as trees, have chemical defences only during juvenile stages and are characterized by faster regrowth rates (Bryant *et al.*, 1988). In this study, the growth responses following defoliation of woody plants, having different morphological and phenological characteristics, from a low resource environment (semi-arid Karoo, South Africa) are examined.

The semi-arid region of southern Africa is an extensive grazing area of approximately 652 339 km^2 (constituting 31.4% of the area south of 22°S parallel) (Rutherford & Westfall, 1986) known as the Karoo. This region is characterized by low annual rainfall (100 - 400mm), hot dry summers, cold winters and especially low night time winter temperatures. The region is used extensively as natural rangelands for the production of wool and mutton sheep. The overutilization of the natural plant cover by domestic livestock has been recognized as a major cause of the degradation of the rangelands of this region (Acocks, 1955; Roux & Vorster, 1983). Knowledge of Karoo shrub growth responses is therefore essential for determining grazing management models for this ecosystem. The objectives of this study were (1) to determine the variability in the range of vegetative growth patterns of selected Karoo shrubs, (2) to test whether existing models of plant growth responses to defoliation apply to karoo shrubs and, (3) to determine the short-term vegetative growth responses to various frequencies and intensities of defoliation at different times of the year.

Earlier studies of karoo plants have been carried out under irrigation and under optimum growing conditions (Hobson & Sykes, 1980; van der Westhuizen & Joubert, 1983). The relatively stable experimental conditions of these studies make it difficult to extrapolate the results to environmentally variable field conditions where plants also experience inter- and intra-specific competition for resources.

2.2 METHODS

2.2.1 Field Experiments

Plant individuals of similar size and canopy structure were subjectively chosen. Defoliation treatments were carried out to simulate grazing by sheep i.e. only twigs of 1 mm diameter and less, and leaves were clipped. Similar percentages of apical meristems were removed with each treatment to avoid the danger of imposing different meristemetic limitations as illustrated by Richards (1986). In the first set of field experiments, plants were defoliated at different intensities by removing 0% (controls), 40% and 80% of leaf and twig material. Clipping began at the periphery of the plant crown and moved systematically towards the crown centre until the desired percentage of the volume of the canopy had been removed. At two, four and six weeks following defoliation, five individuals per defoliation treatment were harvested. Each plant was divided into five separate organ categories and oven dried at 70°C to constant dry weight. The dry weights of the following categories were determined; leaves, twigs, stems, root-crown and roots. Sections of the stem which extend below the soil surface were considered as the root crown. This procedure was undertaken at the following dates to compare plant growth responses at different times of the year: 9 January 1989 (summer), 9 April 1989 (autumn), 9 July 1989 (winter) and 9 October 1989 (spring). *O. sinuatum* was not defoliated during summer because leaf browning and abscission had occurred at the onset of summer which resulted in the plant individuals of this species being at different stages of senescence. However, plants with similar apparent leaf biomass were marked but were not defoliated (controls) and were harvested at two-weekly intervals.

In a second series of field experiments plant growth responses to different frequencies of

defoliation were investigated. On 8 January 1989 fifteen individuals of each species were defoliated by removing 80% of leaf and twig biomass. Following this initial defoliation three sets of individuals, with each set having five replicates, were defoliated at either six-, thirteen- or twenty-six week intervals and each time removing all the regrowth since the previous defoliation. Control plants were left uncut. Plants were harvested on 3 January 1990, divided into plant organ categories as outlined above, oven-dried to constant weight and the dry weights determined.

2.2.2 Growth Ratio

The replicates, although subjectively chosen, showed considerable variation in biomass. Therefore a proportional increment in biomass, called the growth ratio, was used to indicate growth. Growth ratio (GR) was calculated as the ratio of the dry weight of the potentially grazed material (leaves and twigs) to the dry weight of the ungrazed material (stems, root-crown and roots) for each plant individual harvested.

2.2.3 Statistical Analyses

Growth ratio values were arcsine transformed prior to statistical analyses. Two way analysis of variance was used to (i) compare the change in growth ratios over time (two, four and six weeks) and (ii) to determine the differences between defoliation treatments (control, 40% and 80% treatments) within each of the three species. One-way analyses of variance was used to compare the effects of frequency of defoliation on growth ratio within each species. This was followed by Tukey's multiple range test to determine significant between-treatment differences (Zar, 1974).

2.3 RESULTS

2.3.1 Defoliation Intensities

The undefoliated *O. sinuatum* individuals became increasingly senescent over the six-week monitoring period during summer which is illustrated by the decreasing GR values in Fig 2.1. The increase in the GR values of the controls from the final summer harvest (21

February 1989) to the start of the autumn monitoring period (9 April 1989) indicates that a period of rapid growth occurred during March 1989. The control plants maintained the same growth ratios (GR's) between 6 April and 21 May 1989 (autumn). No regrowth was recorded in either defoliation treatments (40% and 80%) at four weeks following defoliation during autumn. Increases in GR were measured in both defoliation treatments at six weeks after defoliation, which indicates that regrowth had taken place between four to six weeks following defoliation ($P < 0.001$, Table 2.1). The statistical significance of the change in GR values ($P < 0.001$) of *O. sinuatum* during spring (Table 2.1) is due to the considerable decrease in GR values observed in the control and defoliated plants after four weeks (Fig. 2.1) which designates the onset of senescence.

The nearly constant growth ratios of the 40% and 80% treatments of *P. pallens* during summer and winter (Fig. 2.2) indicate that no regrowth occurred in these treatments over the monitoring periods during these seasons. *P. pallens* individuals defoliated at 40% experienced considerable regrowth between two and four weeks after defoliation during autumn and spring. These individuals did not undergo further growth in the four to six week periods following defoliation. An increase in GR was observed in the 80% treatment of this species during spring only.

The GR values of the control treatments of *Ruschia spinosa* remained relatively constant over the six-week monitoring periods during the four seasons (Fig. 2.3). No regrowth was apparent in the 80% defoliation treatments over the six-week monitoring periods at any time of the year. The only regrowth monitored in this species was in the 40% treatment, during spring, in the period four to six weeks after cutting ($P < 0.05$, Table 2.1).

2.3.2 Defoliation Frequencies

The growth ratios of the plants in each of the four defoliation frequency treatments were determined after one year for all study species. Significant between-treatment differences were observed for *O. sinuatum* where GR increased with decreasing defoliation frequency (Fig. 2.4). Control plants had the highest GR values. The least difference between the controls and the 26-week treament was observed in *O. sinuatum*. A gradient of increasing GR with decreasing defoliation frequency was also found in *P. pallens* but between-treatment differences were less significant (Fig. 2.4). In *P. pallens*, however, there was a more

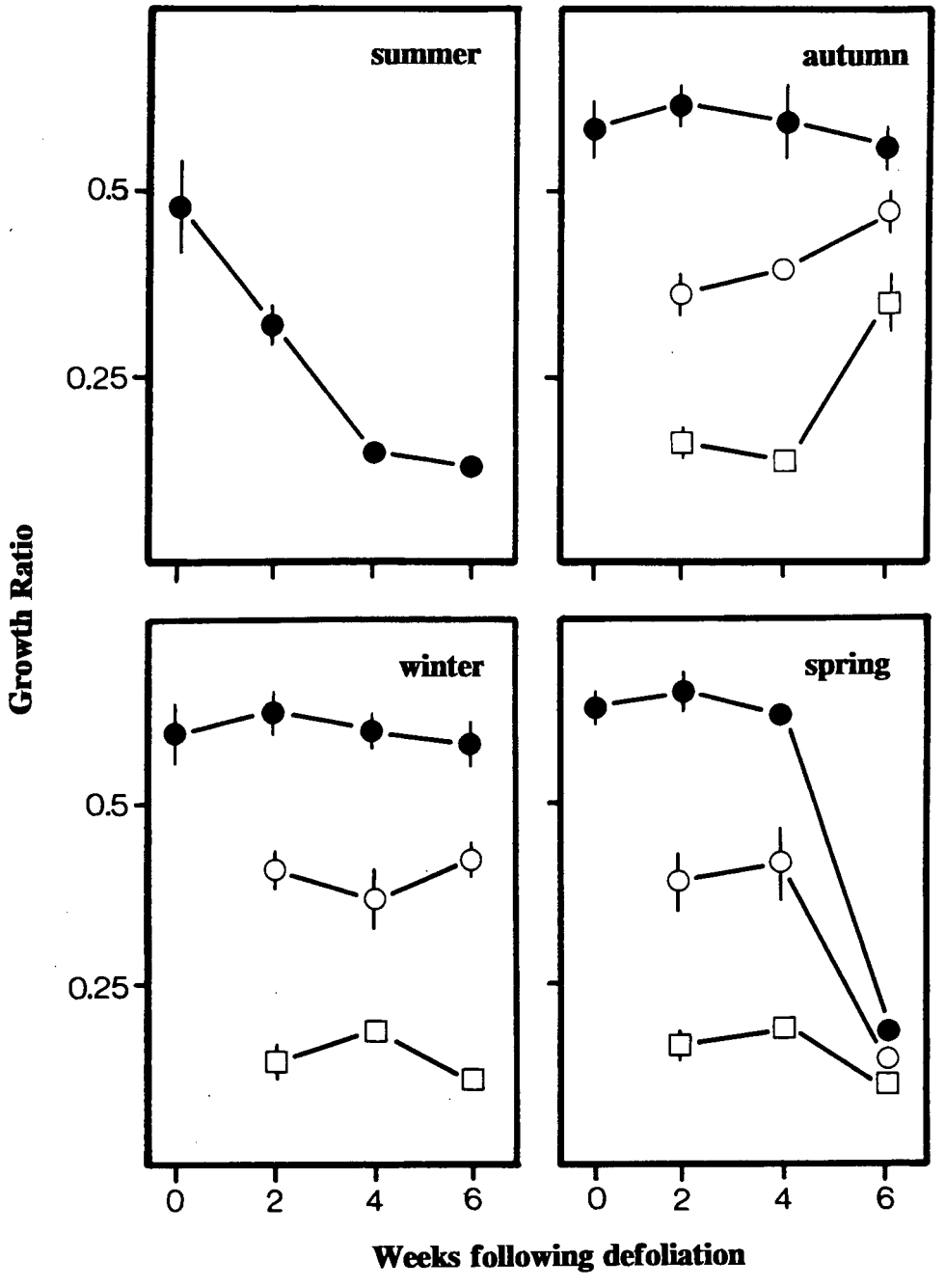

Figure 2.1: Changes in growth ratio measured at two-weekly intervals following 80% defoliation (open squares), 40% defoliation (open circles) and no defoliation (controls, closed circles) of ***Osteospermum sinuatum***. Each point represents the mean of five observations, and the bars = $\pm$ SEM. Where there are no bars, the SEM's are too small to show on the scale.

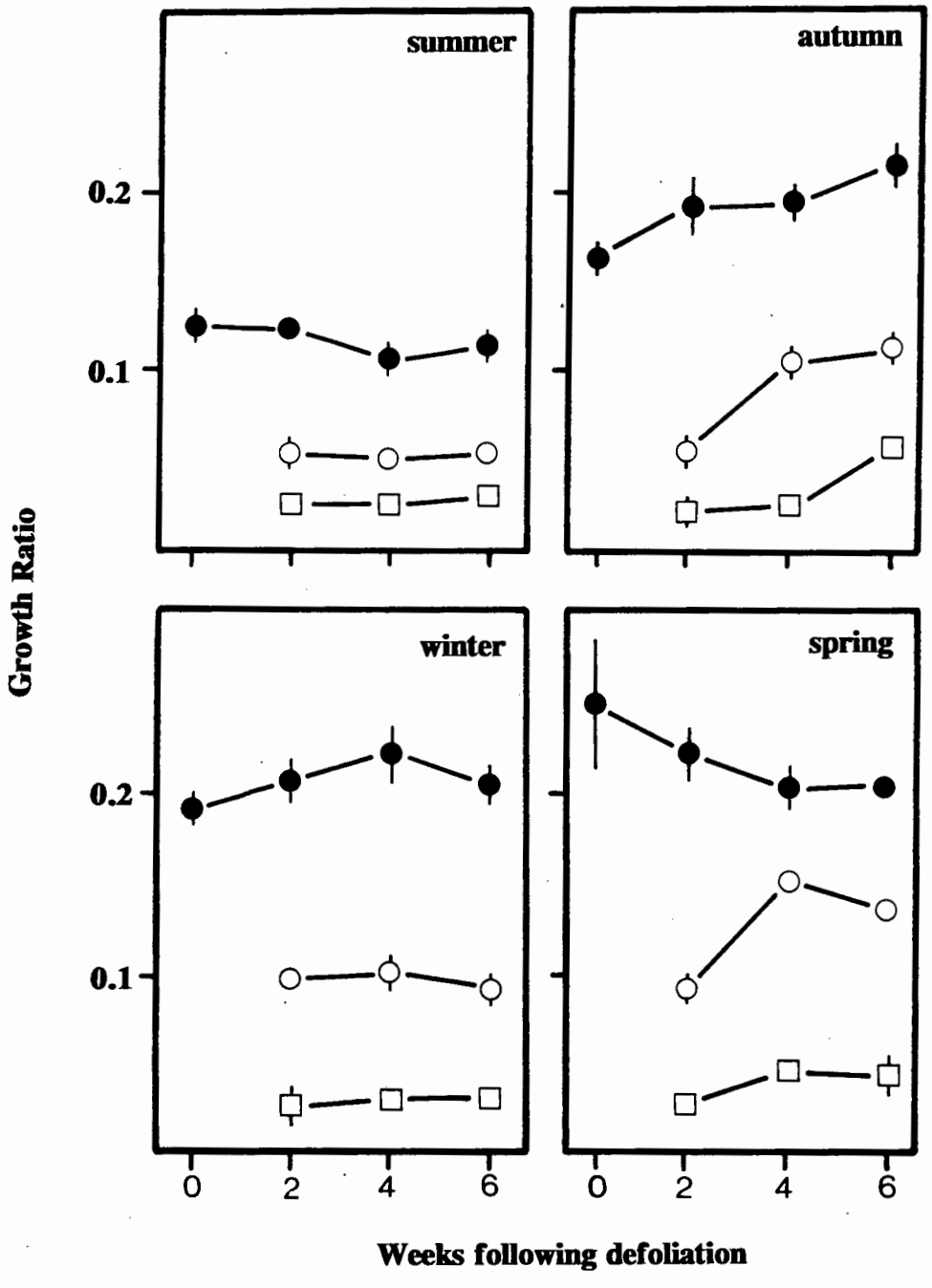

Figure 2.2: Changes in growth ratio measured at two-weekly intervals following 80% defoliation (open squares), 40% defoliation (open circles) and no defoliation (controls, closed circles) of ***Pteronia pallens***. Each point represents the mean of five observations, and the bars = $\pm$ SEM. Where there are no bars, the SEM's are too small to show on the scale.

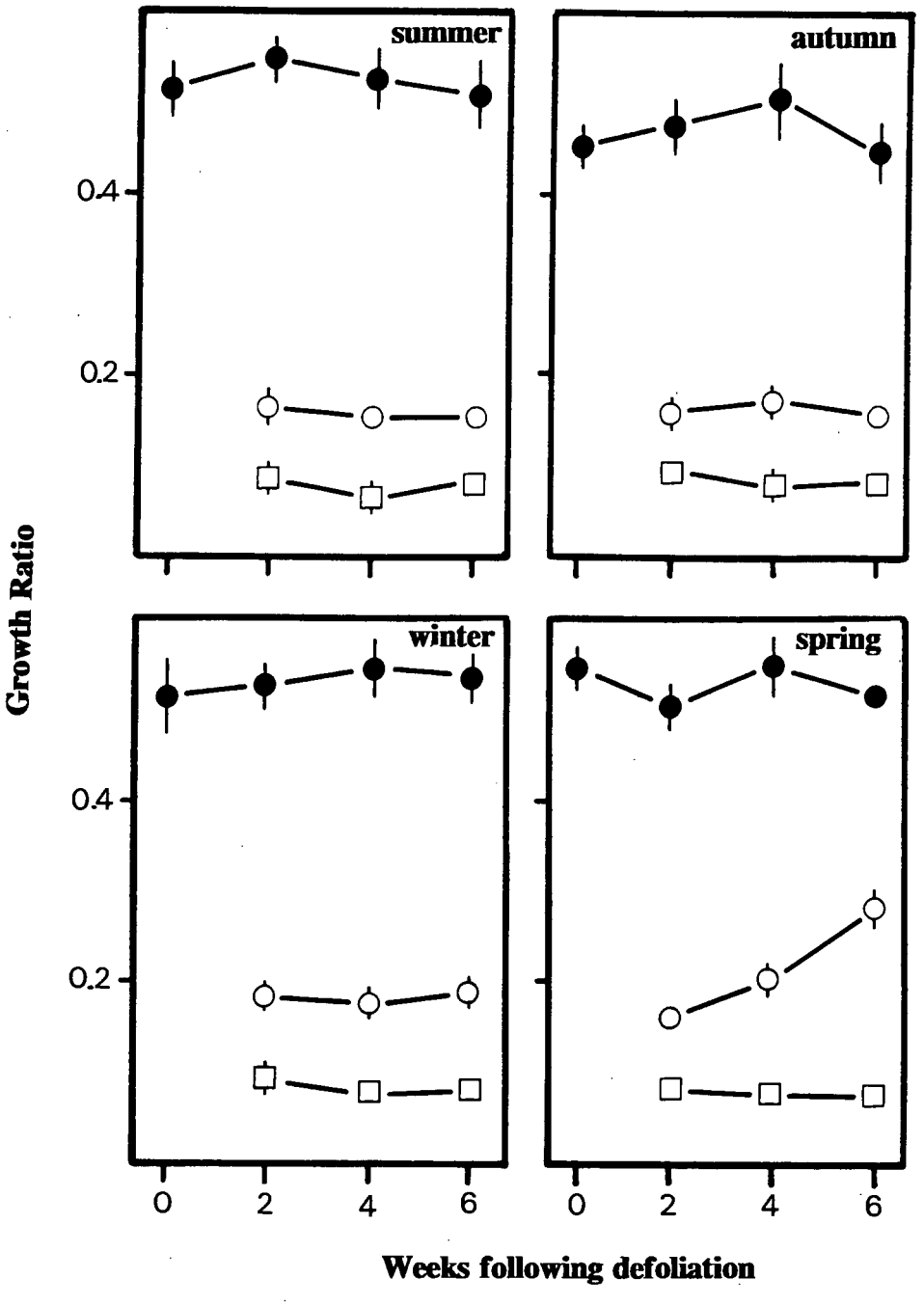

Figure 2.3: Changes in growth ratio measured at two-weekly intervals following 80% defoliation (open squares), 40% defoliation (open circles) and no defoliation (controls, closed circles) of ***Ruschia spinosa***. Each point represents the mean of five observations, and the bars = $\pm$ SEM. Where there are no bars, the SEM's are too small to show on the scale.

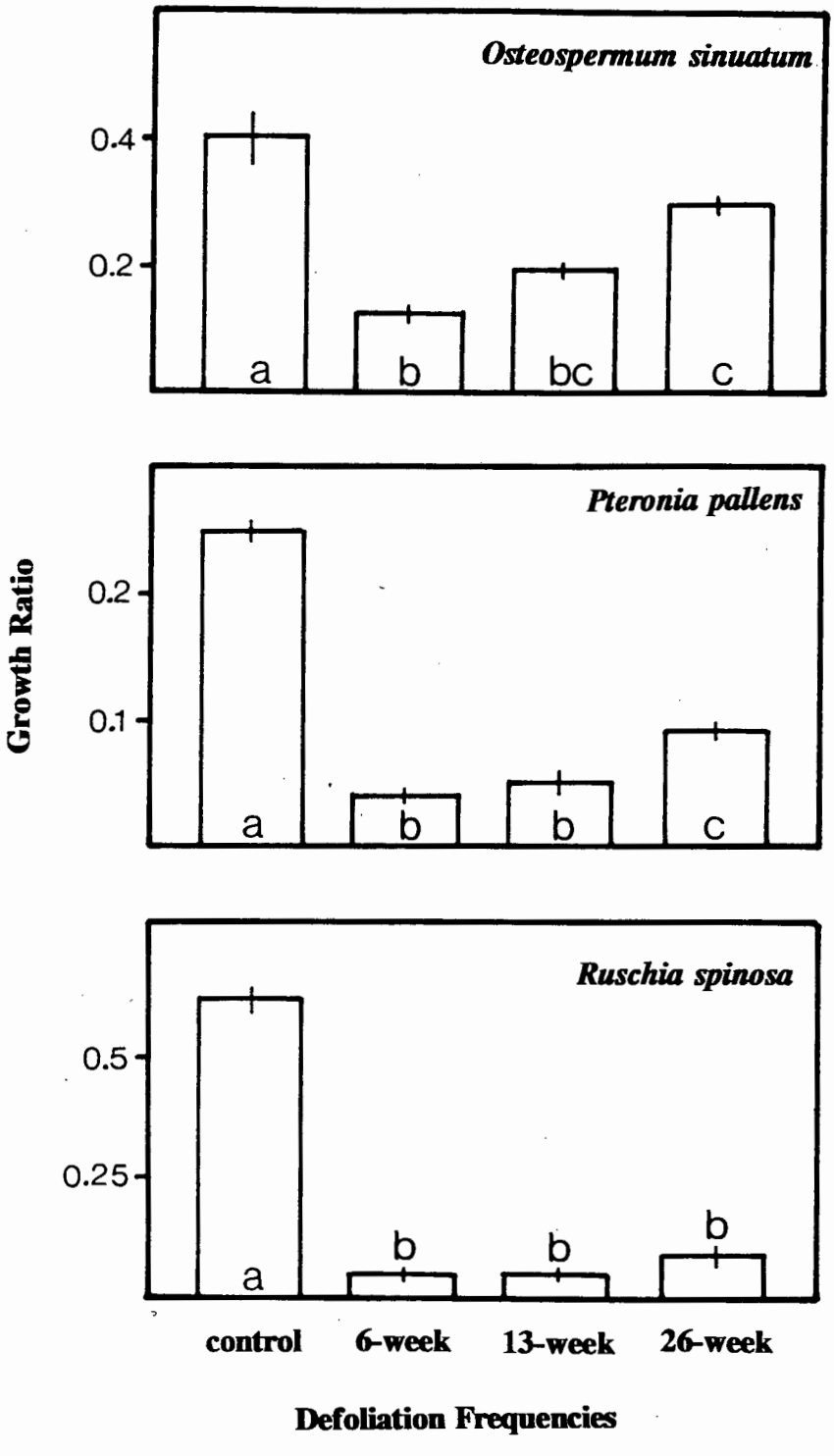

Figure 2.4: Growth ratios of three karoo shrubs subjected to the following frequencies of defoliation over a one-year study period; no defoliation (control), every six weeks (6-week), every thirteen weeks (13-week) and every twenty-six weeks (26-week). Each point represents the mean of five observations, and the bars = $\pm$ SEM. Dissimilar letters designated to the growth ratio values of each species show significant differences at $P < 0.001$

pronounced difference between the controls and the other defoliation treatments and no significant difference between the 6-week and 13-week treatments. The GR values of *P. pallens* and *O. sinuatum* were relatively low and this could be due to the fact that the plants were harvested during summer when the GR values of both species appear to be generally low (Figs. 2.1 & 2.2). The greatest difference between the controls and the defoliation treatments were observed in *R. spinosa* (Fig. 2.4). No significant between-treatments differences were observed for the defoliated individuals of this species.

2.4 DISCUSSION

Defoliation exerted a distinctive negative effect on vegetative regrowth of karoo shrubs. Regrowth varied greatly between species, with defoliation treatment, and at different times of the year. Clipping also reduced the reproductive output of the defoliated plants significantly since no flowers were produced by the defoliated plants at the times when reproductive structures were produced by the control plants. The three species represent a continuum of regrowth responses to defoliation with *O. sinuatum*, the dwarf deciduous shrub, having the greatest regrowth capacity, followed by *P. pallens*, the dwarf evergreen shrub and lastly *R. spinosa*, the dwarf succulent shrub.

Different intensities of defoliation exerted markedly dissimilar regrowth responses with the heavy (80%) intensity of defoliation resulting in mostly no regrowth of Karoo shrubs over the six-week monitoring periods. Similarly, a gradient of increasing regrowth capacity with decreasing defoliation frequency was observed in the two faster growing non-succulent species, *O. sinuatum* and *P. pallens*. The succulent *R. spinosa*, however, did not show any regrowth even at a lenient defoliation frequency of every twenty-six weeks.

Summer and winter represent the periods of greatest susceptibility to grazing for karoo shrubs since no significant regrowth was recorded in any of the defoliation treatments during these seasons. This well-defined seasonal pattern in rates of regrowth suggests that seasonal changes in environmental factors have definite effects on regrowth following defoliation. The extremes of temperatures (summer and winter, Section 1.5, Fig. 1.1), the low water

Table 2.1: Two-way analysis of variance of time since defoliation and defoliation intensity of *Osteospermum sinuatum*, *Pteronia pallens* and *Ruschia spinosa* during the four seasons of the year. Values in the table are F-values which are the main effects and the interaction between the two factors. Degrees of freedom for the time factor is 3,59 and for the defoliation treatment factor, 6,59. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

		TIME	DEFOLIATION	INTERACTION
O.sinuatum	autumn	14.80 ***	122.26 ***	2.62 *
	winter	0.53 NS	318.69 ***	1.24 NS
	spring	83.79 ***	225.72 ***	15.84 ***
P.pallens	summer	1.78 NS	503.32 ***	1.97 NS
	autumn	26.09 ***	503.70 ***	3.07 *
	winter	1.78 NS	558.57 ***	0.99 NS
	spring	4.55 **	408.22 ***	5.37 ***
R.spinosa	summer	0.62 NS	520.41 ***	0.32 NS
	autumn	3.69 NS	397.18 ***	3.28 NS
	winter	0.55 NS	507.48 ***	0.70 NS
	spring	8.44 *	494.88 ***	9.51 **

availability during winter (Section 1.5, Fig. 1.1), and the high evapotranspirative demands during summer due to high temperatures (Section 1.5, Fig. 1.1) may account for the lack of regrowth during these months.

Water stress is an abiotic factor to which semi-arid rangeland plants are often subjected and which has profound negative effects on plant growth and development since plant water status is known to determine rate of carbon assimilation and its use in dry matter production (Hadley & Szarek, 1981). Plant strategies in response to water stress (water use patterns) should therefore be closely associated with the capacity to regrow following defoliation. The morphological and phenological characteristics of the three karoo shrubs present distinct mechanistic water use and carbon acquisition patterns which could explain the regrowth responses of these shrubs. Deciduous leaves (*O. sinuatum*) are inexpensive to produce in terms of carbon and nitrogen, they maintain high stomatal conductances and high transpiration rates and are therefore lost to escape dehydration (Chabot & Hicks, 1982). Defoliation of a deciduous shrub under water stress would therefore be relatively less harmful since the reduction in leaf area (clipping) would decrease the rate of water loss (transpiration) and the resultant improved water status could ameliorate the already poor potential for carbon acquisition of the remaining leaves. Deciduous shrubs should therefore have the capacity to regrow relatively faster tthan evergreen shrubs following defoliation because of the potential increase in carbon acquisition and because deciduous leaves are inexpensive to produce. A feature such as ericoid sclerophyllous leaves of evergreen shrubs (*P. pallens*) have been interpreted to assist in the maintenance of plant water status (van der Heyden & Lewis, 1989) by avoiding dehydration because of their small evapotranspirative area. Regrowth following defoliation would be less efficient than in a deciduous shrub since evergreen leaves are energetically more costly to produce (Bryant *et al.*, 1988). Finally, leaf succulence (*R. spinosa)* is commonly considered as an adaptation to tolerate low plant water status and partial or complete stomatal closure is thought to be maintained which could lower carbon assimilation. Total plant carbon gain and dry matter production would therefore be inherently slow and would decline even further with leaf removal. Of all the karoo growth forms, regrowth following defoliation of the succulent shrub is expected to be the least efficient.

The availability of the water resource is considered to be the principal determinant of plant production in arid ecosystems (Hadley & Szarek, 1981) and affected both inter-specific differences and seasonal differences in growth ratio of the karoo shrubs. Water availability also appears to be the key environmental factor determining the timing of leaf loss in the deciduous species. The onset of senescence in *O. sinuatum* was not a recurring annual phenological event governed by the specific time of year but a plant response closely associated with rainfall events. For example, the start of senescence in *O. sinuatum* was recorded in February 1989 (Fig. 2.1) after a relatively wet late-winter to early-spring period (August to September 1988) followed by an unusually wet early summer (December 1988) (Section 1.5, Fig.1.1). However, during August-September 1989, rainfall was less than half of that measured over the same period in the previous year which possibly caused the earlier spring onset of senescence during 1989 (Fig. 2.1). Management decisions in karoo rangelands, therefore, cannot be rigidly structured and should consider the amount and timing of rainfall events as suggested by Westoby (1980).

Plant responses to defoliation have often been explained in terms of the effects on carbon balance components such as rate of carbon assimilation (Painter & Detling, 1981; Wallace *et al.*, 1984), its distribution to various organs (Ryle & Powell, 1974; Danckwerts & Gordon, 1987) and the level of carbohydrate reserves (Trlica & Cook, 1971; White, 1973). There is now conclusive evidence to show that karoo shrub growth responses following defoliation are similarly related to utilization patterns of the carbon resource. *R. spinosa*, the species with the lowest regrowth ability following defoliation, has high concentrations of polyphenolic compounds, condensed tannins and protein precipitating tannins (Appendix 1). Following defoliation, elevated levels of these compounds were observed in the above-ground tissues of this species which suggest an allocation of carbon to chemical defences as opposed to regrowth structural material. In contrast, *P. pallens* and *O. sinuatum*, the species with the greater regrowth ability, have intermediate to very low levels of polyphenolics and no tannin compounds (Appendix 1). Defoliation had no effect on the production of total polyphenols in these two species. It appears that newly assimilated carbon or reserve carbohydrates were utilized in the latter case for regrowth and not for the production of chemical defence compounds. Van der Meijden *et al.* (1988) indeed proposed that (a)

structural or chemical defenses against herbivores and (b) a high regrowth capacity present two alternative plant strategies against herbivores. The distinct regrowth responses of the three karoo shrubs support this hypothesis in that *O. sinuatum* and *P. pallens*, the species with low levels of, or no chemical defences (Appendix 1) or structural defences, exhibited the highest regrowth capacity. On the other hand, *R. spinosa* with its structural defence (spines) and chemical defence compounds (Appendix 1) exhibited a much lower potential for regrowth following defoliation.

The observations from this study provide support for models predicting that resource allocation controls plant responses to defoliation (Coley *et al.*, 1985). Shallow-rooted succulent shrubs, such as *R. spinosa*, which have an inferior ability to acquire resources such as water and carbon cannot promote rapid growth, and the primary evolutionary response to leaf removal is to increase chemical defences which is less costly in terms of resource use than regrowth. In contrast, deep-rooted shrubs (*O. sinuatum* and *P. pallens*), with water use patterns allowing greater photosynthetic carbon gain, invest carbon preferentially in growth rather than chemical defences (Coley *et al.*, 1985).

Not one of the defoliation treatments reached the growth ratios of the controls over the six week monitoring periods at any time of the year. Management systems should therefore always allow for periods of recovery (following grazing) of much greater than six weeks, even at the most productive times of the year and at the lowest defoliation intensities. Grazing systems associated with high impact defoliations, on the other hand, should accomodate for resting periods of much greater than twenty-six weeks since not one of the 80% defoliated plants attained the biomass levels of the controls, even at the most lenient frequencies of defoliation i.e 26-week intervals (Fig. 2.4).

The results of this study indicate that regrowth following defoliation varies with growth form, intensity and frequency of defoliation, time of the year and the timing and amount of rainfall. The extremes of temperatures, generally low water availability and the resultant limitation on nutrient availability (Hadly & Szarek, 1981) possibly do not favour compensatory vegetative and reproductive regrowth over a one year period. This study focussed on the

short-term effects and provides an essential understanding of the responses of karoo shrubs to defoliation. However, examination of the long-term effects of defoliation is necessary before these results can be reliably intergrated into a management model.

CHAPTER 3

FORMS AND SITES OF CARBOHYDRATE STORAGE IN KAROO SHRUBS

3.1 INTRODUCTION

Nonstructural carbohydrates of economically important crops such as fruit trees and rangeland plants, have been the subject of considerable research because of the close association between plant growth and the production and utilization of carbohydrates. Plant carbohydrate concentrations are therefore often measured in response to stresses which affect plant growth including water stress (Drossopoulos *et al.*, 1987), ozone (Miller *et al.*, 1989), heavy metal and SO_2 pollution (Balsberg-Pahlsson, 1989), elevated CO_2 levels (Wong, 1990), temperature (Frossard & Friaud, 1989; Livingstone *et al.*, 1989) and defoliation practices (Kinsinger & Hopkins, 1961; Trlica & Cook, 1971; Richards & Caldwell, 1985). Of the storage carbohydrates found in vascular plants most is known about starch and sucrose in terms of their chemistry and biochemistry (Duffus, 1984; Hawker, 1985; Manners, 1985; Beck & Ziegler, 1989). Polymers of fructose (fructans) are an alternative form of carbohydrate often accumulated (Brocklebank & Hendry, 1989), and have been reported to be the principal reserve carbohydrates in about 12% of the angiosperm flora (Hendry, 1987). Fructans have recently been intensively studied because of their crucial role in the metabolism of cool-season plants (Pontis & del Campillo, 1985; Hendry, 1987; Cairns & Pollock, 1988; Chatterton *et al.*, 1990). The physiological significance of fructans appears to be associated with cellular osmoregulation, adaptation to low temperature photosynthesis and resistance to freezing (Hendry, 1987; Pontis, 1989; Tognetti *et al.*, 1989; Bancal & Gaudillere, 1990). The wide range of functions attributed to fructans in plants has led to the suggestion that fructan occurrence, in predominantly highly evolved families, is a recent evolutionary response to more than one selective pressure (Hendry, 1987).

The present understanding of plant carbohydrate metabolism is derived primarily from studies of agricultural crops, pasture grasses and legumes. Special emphasis has been placed on carbohydrate allocation of leaves and/or underground plant parts, and less information is available on the carbohydrate contents or concentrations of vegetative organs such as twigs and stems. In fact, many rangeland and pasture management practices are based on the assumption that underground parts of perennial plants are the primary storage organs of carbohydrates (Weinmann, 1948; Weinmann, 1961; Loescher *et al.*, 1990). Aerial parts are thought to act only as temporary or insignificant sites of carbohydrate storage. Of the few studies undertaken on shrubs, emphasis has been placed on the carbohydrate status of branches (Diamantoglou *et al.*, 1989), roots (Lym & Messersmith, 1987) or on broad aboveground and belowground categories (Menke & Trlica, 1981). However, no detailed studies have been undertaken to quantitatively compare the carbohydrate levels in the various organs of rangeland shrubs, despite the obvious management implications.

This chapter reports on the relationships between karoo shrub growth form and the location, quantities and forms of carbohydrates accumulated in these species. The specific objectives were (1) to identify the spatial patterns and size of carbohydrate pools in evergreen, deciduous and succulent karoo shrubs, (2) to determine which of the reserve carbohydrate groups (glucans or fructans) is the major storage carbohydrate in karoo shrub growth forms, (3) to determine the range of fructan chain lengths in karoo shrubs, and (4) to assess the ecological significance of the form of carbohydrate accumulation and spatial variation in pool sizes within each growth form.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material

Five replicate plant individuals of each species were collected on 10 and 11 January 1989. Plants were collected between 9 am and 11 am to minimize diurnal variability. Immediately following the harvest, plant material was frozen in liquid nitrogen to retard enzyme activity and then packed in an insulated freezer box packed with dry ice for transportation to the laboratory. Plants were divided into the following organ categories: (1) leaves (2) twigs,

characterized by a slightly green colour and the distinct absence of bark tissue (3) stems with distinct bark tissue (4) root-crown, section of stem which extends below the soil surface and (5) roots. Plant material was oven dried at 70°C to constant dry weight. The dried samples were ground in a Wiley mill to pass a 40-mesh screen prior to carbohydrate analyses.

3.2.2 Glucose, Fructose and Sucrose Assays

A 300 mg sample (leaves, stems or roots) was mechanically shaken in 100 ml distilled water for 1 hour and then filtered (Whatman No 1). Proteins were precipitated by treating 10 ml of the filtrate with 5 ml 0.085 N $K[Fe(CN)_6].3H_2O$, 5 ml 0.25 N $ZnSO_4.7H_2O$ and 10 ml 0.1 N NaOH. The solution was then made up to 25 ml from which 0.1 ml aliquots were used for the following glucose, fructose and sucrose assays (Bergmeyer, 1974). At pH 7 the enzyme hexokinase (Boehringer Mannheim) was used to catalyze the phosphorylation of glucose by ATP. In the presence of glucose-6-phosphate dehydrogenase (Boehringer Mannheim) the glucose-6-phosphate formed is oxidized by NADP to gluconate-6-phosphate with the simultaneous formation of NADPH. The NADPH formed was measured by means of its absorbance at 340 nm, and the amount of glucose determined stoichiometrically. Fructose was converted to fructose-6-phosphate (Hexokinase & ATP) and then to glucose-6-phosphate (phosphoglucose isomerase, Boehringer Mannheim) which was then taken through the same analytical procedures as glucose so that the amount of NADPH formed was related to the amount of fructose present in the sample (Bergmeyer, 1974). Sucrose was hydrolyzed by *B*-fructosidase (Boehringer Mannheim) and the content in the sample was calculated from the difference in the glucose concentration before and after enzymatic hydrolysis of sucrose. Results were expressed as mg/g DW.

3.2.3 Total Nonstructural Carbohydrate (TNC) Measurement

Colorimetric methods such as the phenol-sulphuric acid method (Dubois *et al.*, 1956) are not valid for accurate TNC concentration measurements of mixtures of carbohydrates because the absorbance values of different monosaccharides of the same concentration differ substantially at the same wavelength. The advantage of TNC concentration measurements as used in this study is that reducing power determined by means of volumetric titration changes (Smith, 1981), allows the simultaneous measurement of both glucan and fructan contents of the same sample.

Total nonstructural carbohydrates were extracted from 100 - 500 mg samples by boiling in 25 ml distilled water for 5 minutes followed by adding 400 units amyloglucosidase enzyme (from *Asperilligus niger*, Sigma Chemical Co), buffered to pH 4.5, and incubated at 55°C for 24 hours. Preliminary investigations indicated that the temperature and the incubation period were sufficient for the enzyme to completely hydrolyze all disaccharides and starch. Samples were filtered through Whatman No 1 filter paper, treated with lead acetate to precipitate proteins, pigments and other contaminants. Samples were centrifuged at 8000 rpm for 20 minutes and the supernatant made up to 100 ml. Reducing power of a 1 - 10ml aliquot was determined by the Shaeffer-Somogyi copper-idiometric titration method described by Smith (1981). This value gives an indication of starch and sugar content. A second aliquot was acid hydrolized (1 N H_2SO_4, 100°C water bath, 15 minutes), neutralized with 1N NaOH and tested for reducing power to obtain a value for sugars, starch and fructans (Smith, 1981). Results are expressed as glucose equivalents on a dry weight basis.

3.2.4 Fructan Degree of Polymerization

The degree of polymerization of fructans was determined by using the differential solubility of fructans in ethanol solutions as demonstrated by Smith & Grotelueschen (1966). Stem samples were extracted serially with water-ethanol mixtures, from 95% to 0% (water), by mechanically shaking for one hour at room temperature, with repeated extraction at each ethanol strength. Ethanol was evaporated from extracts and replaced with water while avoiding complete dessication. Soluble carbohydrate concentrations at each ethanol extraction were determined by means of the reducing power titration technique (Smith, 1981).

3.2.5 Thin Layer Chromatography Procedure

A TLC method (Collins & Chandorkar, 1971) was employed to characterize the fructo-oligosaccharides in two plant tissue categories, the aboveground (leaves, twigs and stems) and belowground (root-crown and roots) tissues. Milled samples were boiled for 5 minutes in distilled water and filtered through Whatman No 1 filter paper. The filtrate was centrifuged (5000 rpm), passed through a mixed-bed ion exchange column (Collins & Chandorkar, 1971), decolourized with charcoal and filtered through a 0.45 μ filter (Millipore) (Collins & Chandorkar, 1971). The filtrate was evaporated to dryness at room

temperature and the residue dissolved in 50% ethanol to achieve a final concentration of 2 μg fructose equivalents per μl (Collins & Chandorkar, 1971), determined by the reducing power titration technique (Smith, 1981). Two μl of each extract was spotted on Kieselguhr-coated TLC plates with fluorescent indicator (DC-Fertigplatter, Germany) and developed in a 1-propanol-ethyl acetate-water (40:45:15 v/v) solvent for 90 minutes (Collins & Chandorkar, 1971). Fructans were located by a urea-metaphosphoric acid spray (Wise *et al.*, 1955). To assess the efficiency of the TLC procedure, onion bulb (*Allium cepa* L.) extracts were also tested since the range of oligosaccharides present in this species has previously been identified (Darbyshire & Henry, 1981). Fructose, sucrose, inulin and the oligofructans separated from onion were used as reference compounds.

3.3 RESULTS

3.3.1 Mono- and Di-saccharide Concentrations

Similar concentrations of glucose, fructose and sucrose were found in the leaves, stems and root tissues of *O.sinuatum* (Fig.3.1). In *P. pallens* tissues, the highest concentrations of fructose were measured of all the species' tissues analyzed, with particularly high values in the leaves (Fig.3.1). Sucrose and glucose values in the roots and stems of *P.pallens* were comparable with the results for the same tissues of *O.sinuatum.* However, in the leaves of *P. pallens*, insignificant quantities of sucrose and particularly high levels of monosaccharides (glucose and fructose) were found. Highest sucrose levels of all plant tissues analyzed, were measured in *R. spinosa* tissues, with particularly high levels in the stems and roots (Fig.3.1). Sucrose was present in greater quantities than glucose or fructose in the roots of *O. sinuatum* and *R.spinosa.* Fructose contributed substantially to the total sugars of *O.sinuatum* and *P.pallens*, which is in contrast to the pattern found for *R.spinosa*, which did not have significant concentrations of fructose.

3.3.2 Total Nonstructural Carbohydrate (TNC) Concentration

The highest TNC concentrations were recorded in the twigs of all species, followed by the leaves and stems with the belowground organs generally having the lowest TNC concentrations (Fig.3.2). No differences were observed between the carbohydrate

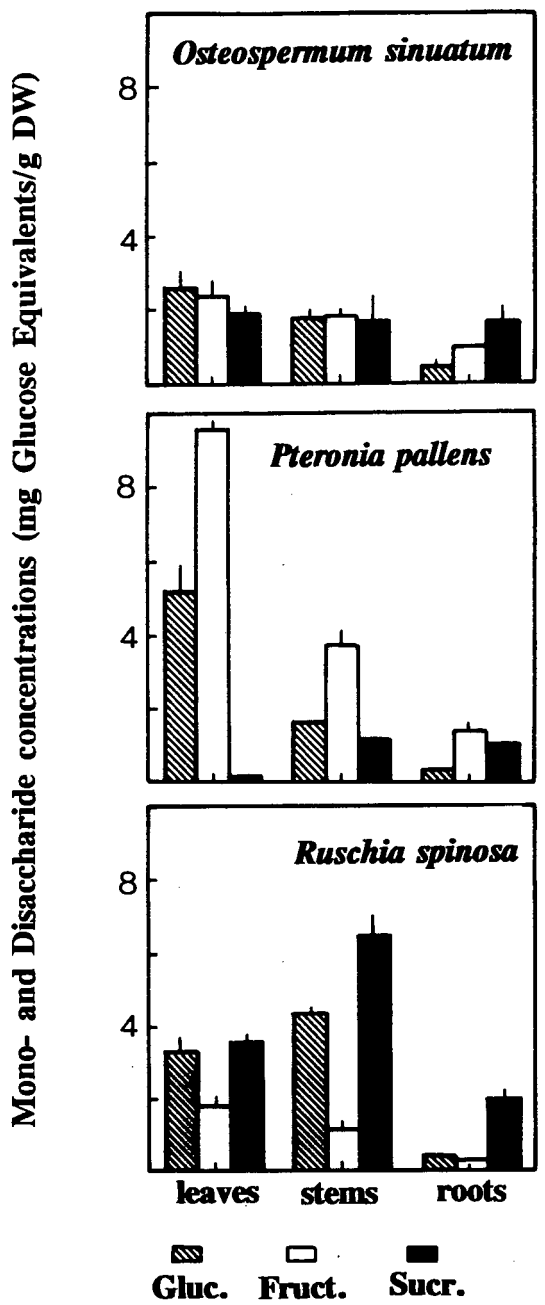

Figure 3.1: Glucose, fructose and sucrose concentrations (mg Glucose equivalents/g DW) of leaves, stems and roots of *Osteospermum sinuatum*, *Pteronia pallens* and *Ruschia spinosa*. Values are mean $\pm$ SE.

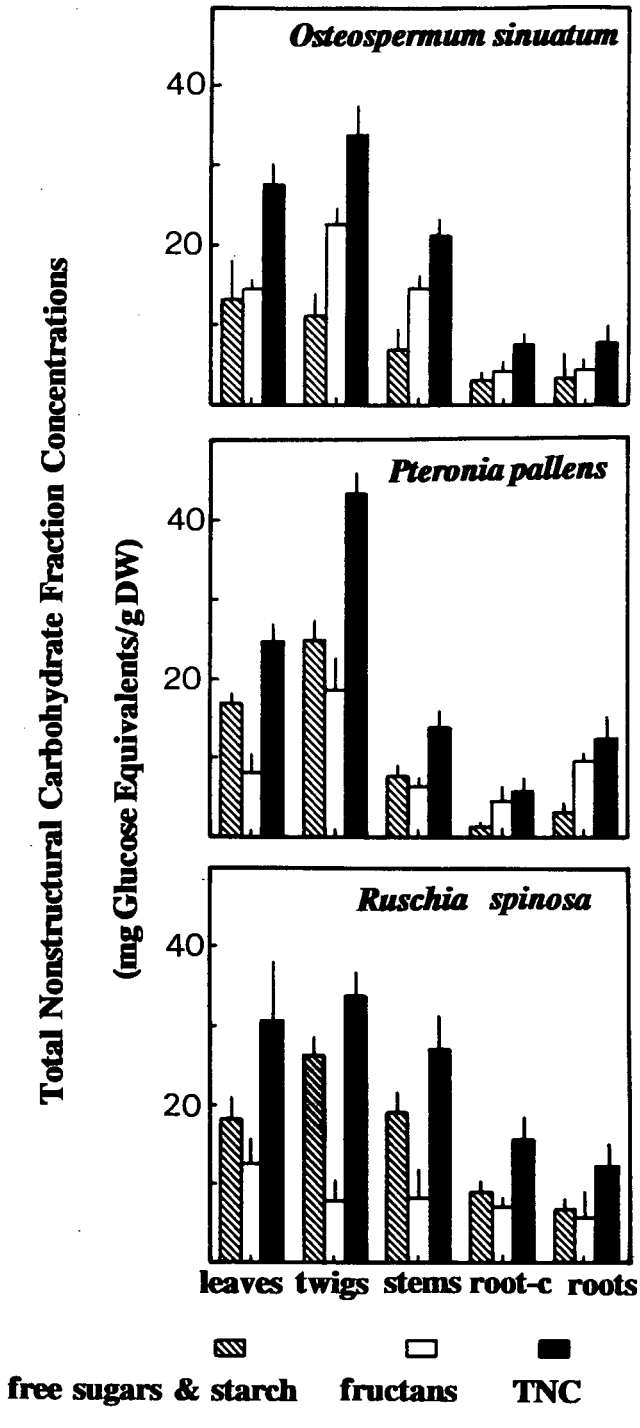

Figure 3.2: Concentrations (mg Glucose equivalents/g DW) of sugars & starch, fructans and total nonstructural carbohydrates (TNC) in five plant organ categories of *Osteospermum sinuatum*, *Pteronia pallens* and *Ruschia spinosa*. Values are expressed as mean $\pm$ SE.

* root-c = root-crown

concentrations in roots and root-crowns in all three shrubs (Fig.3.2). Fructans comprised the major part of the TNC fractions in all *O. sinuatum* tissues and appeared to be the primary form of carbohydrate accumulated in this species. Fructan concentrations were also higher than starch and sugar concentrations in the below-ground organs of *P. pallens* with relatively low fructan concentrations present in aboveground *P.pallens* tissues. Fructans were of minor significance in *R. spinosa* organs. However, this does not indicate that starch is the major form of carbohydrate accumulation in these tissues since mono- and disaccharides were also present in relatively high concentrations (Fig.3.1) in the sugar & starch fraction. To determine the contribution of starch to TNC, the sugar concentrations (Fig.3.1) were subtracted from the sugar & starch concentrations of the leaf, stem and root tissues (Fig.3.2) and the results presented in Table 3.1. From Table 3.1 it is apparent that fructans accumulated in far greater quantities than starch or sucrose in the leaves, stems and roots of *O. sinuatum* and *P. pallens*. Although the concentration of starch is generally low in these two species, it appears to be present at higher levels in the leaves than in the other tissues. In the roots of these species, however, sucrose appears to be accumulated at concentrations in excess of four (*O. sinuatum*) and twenty (*P. pallens*) times the levels of starch. In *R. spinosa*, however, there appears to be similar accumulation patterns of starch and fructans in the leaves, stems and roots (Table 3.1).

3.3.3 Proportional Contribution of Plant Organs to TNC

Nonstructural carbohydrate pool sizes were the highest in the stems (40 - 58 %) of all karoo species studied (Fig.3.3). Despite the twigs having the highest TNC concentrations (Fig.3.2), this organ is not the site of greatest carbohydrate storage in any of the species. In *O. sinuatum* and *P. pallens*, high levels of carbohydrate storage had taken place in the twigs, but to a lesser degree than in the stems. Below-ground organs (root-crown and roots) accumulate surprisingly low levels of TNC (14.5%, *P. pallens*; 7.8% *O. sinuatum*). Similarly, leaves of *O. sinuatum* and *P. pallens* contributed very little (4.2% and 9% respectively) to total plant TNC. Leaves and roots of *R. spinosa* contributed almost twice as much carbohydrate to TNC in comparison with the same organs of the non-succulent species. However, the twigs of *R. spinosa* accumulated (relative to the other plant parts) low levels of TNC, being overshadowed by both the underground tissues and the leaves.

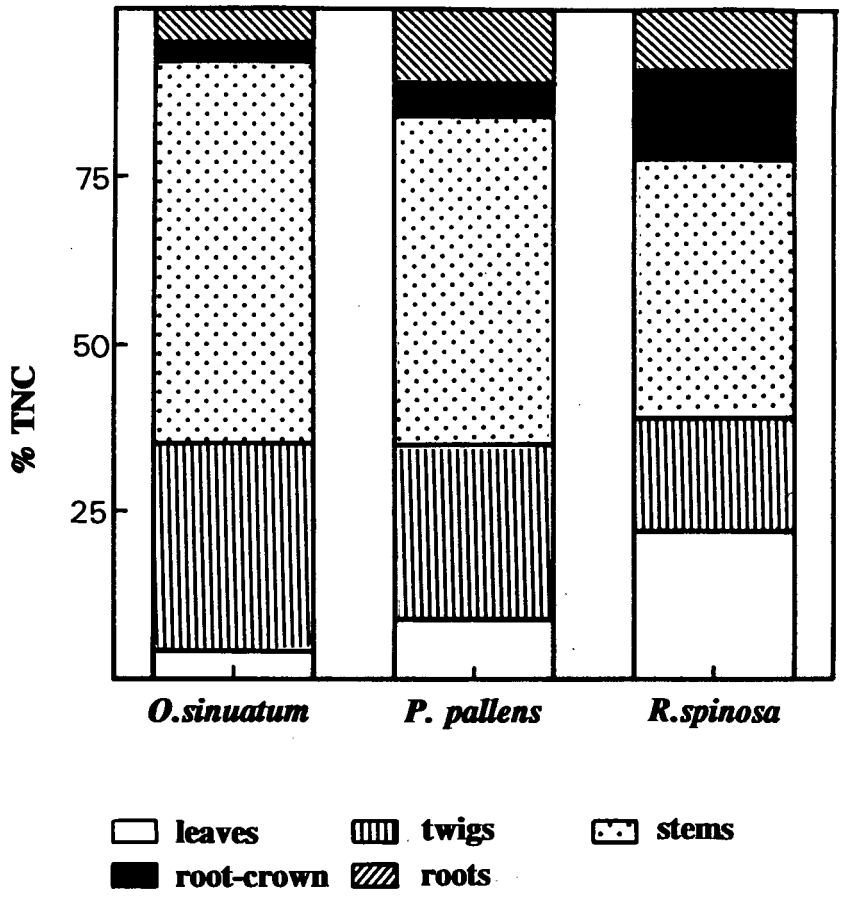

Figure 3.3: Proportional contribution (%) of five plant organ categories to the total nonstructural carbohydrate (TNC) pool of *Osteospermum sinuatum*, *Pteronia pallens* and *Ruschia spinosa*.

3.3.4 Degree of Fructan Polymerization

Concentrations of carbohydrates extracted from the stems of karoo shrubs increased steadily as ethanol concentrations were decreased from 95% to 65% (Fig.3.4). Almost no additional carbohydrates were extracted with ethanol concentrations of less than 65%. The highest carbohydrate concentrations were extracted from *R. spinosa*, followed by *P. pallens* and *O. sinuatum*. Since short-chain polymers of fructose are removed with high ethanol concentrations (65 to 80 %), and long-chain molecules with lower ethanol concentrations (> 20%) (Smith & Groteleuschen, 1966; Smith, 1973), the fructans of karoo shrubs are probably of the short-chain variety, comparable in chain length with amylopectin (Smith, 1973).

3.3.5 Characterization of Oligofructans

Thin layer chromatographic (TLC) procedures confirmed the presence of fructose, sucrose and a range of oligofructans in onion bulbs (Fig.3.5) as reported by Darbyshire & Henry (1981) and Suzuki & Cutcliffe (1989) using HPLC methods. Homologs of DP greater than 7 could not be separated with this TLC system. Inulin (DP > 26) remained stationary at the point of spot application. Stems of all three karoo shrubs contained fructose and sucrose, and contained no oligofructans with a DP of less than 7. The presence of longer chain polymers of fructose in these tissues was confirmed by the characterisation of combined fructose units at the point of spot application, similar to the observation for inulin (Fig.3.5). The incompleteness of the colour-forming reaction of the *R. spinosa* spot at the point of application suggests that the fructans of this species were present at lower levels than in *O. sinuatum* and *P. pallens*. This chromatographic technique was also applied to below-ground tissue samples and also showed the absence of oligofructans and the presence of fructans with a DP of greater than 7 in these tissues.

3.4 DISCUSSION

All karoo shrubs considered in this study contained sucrose, starch and fructans. However, the contribution of each form of carbohydrate to the total nonstructural carbohydrate pool differed among species. Not one of the species stored sucrose in any appreciable amounts.

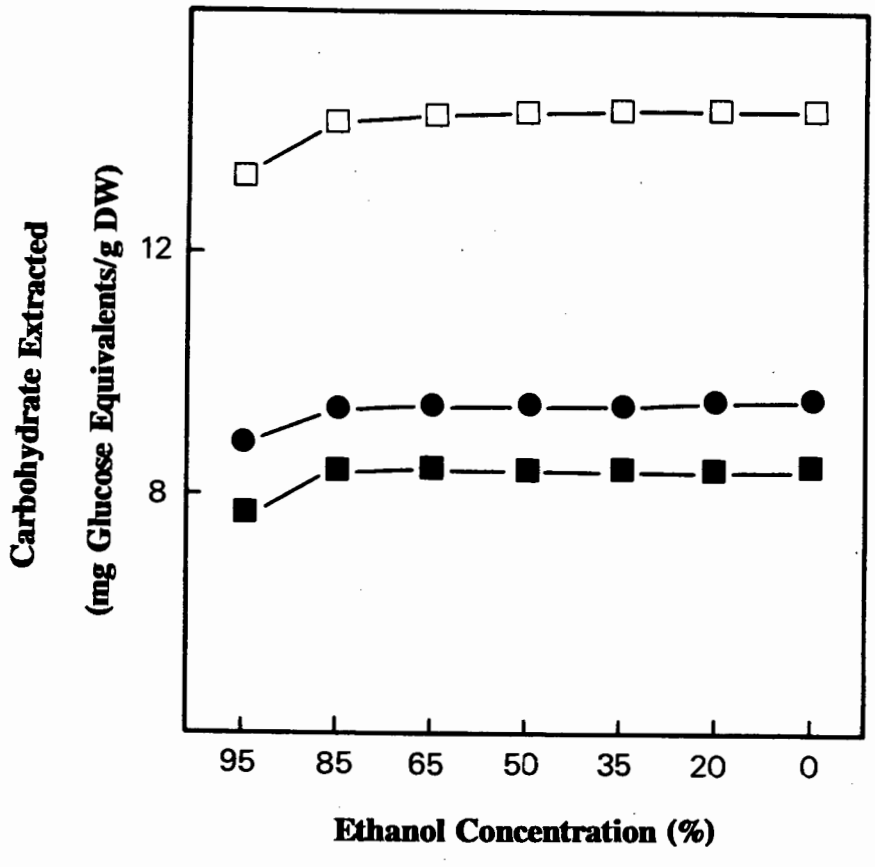

Figure 3.4: Concentrations (mg Glucose equivalents/g DW) of nonstructural carbohydrates extracted with various ethanol concentrations (%) from *Osteospermum sinuatum* (closed squares), *Pteronia pallens* (closed circles) and *Ruschia spinosa* (open squares) stem samples. Values are mean $\pm$ SE. Absence of bars indicates that the standard error is too small to show on the scale of the graph.

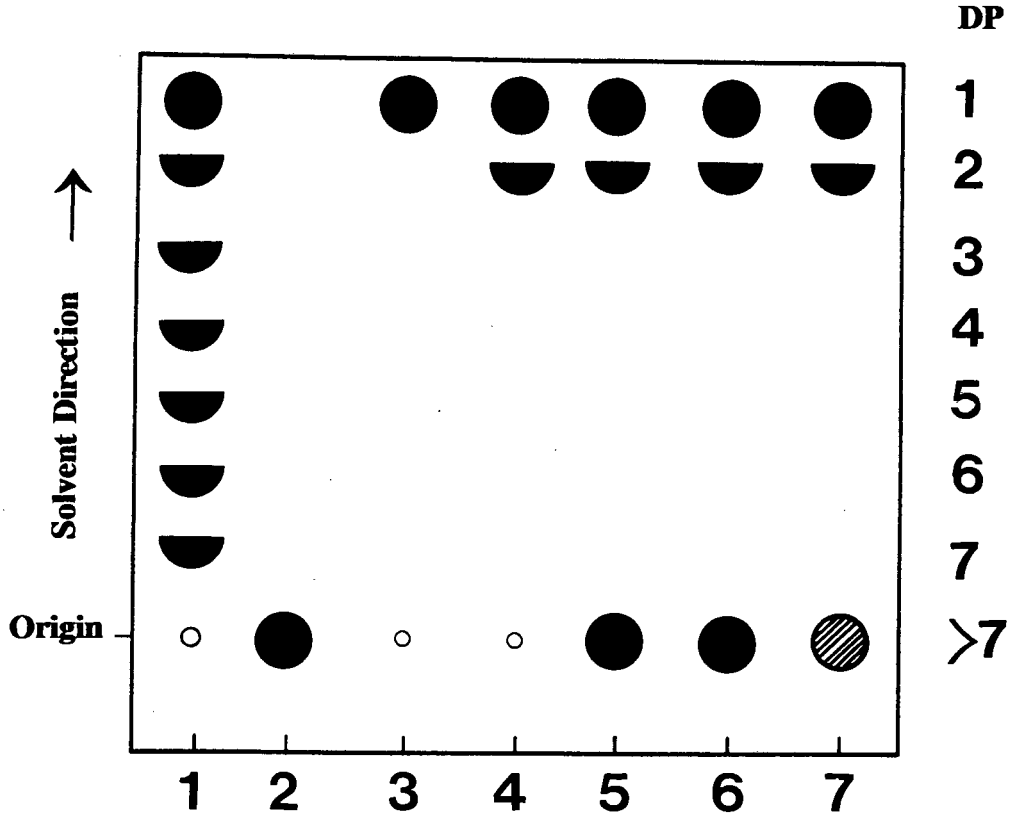

Figure 3.5: Diagramatic representation of the seperation of oligofructans by thin-layer chromatography from stem tissue of *Osteospermum sinuatum*, *Pteronia pallens* and *Ruschia spinosa* and from *Allium cepa* bulb tissue. 1 = *Allium cepa*; 2 = inulin standard; 3 = fructose standard; 4 = fructose/sucrose standard mixture; 5 = *Osteospermum sinuatum*; 6 = *Pteronia pallens*; 7 = *Ruschia spinosa*. ● denotes complete spot development at the point of application (DP > 7) or the complete seperation of fructose (DP = 1), ◡ denotes the seperated oligofructans, o is the point of spot application and ▨ denotes inferior spot development.

Table 3.1: Reserve carbohydrate fractions (mg Glucose equivalents/g DW) in plant tissues of *Osteospermum sinuatum*, *Pteronia pallens*, and *Ruschia spinosa*. The values in brackets are the contributions (%) of carbohydrate fractions to total nonstructural carbohydrates of that particular organ.

		LEAVES	STEMS	ROOTS
	STARCH	6.20 (28)	1.59 (9)	0.39(6)
O. sinuatum	SUCROSE	1.89 (8)	1.65 (9)	1.65 (25)
	FRUCTANS	14.4 (64)	14.5 (82)	4.58 (69)
	STARCH	1.88 (19)	1.03 (12)	0.05 (0.5)
P. pallens	SUCROSE	0.165 (2)	1.16 (14)	1.05 (10)
	FRUCTANS	7.94 (79)	6.13 (74)	9.3 (89.5)
	STARCH	9.38 (38)	6.92 (45)	3.88 (40)
R. spinosa	SUCROSE	3.0 (12)	0.40 (3)	0.35 (3)
	FRUCTANS	12.3 (50)	8.00 (52)	5.54 (57)

The succulent *R. spinosa*, was the only species to accumulate fructans and starch in equal quantities and also contained the highest leaf TNC concentrations and pool sizes of all the species studied. This unique pattern of carbohydrate accumulation is probably associated with the structural features and possibly distinctive physiological properties associated with the succulent leaf type (von Willert *et al.*, 1990). In the two non-succulent species (*O.sinuatum* and *P.pallens*), carbohydrates were accumulated predominantly in the form of fructans. This result is not unusual since both species belong to the Asteraceae, which is a plant family containing many fructan-accumulating species (Hendry, 1987). Since the deciduous *O.sinuatum* and the evergreen *P.pallens* (both Asteraceae) exhibited almost identical patterns in the form and location of carbohydrate stores, there is no relationship between leaf longevity and form of carbohydrate accumulation. Although the spatial patterns and the forms of carbohydrate accumulation were comparable in these two Asteraceous shrubs, the absolute concentrations of TNC components varied between these species. Generally, *O. sinuatum* tissues had higher concentrations of starch and fructans than *P. pallens* tissues. For example, the deciduous leaves of *O. sinuatum* had higher concentrations of sucrose, starch and fructans than the evergreen leaves of *P. pallens* which is in contrast with the traditional belief that evergreen leaves act as carbon and nutrient stores (Chabot & Hicks, 1982). The evergreen *P. pallens*, however, had higher concentrations of fructose and glucose but these sugars are not considered as carbon storage compounds. The elevated monosaccharide concentrations of *P. pallens* leaves suggests a different diurnal pattern (with respect to *O. sinuatum*) of regulating the partitioning between storage carbon compounds and the constituent monosaccharides.

Irrespective of phylogenetic relationships and growth form, the twigs of karoo shrubs contained the highest concentrations, and the stems the highest total contents of TNC than any other organ in the plant. This is in distinct contrast to the patterns reported for other woody species (Loescher *et al.*, 1990) and grasses (White, 1973) where the root, root crown and stem bases (grasses) have been considered as the main sites of carbohydrate accumulation. High intensity stocking rate grazing systems, where trampling is a frequent occurrence, could result in the destruction and loss of karoo shrub carbohydrate reserves which would decrease or annul regrowth potential.

The role of fructan as storage material is well documented and there is also some evidence for its contribution to cold-season adaptations and drought tolerance (Brocklebank & Hendry, 1989). It is therefore tempting to suggest that the occurrence of fructans in karoo shrubs is a requirement or contributes to their ability to survive exceptionally cold winters and particularly dry summers. However, detailed analyses of floras have shown that fructan accumulating species and nonaccumulators successfully compete in the same environment (Pollock & Cairns, 1991). This study of karoo shrubs indicates that form of carbohydrate accumulation can in fact be of selective advantage in that the predominantly fructan accumulator (*O. sinuatum*) is a relatively fast grower, whereas the partial fructan accumulator (*R. spinosa*) regrows poorly following defoliation (Chapter 2). The relationship between a rapid regrowth capacity and fructan accumulation is probably not a direct one but is mediated via the superior ability of the fructan accumulating plant to survive and grow under adverse environmental conditions.

This study revealed the absence of oligofructans (DP 1 - 7) in the three karoo shrubs studied, which is not uncommon among plant species since glucosyl transferase, the enzyme responsible for the synthesis of sucrosyl oligosaccharides, has evolved only once during the evolution of the angiosperms (Kandler & Hopf, 1982), which would explain the restricted occurrence of oligofructans in a few highly derived groups (Kandler & Hopf, 1982). The abundance of the different fructan compounds in the plant kingdom and their close plant familial relationships suggest that phylogenetic factors determine the form of fructan series accumulated in plants (Kandler & Hopf, 1982). For example, the Compositeae is thought to be an inulin (β2-1 linked fructose polymers) containing family while the fructans of grasses are β2-6 linked polymers (levans) (Smith, 1973). In addition to the variability in plants with respect to the fructan branch type accumulated (eg. levan or inulin), fructan degree of polymerization also varies greatly among plant species (Pollock & Cairns, 1991). The differential solubility of karoo shrub fructans suggests that they are of the short-chain variety, probably of a DP somewhere between 7 and 26 (Smith, 1973), similar to the short-chain fructans identified in *Festuca arundinacea* by Smith & Grotesleushen (1966). Short-chain fructan accumulating species do not always share common environmental pressures which suggests that fructan chain length has no selective advantage and is probably

determined by phylogenetic factors. Present understanding of the mechanisms of degradation and synthesis of fructans cannot explain why a single enzyme, fructan-fructan fructosyltransferase, should terminate chain elongation in some species at a DP of 30 and at a DP of 260 in other species (Pontis & del Campillo, 1985).

O. sinuatum and *P. pallens*, the Asteraceous species, accumulate predominantly fructans, while the succulent species, *R. spinosa*, accumulates starch and fructans in equal proportions. Some attributes of fructans could possibly ensure that the predominantly fructan accumulators regrow better following defoliation. The apparent relationship between fructan accumulation and cellular osmoregulation (Hendry, 1987), for example, could contribute to a superior physiological adaptation of fructan accumulators, and hence the greater regrowth (compared to the succulent species) following defoliation (Chapter 2). The role of fructans in karoo plant function need to be examined in order to understand the ecological significance of predominantly fructan accumulation in some of the karoo shrubs.

CHAPTER 4

NONSTRUCTURAL CARBOHYDRATES: EFFECTS OF FREQUENCY OF DEFOLIATION

4.1 INTRODUCTION

Nonstructural carbohydrates accumulated by plants function as a readily available source of energy which is used for cell metabolic processes and plant growth (Smith, 1973). Utilization of nonstructural carbohydrates should therefore be particularly marked during periods when environmental conditions (drought) or management practices (grazing) reduce or annul photosynthetic carbon assimilation. Studies on grasses (Davidson & Milthorpe, 1966b), lucerne (Hodgkinson, 1969), clover (Culvenor, Davidson & Simpson, 1989) and shrubs (Buwai and Trlica, 1977) for example have shown that regrowth following defoliation depends, at least in part, on mobilization of stored nonstructural carbohydrates. The importance of stored carbohydrates as resources for regrowth following defoliation has been questioned and it has been suggested that the contribution of recently produced photosynthates (by remaining leaf biomass) to subsequent regrowth exceed that of reserve carbohydrates (Davidson & Milthorpe, 1966b; Richards, 1986; Richards & Caldwell, 1985). Present understanding of the effects of defoliation on the carbon economy of plants is based on numerous detailed investigations covering aspects such as changes in reserve carbohydrate status (White, 1973; Buwai and Trlica, 1977), translocation of stored nonstructural carbohydrates (Danckwerts & Gordon, 1987), net photosynthetic rates (Ryle and Powell, 1975; Painter and Detling, 1981) and partitioning of recently fixed photosynthates (Danckwerts and Gordon, 1987). Although the carbon allocation patterns observed in these studies do not provide a model which is generalizable for all plant species, there is general concensus that regrowth following defoliation is a function of the plant carbon resource and that this physiological understanding of plant responses to defoliation should be an essential consideration in the planning of management strategies for the utilization of plants (harvesting

or grazing). Almost no research has been undertaken on the effects of defoliation practices on the carbon economy of semi-arid rangeland shrubs of the Karoo. An earlier study of a karoo grass species and a shrub species was undertaken on irrigated and fertilized plant inidividuals (van der Westhuizen, 1980) but the results cannot easily be applied to plants growing naturally in this resource constrained environment.

The objective of the study, reported on in this Chapter, was to determine whether different degrees of consistent and repeated removal of photosynthetic tissue (frequencies of defoliation) have similar effects on carbohydrate stores of karoo shrubs. In particular, the effects on allocation patterns of nonstructural carbohydrates in different karoo growth forms are examined and related to the known regrowth capacities of these species (Chapter 2).

4.2 MATERIALS AND METHODS

4.2.1 Experimental Procedures

Fifteen individuals of each species were defoliated on 8 January 1989. A defoliation intensity of 80% was applied by removal of leaf and twig (< 1mm) material by clipping starting at the periphery of the plant crown, moving systematically towards the centre until the desired percentage of the volume of the canopy had been removed. Clipping removed all apical meristems to avoid imposing different meristemetic limitations on replicated plant individuals of the same treatment (Richards, 1986). Following this initial defoliation treatment, plants were repeatedly defoliated over a year period at either six, thirteen or twenty-six week intervals (five replicates) by removing all regrowth that had occurred since the previous defoliation. Control plants were left uncut thoughout the experiment. Plants were harvested on 3 January 1990 and divided into the following plant part categories: leaves, twigs, stems, root-crown and roots. Sections of the stem which extend just below the soil surface were considered as the root-crown. Plant material was frozen in liquid nitrogen to retard enzyme activity and then transported to the laboratory in insulated freezer boxes packed with dry ice (-78°C). Plant material was oven-dried to constant weight at 70°C and dry weights were determined prior to chemical analyses.

4.2.2 Chemical Analysis

Since the three Karoo shrubs accumulate nonstructural carbohydrates in varying mixtures of starch, sucrose and fructans (Chapter 3), a modified Shaeffer-Somogyi copper-idiometric titration technique (Smith, 1981) was used because it includes all glucose and fructose units in the quantification of total nonstructural carbohydrates (TNC) concentrations. Nonstructural carbohydrates were extracted from 100 - 500 mg tissue samples by boiling in 25 ml distilled water for 5 minutes followed by cooling to room temperature and the addition of 400 units amyloglucosidase enzyme extracted from *Asperilligus niger* (Sigma Chemical Co.). The mixture was buffered to pH 4.5 by addition of 10ml of an acetic acid/sodium acetate solution and incubated at 55°C for 24 hours. Samples were filtered through Whatman No 1 filter paper, treated with 10 % neutral lead actetate to precipitate proteins, made up to 100 ml with distilled H_2O and centrifuged at 8000 rpm to remove the precipitate. A 1 to 10 ml aliquot was acid hydrolyzed (1 N H_2SO_4, 100°C water bath, 15 minutes), neutralized with 1 N NaOH and tested for reducing power to obtain a total nonstructural carbohydrate (TNC) value which includes mono- and disaccharides, starch and fructans. Results are expressed as glucose equivalents on a dry weight basis. All data were analyzed using a one-way analysis of variance technique (Zar, 1974) followed by Tukey's multiple range test to identify significant differences between treatments.

4.3 RESULTS

4.3.1 Organ TNC Concentrations

The total nonstructural carbohydrate (TNC) concentrations of the leaf tissues of *Osteospermum sinuatum* plants subjected to different frequencies of defoliation were of the same order of magnitude (Fig. 4.1) with no significant differences ($P > 0.05$) between treatments. A significant increase ($P < 0.001$) in TNC concentration was, however, observed in the twigs of the 6-week defoliation treatment with no significant differences between the twigs of control plants, 13-week and 26-week interval defoliated plants. In the stems of *O.sinuatum* a significant increase ($P < 0.05$) in TNC concentration was found only in the 26-week treatment (Fig. 4.1) while in the roots and root-crown, significant increases ($P < 0.05$) were observed in all defoliation treatments, relative to the values of the controls.

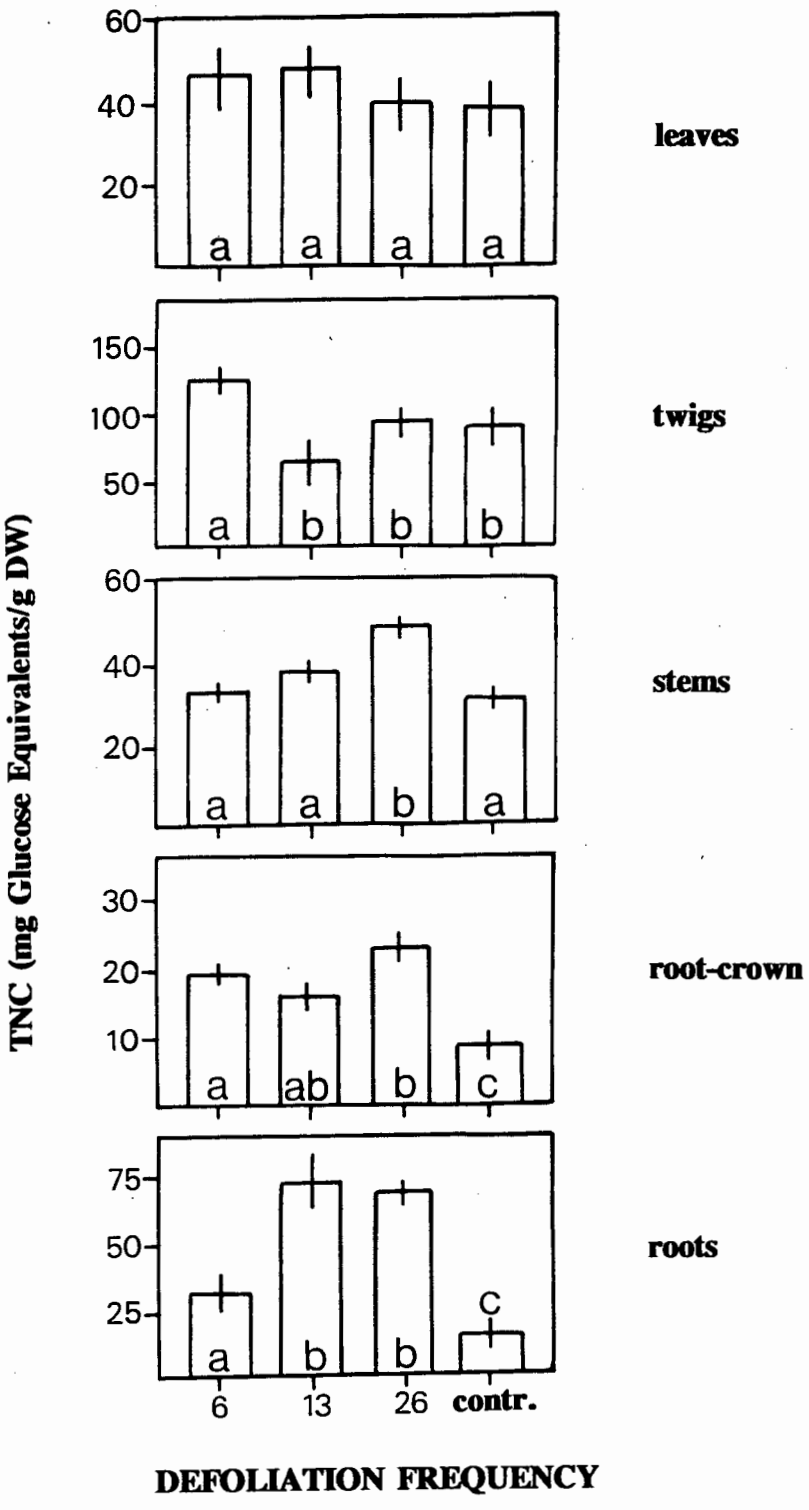

Figure 4.1: Total nonstructural carbohydrate (TNC) concentrations in five organs of ***Osteospermum sinuatum*** plants subjected to the following frequencies of defoliation over a one year study period: every six weeks (6), every thirteen weeks (13), every twenty-six weeks (26) and no defoliation (contr.). Each point represents the mean of five measurements, and the bars = $\pm$ SEM. Dissimilar letters designated to TNC concentrations show significant differences at $P < 0.01$.

Particularly marked increases in TNC concentrations in the roots of the 13-week and 26-week treatments were evident (Fig. 4.1). Leaves of the 13-week and 26-week interval defoliated *Pteronia pallens* plants showed the same TNC concentrations as the leaves of the control plants while in the 6-week treatment, leaf TNC concentrations were significantly lower ($P < 0.05$) than those of the control plants (Fig. 4.2). Significantly lower TNC concentrations were observed in the twigs of all defoliated plants (relative to controls) with no significant differences ($P > 0.05$) between defoliation treatments. In the stems of *P.pallens*, a significant decrease in TNC concentration was observed in the 6-week treatment while the opposite trend was found in the more lenient (13- and 26-week) defoliation treatments (Fig. 4.2). No significant differences were found between the TNC concentrations in the root-crowns of all treatments of *P. pallens* (Fig. 4.2). In the roots, however, decreases in the 6-week and 13-week treatments were detected with significant increases ($P < 0.05$) being observed in the roots of the 26-week interval defoliated plants of *P.pallens* (Fig. 4.2). TNC concentrations in the leaves, twigs and root-crowns of defoliated plants and the controls of *Ruschia spinosa* (Fig. 4.3) did not differ significantly ($P > 0.05$). TNC concentrations of the stems and roots did not differ between defoliation treatments but they were all considerably lower than the concentrations measured in the same organs of the control plants (Fig. 4.3).

4.3.2 Total Plant TNC Levels

Plant TNC pool sizes (dry weight x concentration) increased with decreasing defoliation frequency in *O.sinuatum* and *P.pallens* (Table 4.1). Plant TNC levels of the most lenient defoliation treatments (26-week interval) of both species were significantly greater than TNC values of the control treatment. In *O.sinuatum*, the 13-week treatment values were not significantly different ($P > 0.05$) from the TNC values of the control plants. The 13-week interval defoliated *P.pallens* plants , however, had significantly lower ($P < 0.05$) plant TNC levels than the undefoliated control plants. No differences were observed between defoliated treatments of *R.spinosa* in terms of TNC levels per plant (Table 4.1). The TNC values of the control plants were considerably greater than the values for the defoliation treatments of this species.

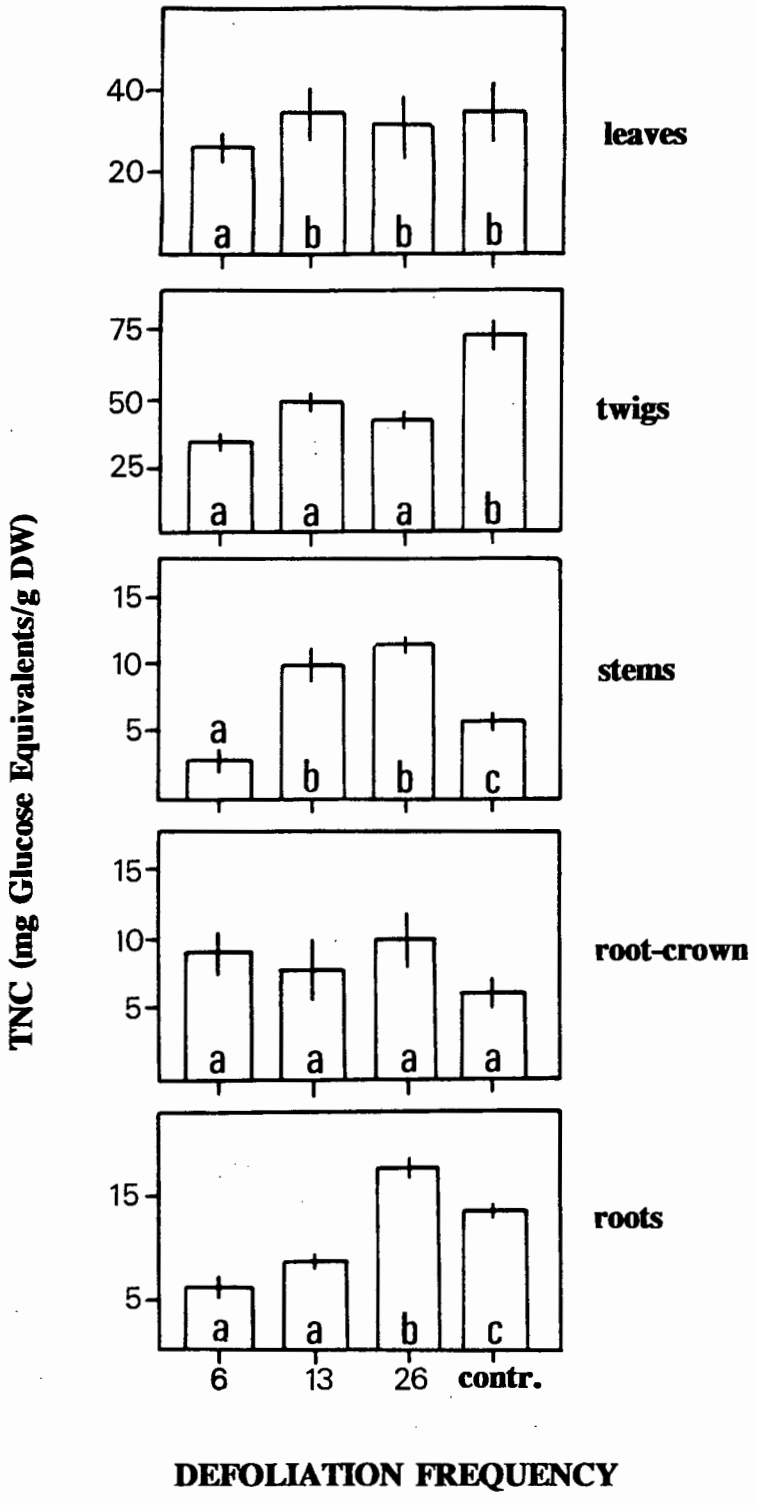

Figure 4.2: Total nonstructural carbohydrate (TNC) concentrations in five organs of ***Pteronia pallens*** plants subjected to the following frequencies of defoliation over a one year study period: every six weeks (6), every thirteen weeks (13), every twenty-six weeks (26) and no defoliation (contr.). Each point represents the mean of five measurements, and the bars = $\pm$ SEM. Dissimilar letters designated to TNC concentrations show significant differences at $P < 0.01$.

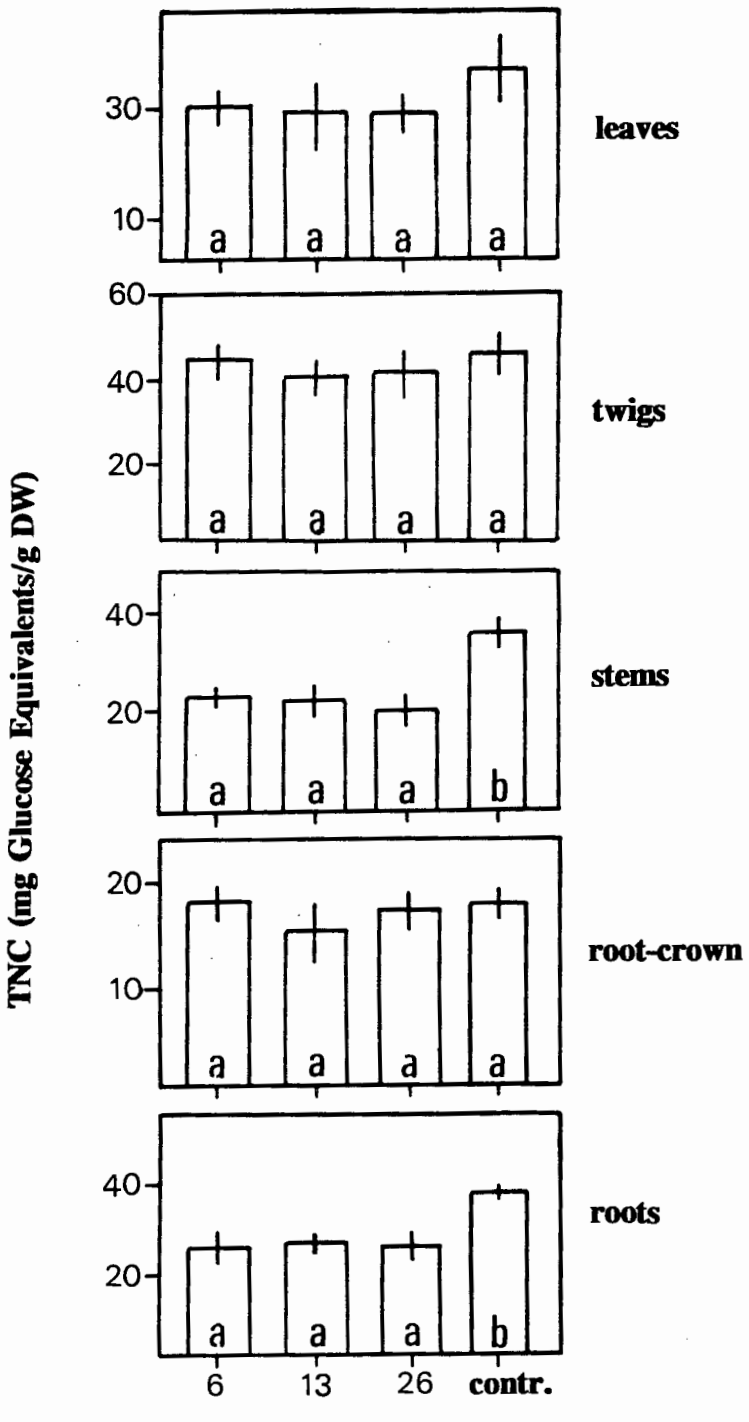

Figure 4.3: Total nonstructural carbohydrate (TNC) concentrations in five organs of ***Ruschia spinosa*** plants subjected to the following frequencies of defoliation over a one year study period: every six weeks (6), every thirteen weeks (13), every twenty-six weeks (26) and no defoliation (contr.). Each point represents the mean of five measurements, and the bars = $\pm$ SEM. Dissimilar letters designated to TNC concentrations show significant differences at $P < 0.01$.

Table 4.1: Total nonstructural carbohydrate content (mg Glucose Equivalents/plant) of *Osteospermum sinuatum*, *Pteronia pallens* and *Ruschia spinosa* plants defoliated at different frequencies over a one year period (n = 5). Values are mean ± SEM. Means in the same row followed by a similar letter are not significantly different at the 0.05 level of probability.

	6-week	13-week	26-week	control
O. sinuatum	711±40 **a**	863±14 **b**	1095±26 **c**	963±26 **b**
P. pallens	1634±96 **a**	2763±211 **b**	5062±87 **c**	4009±233 **d**
R. spinosa	1635±93 **a**	1640±90 **a**	1950±137 **a**	2640±70 **a**

4.4 DISCUSSION

The effects of multiple defoliations on total nonstructural carbohydrate (TNC) concentrations of karoo shrubs varied greatly among species and with defoliation frequency. *Osteospermum sinuatum*, the deciduous non-succulent species, was the only species where repeated defoliations did not result in the decrease of TNC concentrations in any of the plant tissues. Defoliation resulted in the elevation or had no effect on TNC concentrations of *O. sinuatum* tissues. The evergreen succulent species, *Ruschia spinosa* represents the other extreme where defoliations at heavy and at lenient frequencies caused a decrease in TNC concentrations (stems and roots) or had no effect (leaves, twigs and root-crown) on some organs. The responses in organ TNC concentrations of *Pteronia pallens*, the evergreen non-succulent species, were intermediate and increases as well as reductions were observed, depending on the frequency of defoliation. Frequent defoliations mostly caused decreases and the lenient defoliation treatment resulted largely in increases in TNC concentration of *P.pallens* tissues. Degree of defoliation resulted in a generalized pattern in the two non-succulent species (*O. sinuatum* and *P. pallens*) in that the effects of the more frequent defoliation treatments were more severe in terms of TNC concentrations. In the succulent *R. spinosa*, different degrees of repeated defoliation exerted the same effect on the TNC levels of all plant organs.

In this study it was not possible to determine from which sites nonstructural carbohydrates were mobilized in the case of *O. sinuatum*, since all the potential storage organs enlarged their TNC levels in response to defoliation. Analyses of plant organ TNC levels should be undertaken at intervals closer to the time of leaf removal (eg. after 2 weeks) to assess from which organs nonstructural carbohydrates are preferentially used in *O. sinuatum* tissues (refer to Chapter 5). Stems and roots appear to be the sites of primary TNC storage for the other two species since drastic reductions in TNC concentrations were observed in these organs in the heavier defoliation treatment of *P. pallens* and in all treatments of *R. spinosa*. Surprisingly, the root-crowns of these species did not show changes in TNC levels in response to defoliation. The root-crown therefore does not appear to have an important carbohydrate storage function, which is also supported by the observation that this organ consistently accumulated the lowest TNC concentrations in all species.

All the defoliated *O. sinuatum* plants, irrespective of degree of multiple defoliations, as well as the most leniently defoliated *P. pallens* plants, positively readjusted nonstructural carbohydrate allocation patterns after one year. It was therefore expected that the total plant TNC pool sizes of these defoliated plants would be greater than the levels of the control plants. However, only the 26-week interval defoliated plants of these species had higher TNC pool sizes than their respective control plants (Table 4.1). This phenomenon can be explained by the low leaf and twig biomass values of severely defoliated plants (Chapter 2) which, although having high or elevated TNC concentrations, would contribute minimally to plant TNC levels, thereby reducing TNC pool sizes to well below the values of control plants. This illustrates how interpretation of data on the basis of TNC concentrations only, can alter the true significance of the results.

The question has often been debated whether defoliation benefits the productivity potential of plants (McNaughton, 1983; Belsky, 1986). As evidence for these arguments, vegetative and reproductive growth parameters have almost exclusively been used. Biomass allocation studies have shown that defoliation of karoo shrubs, even at a lenient frequency of every 26 weeks, does not benefit the plants in terms of vegetative or reproductive compensatory growth (Chapter 2). However, in the two non-succulent species, restoration of plant storage TNC levels to levels in excess of undefoliated plant TNC levels was observed, and this

readjustment occurred prior to complete vegetative regrowth. Defoliated non-succulent Karoo shrubs therefore overcompensated in terms of carbohydrate allocation to storage as opposed to vegetative or reproductive growth overcompensation as was noted for some grass species. Some factor other than the carbon resource was therefore limiting regrowth in these karoo shrubs. Low decomposition and leaching rates which curtail nutrient availability in arid ecoystems (Hadley & Szarek, 1981) possibly limited production of new photosynthetic tissue following defoliation. Baas (1989) proposed that under suboptimal conditions such as drought or low nutrient conditions, vegetative growth is inhibited due to limitations of nutrient supply. The continuation of photosynthetic processes despite the lack of vegetative growth would result in the formation of carbon based compounds from the excess photosynthates. The results of this study support the predictions from Baas's (1989) carbon/nutrient cycle theory in that the three Karoo shrubs represent alternatives of how the excess carbon is utilized. These alternatives are (i) accumulation of storage carbohydrates and (ii) production of secondary carbon defense compounds. In *O. sinuatum* and *P. pallens* surplus photosynthates are stored as nonstructural carbohydrates as shown by this study. These species, however, do not contain significant quantities of polyphenolic or tannin (secondary carbon) compounds and did not accumulate these defense compounds following defoliation (Appendix 1). *R. spinosa*, representing the second alternative, did not adjust its storage nonstructural carbohydrate concentrations following defoliation. Excess carbon appears to be used for the production of secondary compounds (Appendix 1).

The three karoo shrubs have distinct regrowth capacities (Chapter 2), albeit at low rates: *O. sinuatum* having the highest regrowth capacity, followed by *P. pallens* and lastly *R. spinosa* which exhibited almost no regrowth over a one year period (Chapter 2). Since water and nutrients appear to limit regrowth in this ecosystem, the nutrient relations of karoo shrubs studied should differ and should be species specific to be able to effect such discrete regrowth capacities. Nutrient return in arid ecosystems through mineralization and decay are in fact thought to be strongly localized (Garzia-Moya & McKell, 1970) i.e. concentric plant specific nutrient cycling. The physical nature and chemistry (high in polyphenolics and tannins, Appendix 1) of the evergreen leaves of *R. spinosa* (and of *P. pallens* to a lesser extent) would reduce mineralization processes once litter has fallen, thereby reducing nutrient release rates. In contrast, the absence of secondary compounds (Appendix 1), and the physical

properties of the deciduous *O. sinuatum* leaf would attract the mineralizing soil biota which would lead to a faster nutrient release from the litter. The resultant relatively higher nutrient availability in the soil environment of *O. sinuatum* shrubs would allow the superior regrowth capacity of this species as reported in Chapter 2.

It has been shown that the three karoo shrubs are unable to compensate for defoliation by vegetative regrowth over a one year period (Chapter 2). It has been argued that this inability to completely replace leaf tissue was due to nutrient limitations in the suboptimal semi-arid growth environment of karoo shrubs. No mineralization studies have been undertaken in the Karoo. The evidence that nitrification, for example, in the soils of an adjacent biome (fynbos ecosystem, Stock *et al.*,1988) is limited by low soil moisture content, could also apply to the Karoo. The close association between soil moisture, nutrient factors and plant regrowth, illustrates that plant responses to defoliation cannot be separated from plant responses to the complex of environmental factors. The nature of the response is finally determined by the genetics of the plant, which in turn defines the species specific physiological responses and ultimately the vegetative regrowth of the plant.

CHAPTER 5

NONSTRUCTURAL CARBOHYDRATES: EFFECTS OF SEASON AND INTENSITY OF DEFOLIATION

5.1 INTRODUCTION

Nonstructural carbohydrate reserves have been accredited with the role of providing resources which enable regrowth following defoliation of grasses. Support for this view is based on numerous observations that nonstructural carbohydrate concentrations in storage organs decline following defoliation (Weinmann, 1948; May, 1960; Kinsinger & Hopkins, 1961; Davidson & Milthorpe, 1966b). The importance of reserve carbohydrates in governing regrowth has been questioned by many and the role of current photosynthate has received more recognition (May, 1960; Ryle & Powell, 1974; Richards, 1986). Recent evidence suggests that both photosynthate produced by remaining leaves as well as storage carbohydrates determine regrowth potential of grasses following defoliation (Richards, 1986; Danckwerts & Gordon, 1987). Carbohydrate reserves are thought to be important for a limited period only following defoliation and that carbon required for subsequent regrowth is acquired from photosynthates produced by the new and remaining leaves. Nonstructural carbohydrates in the leaf bases of *Dactylis glomerata*, for example, were found to be only important during the first two days following defoliation (Davidson & Milthorpe, 1966b). Recently, Gonzales *et al.*, (1989) studying perennial ryegrass suggested that regrowth at non-limiting nitrogen concentrations involve the mobilization of up to 90% of stored carbohydrates only during the first six days following defoliation, after which time replenishment of reserves occur. Knowledge of the absolute time taken for a specific plant part to replenish its carbohydrate stores following defoliation is important (i) in order to understand plant processes (in response to defoliation) in stressful environments such as the Karoo and (ii) to predict shrub responses to a possible subsequent defoliation. Short-term changes in carbohydrate concentrations immediatly following defoliation of karoo shrubs are

reported on in this chapter and are intended to provide an estimate of how long shrubs are dependent on their stored carbohydrates.

The paucity of shrub defoliation studies is probably related to the complexity presented by the multiplicity of potential carbohydrate storage sites. For example, nonstructural carbohydrates of karoo shrubs are accumulated in four plant parts (excluding leaves) where they are stored in distinctly different concentrations (Chapter 3). When and to what extent carbohydrates are utilized from storage sites and the relative importance of these sites remain unclear and will be addressed in this study.

Guidelines for a successful grazing practice in most ecosystems, including the Karoo (Danckwerts & Teague, 1989), almost always involve a warning that severe grazing negatively affects plant regrowth potential. This commonly held view is based on the reasoning that defoliation removes the photosynthetic organs of a plant, thereby reducing absolute carbon gain. Furthermore, a heavy defoliation should theoretically have a more severe effect on the carbon economy of the plant, thereby reducing the potential for plant regrowth. However, the changes in stored carbohydrate levels as a result of different intensities of defoliation are not always decisive (Caldwell *et al.*, 1981), probably because studies have not always included an assessment of (i) changes over time (weeks or days) or (ii) the changes occurring in all sites of carbohydrate accumulation.

The principal aim of this study was to determine the effects of different intensities of defoliation on total nonstructural carbohydrate (TNC) concentrations in order to improve the understanding of karoo shrub regrowth responses as affected by different degrees of foliage removal (reported in Chapter 2). TNC concentrations in five plant parts were analyzed to assess the relative importance of the sites of carbohydrate accumulation in shrubs. Changes in TNC concentrations were determined at 2-weekly intervals over a 6 week period to establish the length of time during which the defoliated plant is dependent on stored carbohydrates. These responses were determined during different seasons to assess whether seasonal climatic changes and the concommitant plant physiological changes (eg. photosynthesis) bring about different TNC responses. Seasonal changes in carbohydrate concentrations of undefoliated plants were also studied so that seasonal patterns could be

separated from the TNC changes caused by defoliation.

5.2 METHODS

The experimental procedure for the assessment of the effects of defoliation intensities on plant growth, described in section 2.2, also applies to the present study, since the same plant material was used for total nonstructural carbohydrate (TNC) analyses, reported upon in this chapter. Dried samples were ground in a Wiley mill to pass a 40-mesh screen. Ground tissue was placed in glass vials, redried (70°C) before sealing, and stored in desiccators untill samples were analyzed for TNC. Extraction and determination of total nonstructural carbohydrates were made using procedures described by Smith (1981) (see Section 3.2.3). A total of three replications were used for TNC analyses at each time interval following defoliation. Data were analyzed using a three-factor analysis of variance technique (Zar, 1974) to assess the effects of defoliation intensity, time (weeks) since defoliation and the plant part factor on TNC concentrations. This analysis of variance technique was also employed to establish whether the interactions among factors had a significant effect on TNC concentrations. Three-factor analysis of variance could not be used for the summer data set of *Osteospermum sinuatum* since plants were not subjected to defoliation during this season, as this drought deciduous species had undergone almost complete leaf loss (Chapter 2). Two-way analysis of variance (Zar, 1974) was therefore used to compare TNC concentrations (i) over time (2-weekly intervals) and (ii) among plant parts of undefoliated *O. sinuatum* during summer.

5.3 RESULTS

O. sinuatum leaf TNC analyses could only be undertaken at 0 and 2 weeks following the start of the summer study period since *O. sinuatum* plants were gradually senescing (Chapter 2) and complete leaf abscission had taken place between 2 and 4 weeks after the start of the summer study period. The decrease in leaf TNC concentrations to zero values in the 2 to 4-week period therefore signifies leaf loss as opposed to a drastic decline in leaf TNC

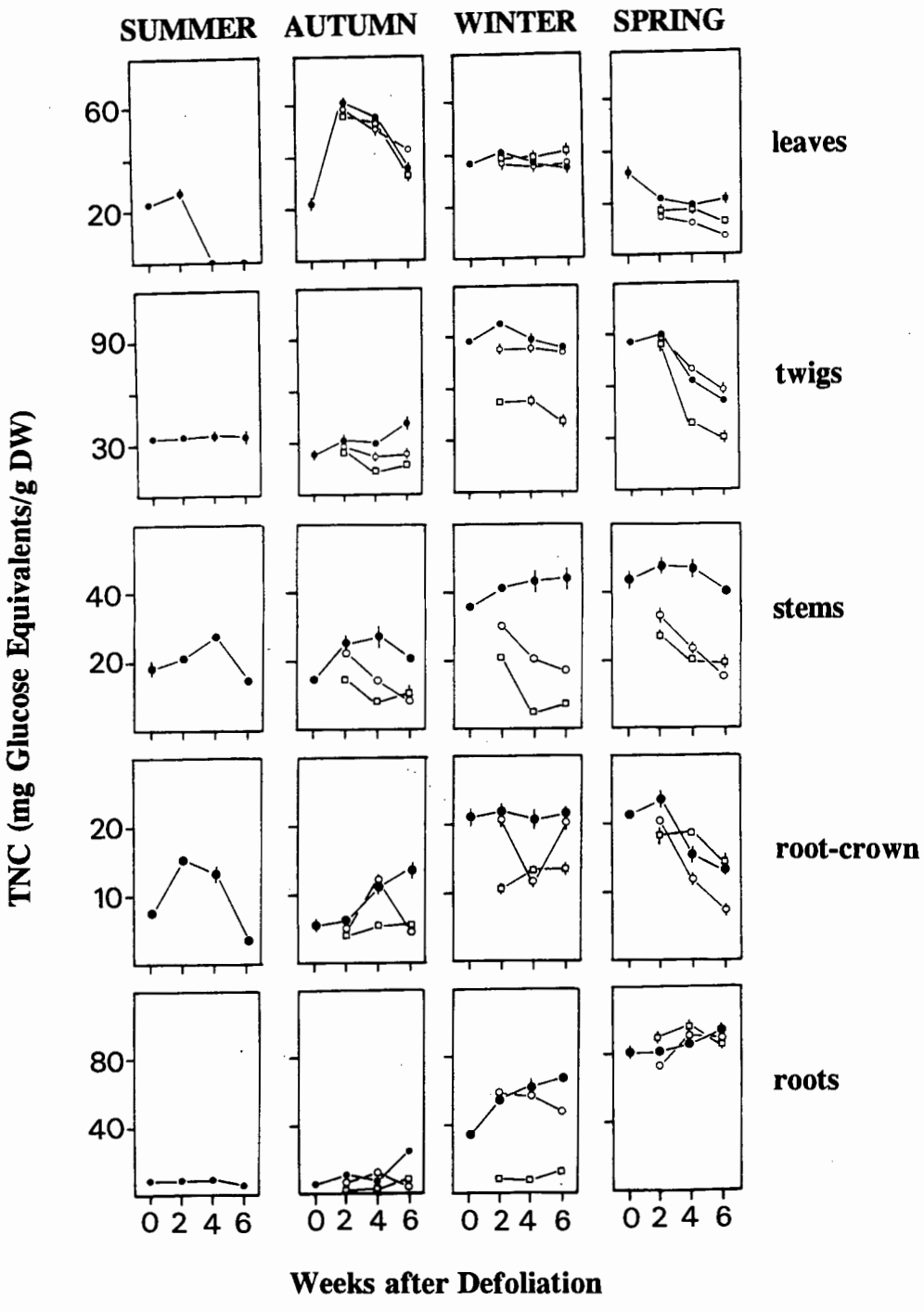

Figure 5.1: Total nonstructural carbohydrate (TNC) concentrations of ***Osteospermum sinuatum*** plant parts, measured at two-weekly intervals following 80% defoliation (open squares), 40% defoliation (open circles) and no defoliation (controls, closed circles). Each point represents the mean of three observations, and the bars = $\pm$ SEM. Where there are no bars, the SEM's are too small to show on the scale.

concentrations (Fig. 5.1). During summer, the concentrations of most *O. sinuatum* plant parts of the controls declined throughout the 6-week study period and reached their lowest values recorded throughout the year (Fig. 5.1). Although TNC concentrations of the twigs and the roots remained unchanged throughout the 6-week summer study period, these values were the lowest measured for these plant tissues throughout the year (Fig. 5.1). The highest TNC concentrations in all plant parts of undefoliated *O. sinuatum* plants were measured during autumn for leaves, and during the winter-spring period for twigs, stems, root-crown and roots (Fig. 5.1). A surprisingly high seasonal fluctuation in TNC concentrations was evident in tissues of the control *O. sinuatum* plants. For example, in the roots a fifty fold increase in TNC concentration was observed from the first (summer) to the final (spring) sampling date over the year-long study period, and this enormous shift in TNC concentration was not equalled by any of the other species in their various plant parts.

Leaf removal, irrespective of defoliation intensity, commonly resulted in the decrease in TNC concentration of all plant parts of *O. sinuatum* (Fig. 5.1). Small increases in response to defoliation were only observed in a few instances such as during autumn (40%, leaves), during winter (80%, leaves), and during spring in the twigs (40%), root-crown (80%) and roots (80%). Generally TNC concentrations of *O. sinuatum* tissues decreased gradually with time in the two to six weeks following defoliation (Fig. 5.1). The only exception was the root-crown of the 40% defoliated plants where increases in TNC concentrations were measured during autumn (4-week) and during winter (6-week). Differences between the 40% and 80% defoliated *O. sinuatum* plants were observed in some cases with the more intense defoliation treatment (80%) generally showing a greater decline in TNC concentrations. However, the reverse pattern was also observed in that 40% defoliation occasionally resulted in a greater decline of TNC concentration (eg. stems, root-crown and roots during spring) (Fig. 5.1).

Seasonal changes in TNC concentrations of undefoliated *P. pallens* plant parts were generally similar to the patterns noted for control plants of *O. sinuatum* plant tissues (Fig. 5.1 & 5.2). The patterns for these two Asteraceous species differ in the following respects. Firstly, the lowest TNC concentrations of undefoliated *O. sinuatum* plant tissues were recorded on the first summer sampling date (09/01/1989), at a time when some of the highest values in plant

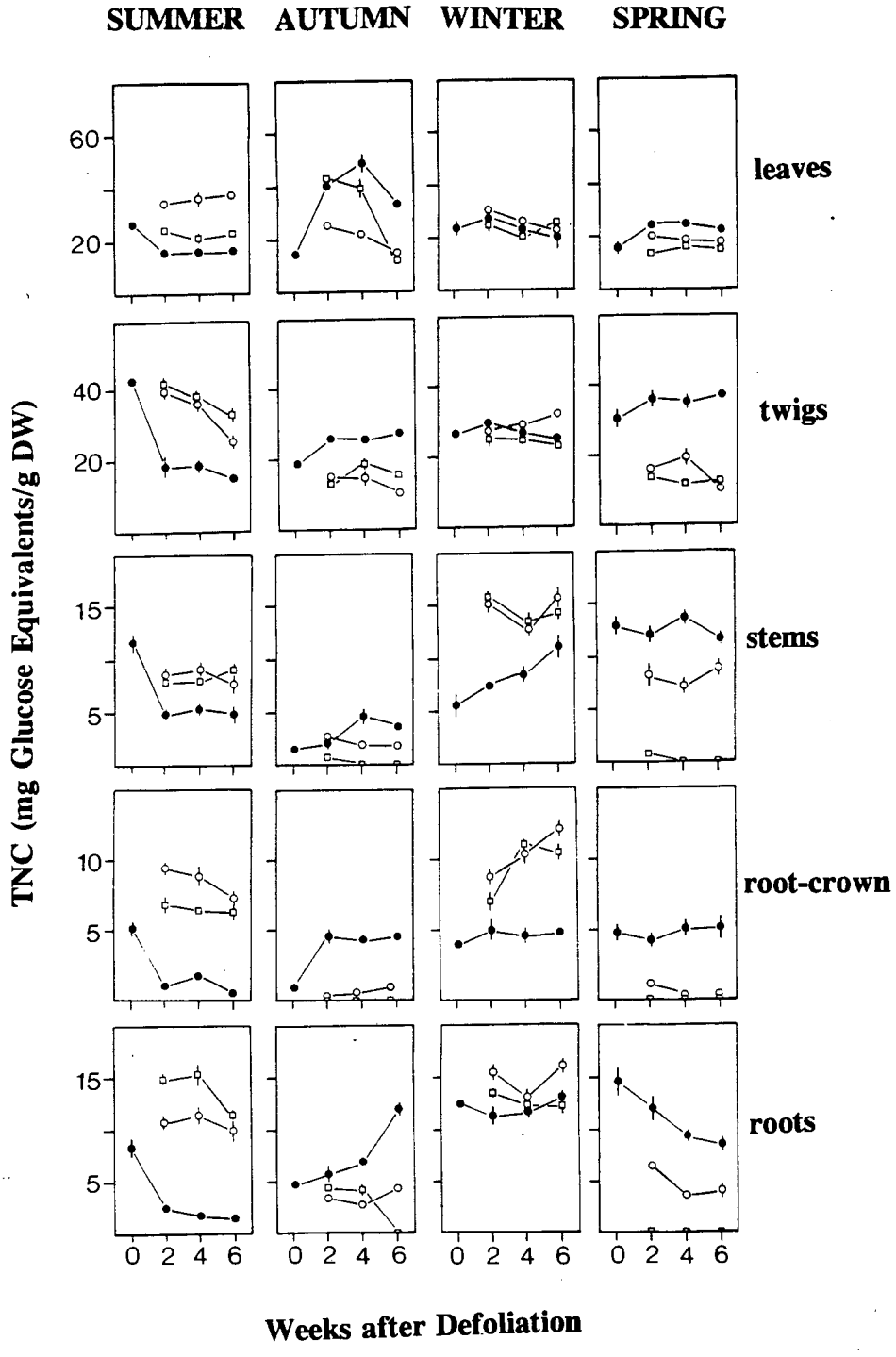

Figure 5.2: Total nonstructural carbohydrate (TNC) concentrations of ***Pteronia pallens*** plant parts, measured at two-weekly intervals following 80% defoliation (open squares), 40% defoliation (open circles) and no defoliation (controls, closed circles). Each point represents the mean of three observations, and the bars = $\pm$ SEM. Where there are no bars, the SEM's are too small to show on the scale.

parts of undefoliated *P. pallens* plants were measured (Fig. 5.1 & 5.2). Secondly, maximum TNC concentrations in *P. pallens* plant parts (excluding stems) was recorded during autumn, whereas TNC concentrations of *O. sinuatum* plant parts (controls) reached their lowest values during autumn (Fig. 5.1 & 5.2).

Summer and winter defoliation commonly resulted in the elevation of TNC concentrations of most *P. pallens* plant parts (Fig. 5.2). However, during winter, leaf and twig TNC concentrations of the controls and the defoliated plants were of a similar order (Fig. 5.2). Autumn and spring defoliation, in contrast, normally resulted in the decline in concentrations of *P. pallens* plant parts. Zero TNC concentrations in response to defoliation were often recorded in *P. pallens* plant parts such as the stems, root-crown and roots (Fig. 5.2).

Differences between the 40% and 80% defoliated plants, in terms of TNC concentrations, were more marked in *P. pallens* than in *O. sinuatum*. In *P. pallens*, for example, 80% defoliation during autumn and spring resulted in no detectable TNC concentrations in the stems, root-crown and roots. In the 40% defoliated plants, however, zero TNC concentrations were only recorded in the root-crown (spring) (Fig. 5.2). Furthermore, TNC concentrations of 40% defoliated plants recovered faster after the initial decline (0 to 2 weeks) than was the case in the 80% defoliated plants.

Differences between TNC responses of the 40% and 80% defoliated *P. pallens* plants were not as evident during the seasons when increases in TNC concentrations were observed i.e. summer and winter. During the latter seasons, relative differences between the 40% and 80% defoliated plants were not consistent and plant part TNC concentrations of the 40% defoliated plants were sometimes greater (eg. summer, root-crown) and were occasionally lower (eg. summer, roots) than in the 80% defoliated plant parts (Fig. 5.2).

Seasonal variation in TNC concentrations of undefoliated *R. spinosa* plants were much less pronounced than the variation noted for the two non-succulent species (Fig. 5.3). Stems, root-crown and roots, in particular, showed relatively little seasonal change. Intensity of defoliation exercised a particularly marked effect on TNC concentrations of *R. spinosa* tissues with the more intense defoliation treatment (80%), resulting in greater decreases

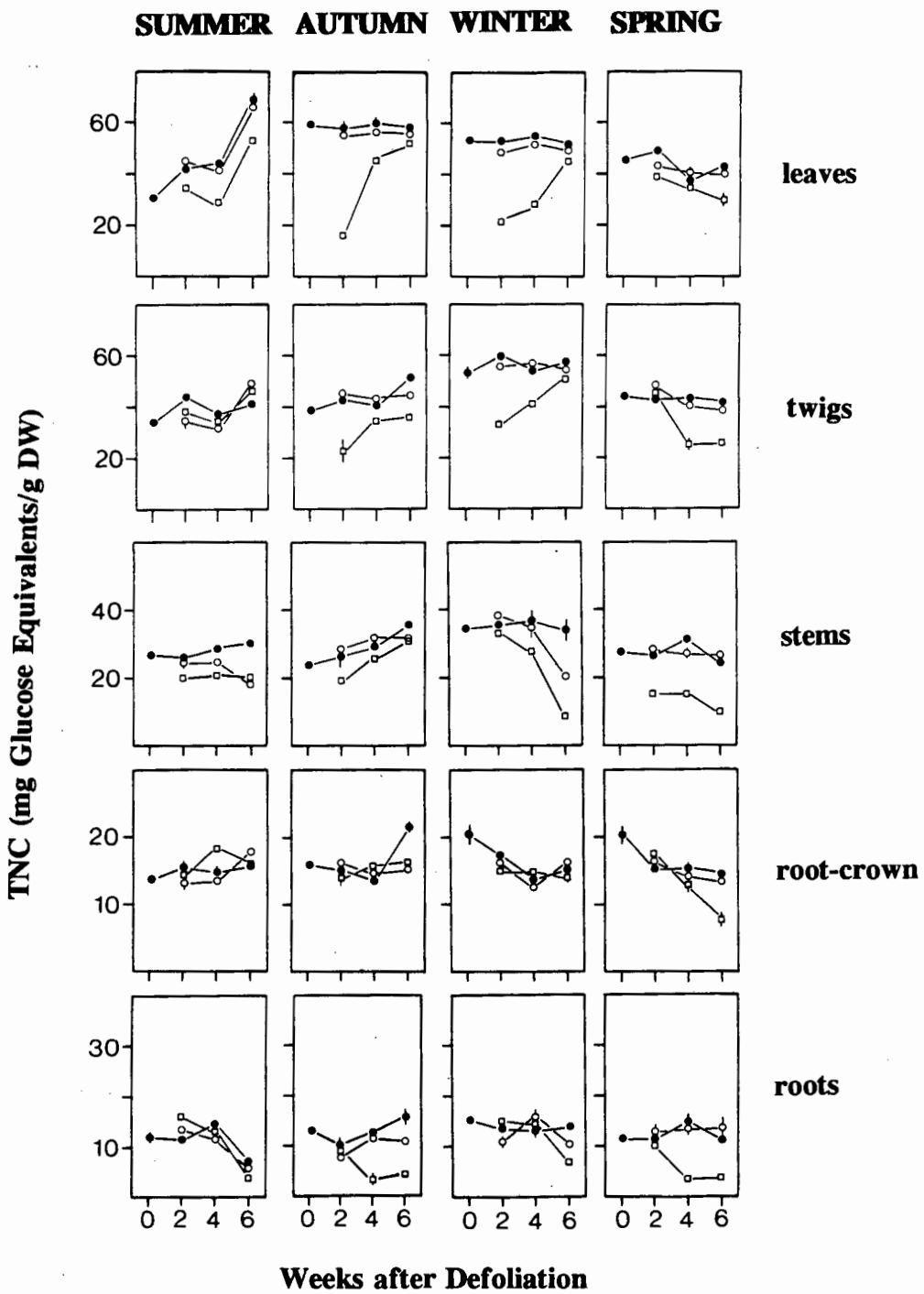

Figure 5.3: Total nonstructural carbohydrate (TNC) concentrations of ***Ruschia spinosa*** plant parts, measured at two-weekly intervals following 80% defoliation (open squares), 40% defoliation (open circles) and no defoliation (controls, closed circles). Each point represents the mean of three observations, and the bars = $\pm$ SEM. Where there are no bars, the SEM's are too small to show on the scale.

(relative to controls) than in the 40% defoliated plants (Fig. 5.3). Distinct differences in the responses of the plant parts were also evident in terms of the time (weeks following defoliation) when a decline in TNC concentration was noted. The leaves and twigs of *R. spinosa* showed an immediate (0 to 2 weeks) decrease in TNC concentration followed by an elevation within six weeks to levels comparable to those of the controls (Fig. 5.3). During spring, however, TNC concentration of the leaves and twigs of the 80% treatment did not show any increases up to six weeks following defoliation. In the stems and roots (root-crown to a lesser extent) the immediate (0 to 2-weeks) decline in TNC concentration of the defoliated plant was not as extreme (relative to leaves and twigs) and noticable decreases were observed only between two and six weeks following defoliation (Fig. 5.3). In these organs, TNC concentrations either levelled off during the 4 to 6 week stage following defoliation or declined even further but rarely showed an increase to levels comparable to those of the controls. Deviations from this generalized pattern were occasionally observed in the stems (autumn), root-crown (summer, autumn and winter) and in the roots (summer) where the concentrations measured did not differ noticably from the concentrations of the undefoliated *R. spinosa* plants (Fig. 5.3).

Analyses of variance illustrated that the effects of the three factors, (i) defoliation intensity, (ii) time since defoliation and (iii) plant part, as well as the effects of interactions between and among these factors on TNC concentrations were significant in all three species studied (Table 5.1 - 5.3). TNC concentrations differed significantly ($P < 0.001$) among the three defoliation treatments, at the intervals following defoliation (2, 4 and 6 weeks), and among the various plant parts (Table 5.1 - 5.3). Furthermore, TNC concentrations of a plant defoliated at a specific intensity were related to the time since defoliation and the plant part measured. TNC concentration at a particular time since defoliation (2, 4 or 6 weeks) was also related to both the plant part analyzed and the defoliation intensity. Finally, in all three species there were statistically significant interactions ($P < 0.001$) of the three factors in determining TNC concentrations (Table 5.1 - 5.3).

TABLE 5.1: Three-way analysis of variance of defoliation intensity (A), time since defoliation (B) and plant part (C) of ***Osteospermum sinuatum*** during autumn, winter, and spring. Values appearing in the table are F-values for the main effect of each factor as well as interactions between and among factors. Two-way analysis of variance was calculated for the summer data since only control (undefoliated) plants were sampled for this species during summer. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	SUMMER	AUTUMN	WINTER	SPRING
MAIN EFFECTS				
DEFOLIATION (A)		110 ***	669 ***	42 ***
TIME (B)	215 ***	134 ***	44 ***	127 ***
PLANT PART (C)	1000 ***	1322 ***	1979 ***	3725 ***
INTERACTIONS				
A X B		27 ***	97 ***	6.3 ***
A X C		8.8 ***	145 ***	26 ***
B X C	100 ***	88 ***	44 ***	62 ***
A X B X C		10 ***	25 ***	7.9 ***

TABLE 5.2: Three-way analysis of variance of defoliation intensity (A), time since defoliation (B) and plant part (C) of ***Pteronia pallens*** during the four seasons of the year. Values appearing in the table are F-values for the main effect of each factor as well as interactions between and among factors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	SUMMER	AUTUMN	WINTER	SPRING
MAIN EFFECTS				
DEFOLIATION (A)	365 ***	344 ***	39 ***	843 ***
TIME (B)	55 ***	118 ***	29 ***	39 ***
PLANT PART (C)	1647 ***	2111 ***	1014 ***	1272 ***
INTERACTIONS				
A X B	48 ***	51 ***	7.3 ***	32 ***
A X C	46 ***	56 ***	5.2 ***	85 ***
B X C	30 ***	102 ***	16 ***	7.0 ***
A X B X C	7.8 ***	15 ***	3.0 ***	11 ***

TABLE 5.3: Three-way analysis of variance of defoliation intensity (A), time since defoliation (B) and plant part (C) of ***Ruschia spinosa*** during the four seasons of the year. Values appearing in the table are F-values for the main effect of each factor as well as interactions between and among factors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	SUMMER	AUTUMN	WINTER	SPRING
MAIN EFFECTS				
DEFOLIATION (A)	38 ***	121 ***	310 ***	208 ***
TIME (B)	154 ***	75 ***	76 ***	135 ***
PLANT PART (C)	2612 ***	2273 ***	3158 ***	2016 ***
INTERACTIONS				
A X B	6.0 ***	52 ***	40 ***	36 ***
A X C	28 ***	47 ***	54 ***	12 ***
B X C	178 ***	33 ***	42 ***	12 ***
A X B X C	9.4 ***	23 ***	26 ***	6.3 ***

5.4 DISCUSSION

Trends of seasonal changes in TNC concentrations of undefoliated karoo shrubs were similar for all three species studied. All species showed a generalized pattern of a decline in TNC concentration from summer to mid-autumn, followed by the replenishment during late-autumn after which time concentrations were kept at relatively high levels throughout the winter and spring periods. However, the magnitude of fluctuation in TNC levels differed among species. The Asteraceous species, *O. sinuatum*, exhibited the greatest fluctuations in both aboveground and belowground tissues, specifically in the roots. This particularly extensive depletion of TNC during the summer-autumn period is probably because the carbohydrate reserves of this species were utilized for maintenance respiratory functions during the summer deciduous phase of the plant. The pronounced decline in TNC reserves during the beginning of autumn coincides with new growth (Chapter 2), and reserves are only replenished during winter when leaf growth has been completed (Chapter 2). In *O. sinuatum*, TNC concentration declined once again towards the end of spring when leaf senescence occurred (Chapter 2). In *P. pallens*, the period of TNC depletion is of a shorter duration and appears to be of a lesser magnitude. Reserves appear to be partially utilized during summer possibly to supplement a water limited low summer photosynthetic carbon gain. Subsequent declines in TNC concentrations at the start of autumn (stems) are associated with new growth occurring in undefoliated plants (Chapter 2). Using the terminology of Menke & Trlica (1981) to catergorize the shape of the annual TNC cycle, the two Asteraceous species can be considered as having a generalized, narrow V-shape TNC cycle. In contrast, the succulent species, *R. spinosa*, exhibited a flat or extended V-shaped TNC cycle since the degree of summer depletion was of a much lower magnitude. The absence of a marked decline in TNC concentrations in *R. spinosa* plant tissues when growth occurs suggests that vegetative growth in undefoliated *R. spinosa* is not dependent on stored carbohydrates but most likely on current photosynthates.

The close relationship between the shape of the annual TNC cycle and regrowth responses following defoliation identified for Colorado rangeland shrubs (Menke & Trlica, 1981) are also pertinent with respect to karoo shrubs. Plants with narrow decline and replenishment TNC cycles, such as *O. sinuatum* amd *P. pallens*, are thought to regrow relatively fast

following defoliation, which has been confirmed by the study reported in Chapter 2. In contrast, plants which are adversely affected by defoliation, such as *R. spinosa* (Chapter 2), are characterized by flat or extended TNC cycles, a feature of *R. spinosa* substantiated by the present study.

A curious phenomenon was the increase in TNC concentrations in *P. pallens* plant parts in response to defoliation during summer and winter. This response was not observed in any of the other species. The deep rooting system and sclerophyllous leaves of *P. pallens* (Chapter 2), are features which probably facilitate moderate rates of photoynthesis during summer drought. Since no regrowth had taken place in this species following defoliation during summer (Chapter 2), surplus carbon from continued photosynthesis, albeit at low rates, resulted in the allocation of carbohydrates to storage. The same reasoning also applies to the winter defoliation responses, when no regrowth occurred in defoliated *P. pallens* plants (Chapter 2) while photosynthetic carbon gain was still proceeding, thereby increasing TNC storage levels. Why were similar responses not observed in the other two species ? In *R. spinosa*, seasonal changes in nonstructural carbohydrates in undefoliated plants were found to be minimal, suggesting that carbohydrates are rarely produced in excess of immediate demand. When excess carbon is available it is allocated to secondary compounds (Appendix 1), as opposed to storage in the form of nonstructural carbohydrates. Leaves remaining following grazing or defoliation are normally the older leaves. Since the age-dependent decline in photosynthetic rates of deciduous species occurs over a much shorter period than in an evergreen plant (Chabot & Hicks, 1982), the remaining leaves of *O. sinuatum* are photosynthetically less active than the leaves remaining following defoliation of an evergreen plant such as *P. pallens*. Relative carbon gain of a defoliated *O. sinuatum* plant would therefore be much lower than that of a defoliated *P. pallens* plant, annulling the possibility of surplus carbon in a defoliated *O. sinuatum* plant over a period as short as 6 weeks.

When regrowth occurred following defoliation (Chapter 2) of *O. sinuatum* (spring), *P. pallens* (autumn & spring) and *R. spinosa* (spring), marked decreases in TNC concentrations were observed during the 6-week monitoring periods. This co-occurrence of regrowth and TNC depletion suggests that regrowth of these karoo shrubs is dependent on stored carbohydrates for periods of at least 6 weeks, which is much longer than the 2 to 6 day

periods quoted for grass species (Davidson & Milthorpe, 1966b; Gonzales *et al.*, 1989). There were indications that TNC concentrations stopped declining or started to increase at 6 weeks following defoliation which suggests that the period of dependency on reserves during regrowth is in the order of 4 to 6 weeks. These estimates of karoo shrub dependency on stored carbohydrates are verified using more sophisticated experimental procedures such as an etiolated regrowth technique (Chapter 7).

Decreased TNC concentrations observed in the twigs, stems, root-crown and roots of defoliated plants suggests that the carbohydrates in these plant tissues had been utilized. It cannot be assumed that the decline in TNC concentrations in the leaves of defoliated plants is a consequence of carbohydrate mobilization. It is more likely that defoliation removed the younger leaves with their inherently higher photosynthetic rates (Chabot & Hicks, 1982), and the remaining older leaves with their lower photosynthetic rates and lower TNC levels erroneously reflect a decline in TNC concentrations following defoliation.

In *R. spinosa* and *P. pallens* (during autumn and spring only), the heavy defoliation intensity resulted in greater declines in TNC concentrations. The differences in TNC concentration between defoliation treatments can therefore be interpreted in terms of dissimilar demands on carbohydrate reserves. During summer and winter, however, *P. pallens* did not regrow, and there was consequently a lower demand for carbon. The ultimate outcome was an increase in TNC concentrations of plant parts and the effects of different intensities of defoliation on TNC concentration became indistinct. In *O. sinuatum*, the effects of intensity of defoliation on TNC concentrations were also lacking a well-defined pattern. It is likely that the moderate defoliation as applied in this study was not very different from the heavy defoliation for this deciduous species since its physiology is adapted to the recurring phenomenon of leaf loss.

In the case of *P. pallens* it was not possible to differentiate a pattern or sequence in which stored carbohydrates were utilized from the plant parts in response to defoliation. For example, no detectable levels of nonstructural carbohydrates were found in the stems, root-crown and roots at approximately the same time following defoliation, which indicates that all three plant parts are similarly important as storage sites for this species. In contrast, there

appears to be a spatial separation in the utilization of nonstructural carbohydrates of *O. sinuatum*. TNC concentrations in the roots of *O. sinuatum* did not decline following moderate defoliations at any time of the year, or following moderate and heavy defoliation during spring. TNC concentrations in the stems, however, decreased drastically at all times of the year, irrespective of intensity of defoliation which suggests that the stem of *O. sinuatum* represents a more important site of carbohydrate storage from where carbon is mobilized preferentially. In the case of *R. spinosa*, TNC concentrations of the twigs of defoliated plants often decreased drastically within 2 weeks following defoliation and then increased towards undefoliated plant levels within 4 to 6 weeks. TNC concentrations of stems and roots decreased later than twig TNC concentrations in defoliated plants and rarely showed a similar rapid replenishment as observed for the twigs. These trends of decline and refilling of storage carbohydrates in *R. spinosa* tissues demonstrate a strong spatial component and an integral temporal element in the carbohydrate utilization patterns of this species following defoliation.

The co-occurrence of regrowth and the depletion of stored carbohydrate reserves following defoliation suggests that grazed karoo shrubs are dependent (for regrowth) on stored carbon for considerable periods possibly even up to 6 weeks following defoliation. The contrasting seasonal patterns of TNC utilization (and TNC accumulation, *P. pallens*) observed in karoo shrubs as well as the differences between heavy and moderate defoliations with respect to TNC utilization patterns, indicate that climate as well as grazing management can greatly affect nonstructural carbohydrate accumulation which in turn influences plant regrowth.

CHAPTER 6

PHOTOSYNTHESIS AND PARTITIONING OF PHOTOSYNTHATES FOLLOWING DEFOLIATION

6.1 INTRODUCTION

The partitioning of photosynthetic products within plants has been studied in some detail for selected grass, cereal and tree species. These studies have most often employed quantitative radiocarbon tracer techniques which involve exposure of plants to $^{14}CO_2$, followed by the determination of ^{14}C-assimilate distribution patterns within the plant after various time intervals (Ryle & Powell, 1974; Ryle & Powell, 1976; Gordon *et al.*, 1977; Gordon *et al.*, 1987; Isebrands & Nelson, 1983; Danckwerts & Gordon, 1987; Danckwerts & Gordon, 1989). In uniculm barley, for example, the bulk of assimilated carbon is thought to be used as respiratory substrate or is utilised for plant structural purposes within a matter of days (Gordon *et al.*, 1977).

Patterns of photosynthate partitioning vary greatly throughout the year because spatial demands for carbon fluctuate with phenological events, such as fruiting or root growth (Oechel *et al.*, 1972; Fick & Sosebee, 1981). Assimilate distribution also appears to be associated with changes in ambient environmental conditions such as light regime (Ryle & Powell, 1976) and temperature (Farrar, 1980).

The effect of defoliation on assimilate partitioning has been studied in graminaceous plants in order to obtain a more exact differentiation between the roles of reserve carbohydrates and (current) photosynthates in regrowth. Evidence indicates that graminaceous plants redistribute current photosynthates from root to leaf meristems following defoliation (Ryle & Powell, 1975), thereby contributing directly to new growth. Patterns of long-term storage and remobilization of ^{14}C, however, indicate that reserve carbohydrates are only partly

utilized for regrowth following defoliation (Danckwerts and Gordon, 1987: Danckwerts & Gordon, 1989). Studies of this nature have enabled grassland scientists to improve the understanding of the relationships between plant growth and plant carbon economy.

Although increased photosynthetic rates have been found for the leaves remaining after defoliation (Gifford & Marshall, 1973; Hodgkinson, 1974; Detling *et al.*, 1979), reduced photosynthetic rates following foliage removal have also been reported (Ryle & Powell, 1975). Elevated photosynthetic rates is one plant physiological response that could account for increased productivity following foliage removal in grasses (McNaughton, 1983; Wallace *et al.*, 1984). It may also account for the different regrowth and carbohydrate allocation patterns following defoliation of karoo shrubs (Chapters 2, 4 & 5).

The objective of this study was to test whether these concepts concerning the effects of defoliation on photosynthesis and photosynthate partitioning in grasses also apply to shrubs. In studying these responses it should be possible to determine whether photosynthetic rates and photosynthate allocation patterns regulate the observed regrowth (Chapter 2) and TNC allocation (Chapters 4 & 5) patterns of karoo shrubs. To elicidate such relationships it was necessary to include analyses of defoliation effects on plant biomass and plant part nonstructural carbohydrate levels. In most studies the partitioning of ^{14}C-assimilates has been investigated in relation to distribution of carbon to broad categories of sinks, such as plant organs. In this study the partitioning of ^{14}C-assimilates to different carbon fractions of each plant organ was also investigated as the precise fate of carbon in allocation studies has been poorly studied in shrubs. Farrar (1980) found it convenient to consider the plant carbon pool as consisting of three carbon fractions (soluble, storage and structural) since it enabled an estimation of the cost of storage and structural carbon synthesis and secondly, the effect of environmental changes on assimilate distribution could also be easily tested by using this 3-compartment model of carbon allocation in a growing plant. The effect of defoliation on assimilate partitioning of karoo shrubs are also studied within the framework of the compartmental analysis used by Farrar (1980) since it has proved to be a simple model which enables an adequate assessment of the fate of photosynthetic products.

6.2 MATERIAL AND METHODS

6.2.1 Plant Material, Growth Conditions and Defoliation

Plants were transplanted from the field into 18-litre pots filled with the field site soil during November 1989. Mature plant individuals of the shallow-rooted *Ruschia spinosa* established easily following transplantation. Transplanting of mature plants of *Osteospermum sinuatum* and *Pteronia pallens*, however, was not effective, probably because the rooting system of these deep-rooted species were inevitably damaged during the transplanting process. Younger plant individuals, with ages ranging from two to five years, were therefore transplanted and used for this study. Plants in their pots, were put back into the soil and irrigated on a weekly basis to encourage rapid establishment in their new surroundings. After one year of growth in their natural environment, the potted plants were transferred to controlled environment rooms with a photoperiod of 12 h light (28°C, 40% RH) and 12 h dark (15°C, 70% RH) cycles. A photosynthetic photon flux density (PPFD) of 1800 μmol m^{-2} s^{-1} was supplied in these rooms. Plants were left for two weeks to acclimate in the environmental rooms before defoliation treatments were applied. Each plant was irrigated with 500 ml of distilled water once every week for the duration of the study period. Four plant individuals of each species were defoliated by removing 40 % of the leaf and twig material. Another set of four plants were left uncut to serve as the control treatment. An additional set of four plants were harvested to determine growth ratios (section 6.2.4) and carbohydrate fraction concentrations (section 6.2.6) in plant parts at the time of defoliation. A moderate defoliation treatment was used to ensure that adequate leaf area remained for $^{14}CO_2$ uptake by a defoliated plant in order to reliably compare the ^{14}C partitioning with that of control plants. $^{14}CO_2$ feeding of a heavily defoliated plant in the modified environment of the assimilation chamber (section 6.2.3) would have taken a much longer period, compared to controls, thereby imposing a different set of environmental conditions on the defoliated plants. Such differences could result in ^{14}C partitioning between the defoliated and control plants not being directly comparable.

6.2.2. Photosynthetic Measurements

Photosynthetic CO_2 assimilation was monitored with a portable infra-red gas analyzer (LCA-2, Analytical Development Company Ltd., Hoddesdon, England). Measurements were

carried out immediately (0 days) following defoliation and at one, two, ten and eleven days following defoliation. All measurements were taken from 8h3O to 10h00 to minimize variation due to diurnal changes in photosynthetic rates. The youngest fully expanded leaves on shoot tips of plants were found suitable for gas exchange measurements in that they did not cause leaks in the air circuit at the point of insertion into the leaf chamber. Repeated photosynthetic meausurements were undertaken on the same set of leaves of 2 shoot tips per plant (total of 8), and the dry weight of these leaves were determined at the end of 3 weeks when all plants were harvested. Shapes of the sclerophyllous leaves of *Pteronia pallens* and the succulent leaves of *Ruschia spinosa* were not suitable for conventional leaf area determinations. Photosynthetic rates of all three species were therefore expressed on a unit dry weight basis. Photosynthetic rates in dry weight units cannot be used to compare absolute photosynthetic rates among species since leaf dry weight of these species is no reflection of potential photosynthetic surface. Defining photosynthetic rates in terms of dry weights facilitates comparisons among species only in terms of the relative changes in photosynthetic rates as affected by defoliation, which is one of the aims of this study. Two-way analysis of variance was used to compare the effects of defoliation on photosynthetic rates measured at various time intervals following defoliation (Zar, 1974).

6.2.3 Labelling with $^{14}CO_2$

Plants were exposed to $^{14}CO_2$ at 3 weeks following defoliation. Plants were transferred to a perspex assimilation chamber designed to be air tight and to provide a steady flow of air over the plants. A fan continuously mixed the air so that the specific activity of $^{14}CO_2$ was supplied uniformly to three plants labelled simultaneously. Air flow through the assimilation chamber was connected in a closed circuit, which included an infra-red gas analyzer (Model 225 MK 3, Analytical Development Company Ltd., Hoddesdon, England) to continuously monitor CO_2 depletion. $^{14}CO_2$ was released into the assimilation chamber by reacting 50% lactic acid with 100 μCi $NaH^{14}CO_3$. To facilitate rapid CO_2 uptake under these conditions the lights of the controlled environment room were switched off and then systematically turned on to simulate the increase in PPFD with the break of day. This sytem ensured that the three plants depleted the $^{14}CO_2/^{12}CO_2$ mixture in the chamber by 200 ppm within 1.5 h. $^{14}CO_2$ labelling was carried out from 08h00 to 10h00.

6.2.4 Plant Harvest and Growth Ratio

Plants were harvested 24 h following $^{14}CO_2$ labelling and each plant was divided into leaves, twigs, stems, root-crown and roots. Plant components were immersed in liquid nitrogen followed by drying at 70°C for 72 h. A growth ratio value (Chapter 2) was calculated for each plant individual. Growth ratio values were arcsine transformed before statistical analyses. Differences between treatments were analyzed using a one-way analysis of variance technique, and the Tukey's multiple range test was used to determine significant between-treatment differences (Zar, 1974).

6.2.5 Extraction and Radioactivity Measurements

Dried plant components were ground in a Wiley Mill to pass a 40 mesh screen. Plant material was mechanically shaken for 1h with 90% ethanol which removed sucrose and hexoses (soluble fraction). A 90% ethanol extraction was used since all three species contain fructans of a relatively short-chain variety (Chapter 3) which would be removed with extractions using more dilute concentrations of ethanol (Smith & Grotelueschen, 1966; Smith, 1973). The ethanol extract was decanted and made up to 50 ml or 100 ml volume depending on organ biomass. The residue was air-dried and then subjected to the enzymatic (amyloglucosidase) procedure used to quantify total nonstructural carbohydrates (described in Chapter 3). This extraction removed the fructans and starch (storage fraction) from the plant tissue. Enzyme extracts were filtered and made up to 50 ml or 100 ml volumes. Absolute radioactivity of subsamples from the ethanol (0.2 ml) and the amyloglucosidase (0.1 ml) extracts was determined with a LS 5000 TD liquid scintillation counter (Beckman Instruments, California, USA), using Ready-Gel scintillation fluid (Beckman Instruments, California, USA). The scintillation counter was set up to provide disintegrations per minute (DPM) values which were automatically corrected for quenching. Plant tissue remaining following the initial labile carbohydrate extractions contained the structural carbon fraction which includes many nitrogenous carbon compounds. This material was dried at 70°C and subsamples of 0.03 g taken for oxidation using a Packard Tricarb Sample Oxidizer (Model 306, Illinois, USA). Gas released during the oxidation process of the subsample (and the filter paper enclosing the sample) was collected in Carbo-sorb (Packard, Illinis, USA) and washed into a scintillation vial with Insta-gel liquid scintillation fluid (Packard, Illinois, USA). The absolute radioactivity of these samples was also determined using the LS 5000

TD liquid scintillation counter. The mean DPM value of three replicate measurements was used to determine the ^{14}C (kBq/g DW) content of each carbon fractions in the various plant parts.

The absolute amount of $^{14}CO_2$ taken up by each plant, however, can vary due to a number of factors. These factors are: (i) variation in environmental conditions within the assimilation chamber, (ii) differences in photosynthetic carbon gain over the labelling period as a result of differences in photosynthetic leaf area per plant and (iii) variation with respect to the absolute quantity of $^{14}CO_2$ generated each time. Due to the resulting variation in $^{14}CO_2$ assimilated by each plant, it was not possible to campare the absolute values of ^{14}C partitioning. The quantity of ^{14}C label in each carbohydrate fraction (within each plant part) was therefore expressed as a percentage of the total ^{14}C-label recovered from the entire plant at the time of harvest. One hundred percent is therefore not based on the initial plant specific $^{14}CO_2$ uptake. Percentage values were arcsine transformed prior to statistical analyses. A one-way analysis of variance technique was used to compare the effects of defoliation on the percentage of ^{14}C-label partitioned to each carbon fraction within each plant part. This was followed by Tukey's multiple range test to determine significant between-treatment differences (Zar, 1974).

6.2.6 Carbohydrate Analyses

Aliquots of the ethanol and enzyme extracts were assayed for carbohydrate content using a modified Shaeffer-Somogyi copper-idiometric titration technique (Chapter, 3; Smith, 1981). Alcohol in the ethanol extract was replaced with water during evaporation on a hot plate prior to reducing power determinations (Chapter 3). Ethanol soluble material included sucrose, glucose and fructose, while the material extracted with amyloglucosidase consisted of starch and fructans, the storage carbon fraction. Two-way analysis of variance was computed to (i) compare the concentrations of the soluble and storage carbohydrate fractions in the defoliated and control plants and (ii) to determine the differences in these carbohydrate concentrations between plant parts (Zar, 1974).

6.3 RESULTS

6.3.1 Growth Ratio

There were no significant differences between the growth ratios of the control plants harvested on day 1 and after 3 weeks (Fig. 6.1) which indicate that no growth had taken place in the control plants in any of the species over the study period. Significant differences ($P < 0.001$) were observed between the control plants and the 40% defoliated plants in all species, which suggests that 40% defoliation significantly decreased leaf and twig biomass. Since regrowth values of the defoliated plants were only measured once during the study period it was not possible to verify, using the data set, whether regrowth had taken place. However, these were no visual signs of new leaves being produced during the 3-week study period (personal observation).

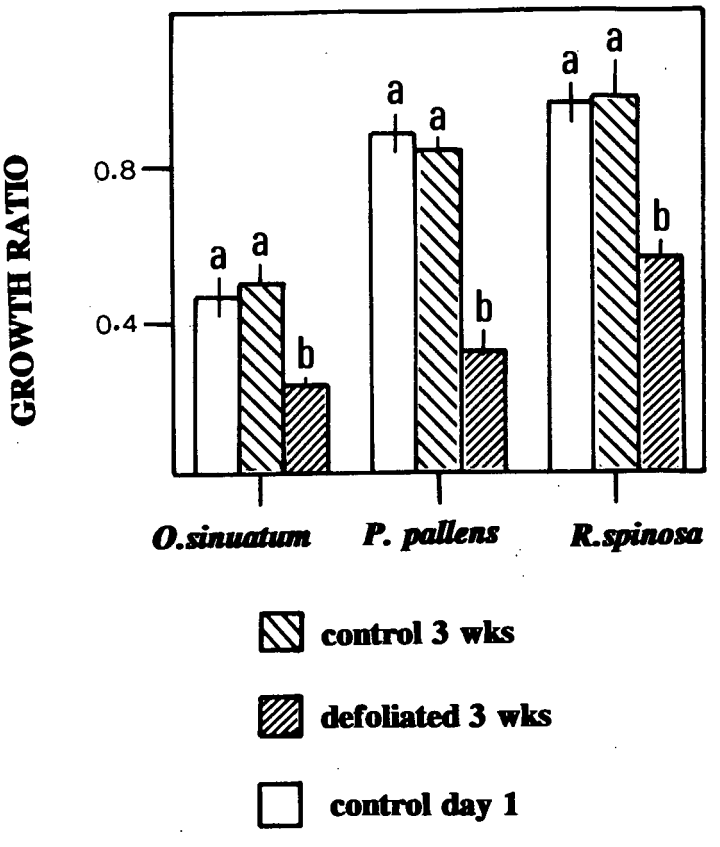

Figure 6.1: Growth ratio of (i) undefoliated plants measured at the start of the study period (day 1), (ii) control plants after 3 weeks (control 3 wks) and (iii) 40% defoliated plants after 3 weeks (defoliated 3 wks). Each point represents the mean of 4 observations, and the bars = $\pm$ SEM. Dissimilar letters designated to the growth ratio values of each species show significant differences at $P < 0.001$.

6.3.2 Photosynthetic Rates

Photosynthetic rates of the defoliated and control plants of *O. sinuatum* and *R. spinosa* did not differ significantly on the 5 days when measurements were made (Fig. 6.2, Table 6.1). Although a considerable increase in net photosynthetic rate was observed in the defoliated plants of *P. pallens* at 3 days following defoliation (Fig. 6.2), the rates of the defoliated plants were not significantly different from the control plants (Table 6.1). However, analysis of variance showed that there was a significant interaction ($P < 0.05$) between the defoliation factor and the time factor (Table 6.1) on the photosynthetic rates of *P. pallens*.

6.3.3 Nonstructural Carbohydrates

Plant part soluble and storage carbohydrate concentrations of the three species remained relatively constant in undefoliated plants over the 3-week study period (Fig.6.3 - Fig.6.5). However, concentrations of carbohydrate fractions differed significantly among plant parts ($P < 0.001$, Table 6.2) of defoliated and control plants of all species. The contribution of these carbohydrate fractions to TNC varied with plant part and with species. In undefoliated plants, sucrose and hexoses generally contributed least to total nonstructural carbohydrates (TNC), except in the leaves of *P. pallens* and the leaves and twigs of *R. spinosa* (Fig.6.4 & Fig.6.5) where the soluble fraction contributed more than the storage component to plant part TNC levels. Defoliation had a significant effect on the storage carbohydrate concentrations of all species ($P < 0.001$, Table 6.2). At three weeks following defoliation decreases in storage carbohydrate concentrations were observed in the twigs, stems, root-crown and roots of all species, exceptions being the root-crown of *O. sinuatum* and twigs of *R. spinosa* (Fig.6.3 - 6.5). Defoliation did not have an effect on the soluble carbohydrate concentrations of *R. spinosa* (Fig. 6.5) in any of its plant parts (no interaction between factors, Table 6.2). In *O. sinuatum*, there were no significant differences between the soluble carbohydrate concentrations of the control and defoliated plants but there appears to be an interaction ($P < 0.01$) between the defoliation and the plant part factors (Table 6.2) in determining soluble carbohydrate concentration of this species. The significance of the effect of defoliation on the soluble carbohydrate concentration of *P. pallens* tissues ($P < 0.05$; Table 6.2) is due to the decrease in concentrations of this carbohydrate fraction observed in the stems following defoliation (Fig. 6.4). However, there was no interaction between defoliation and plant parts in determining the soluble carbohydrate concentrations of this species (Table 6.2).

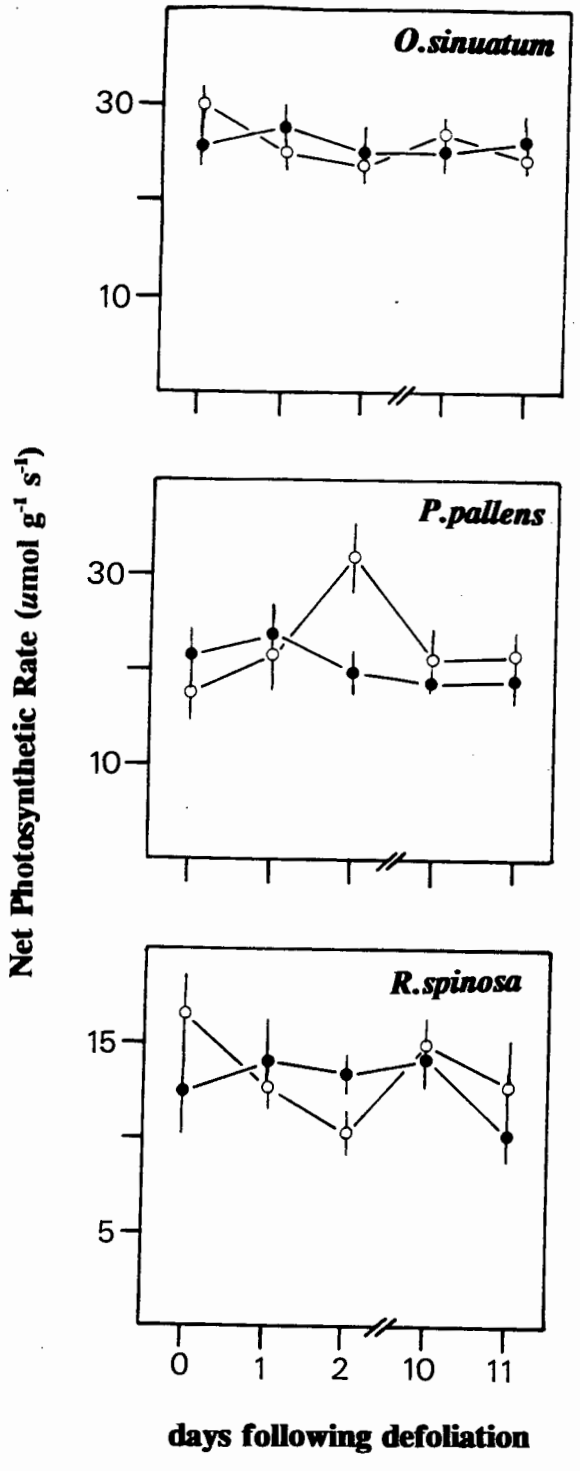

Fig. 6.2: Net photosynthetic rates of youngest fully expanded leaves of undefoliated (closed circles) and 40% defoliated (open circles) plants of *Osteospermum sinuatum*, *Pteronia pallens* and *Ruschia spinosa* at time intervals following defoliation. Each point represents the mean of 8 observations. Vertical bars represent $\pm$ SEM.

TABLE 6.1: Two-way analysis of variance of photosynthetic rates of *Osteospermnum sinuatum*, *Pteronia pallens* and *Ruschia spinosa*. The two factors being simultaneously tested are defoliation treatment and time (days) since defoliation. Values are F-values which are the main effects and the interaction of the two factors. Degrees of freedom for the defoliation treatment factor are 1, 49 and for the time factor are 4, 49. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	DEFOLIATION	**TIME**	**INTERACTION**
O. sinuatum	0.032 ^{NS}	1.297 ^{NS}	5.183 ^{NS}
P. pallens	2.280 ^{NS}	2.514 ^{NS}	3.891 **
R. spinosa	0.193 ^{NS}	2.563 ^{NS}	2.031 ^{NS}

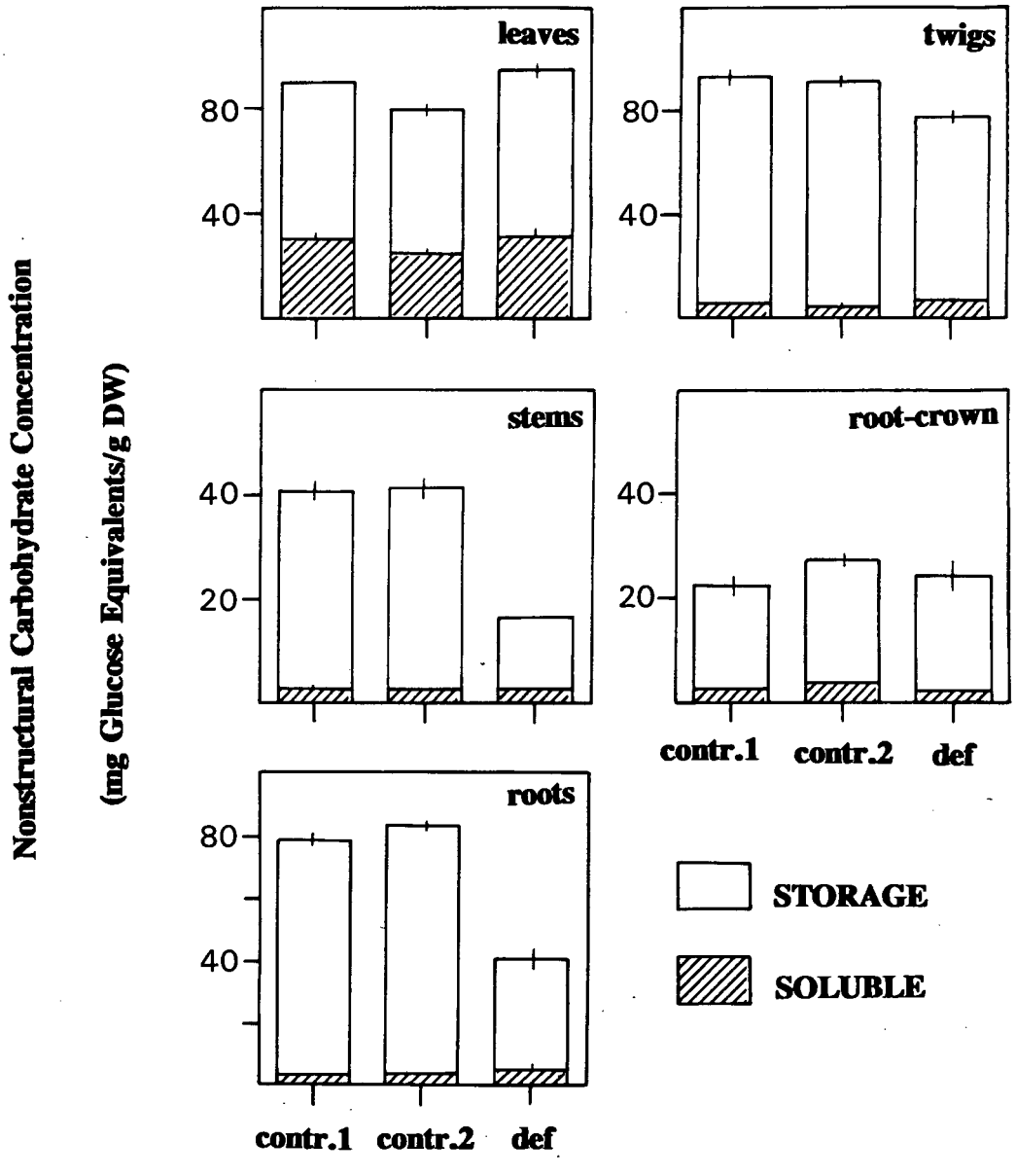

Fig. 6.3: Concentrations of sucrose and hexoses (soluble fraction), and of starch and fructans (storage fraction) in plant parts of undefoliated ***Osteospermum sinuatum*** plants measured on the day of defoliation (contr.1) and again after 3 weeks (contr.2), and of 40% defoliated plants measured 3 weeks following defoliation (def). Each point represents the mean of 3 observations. Vertical bars represent $\pm$ SEM.

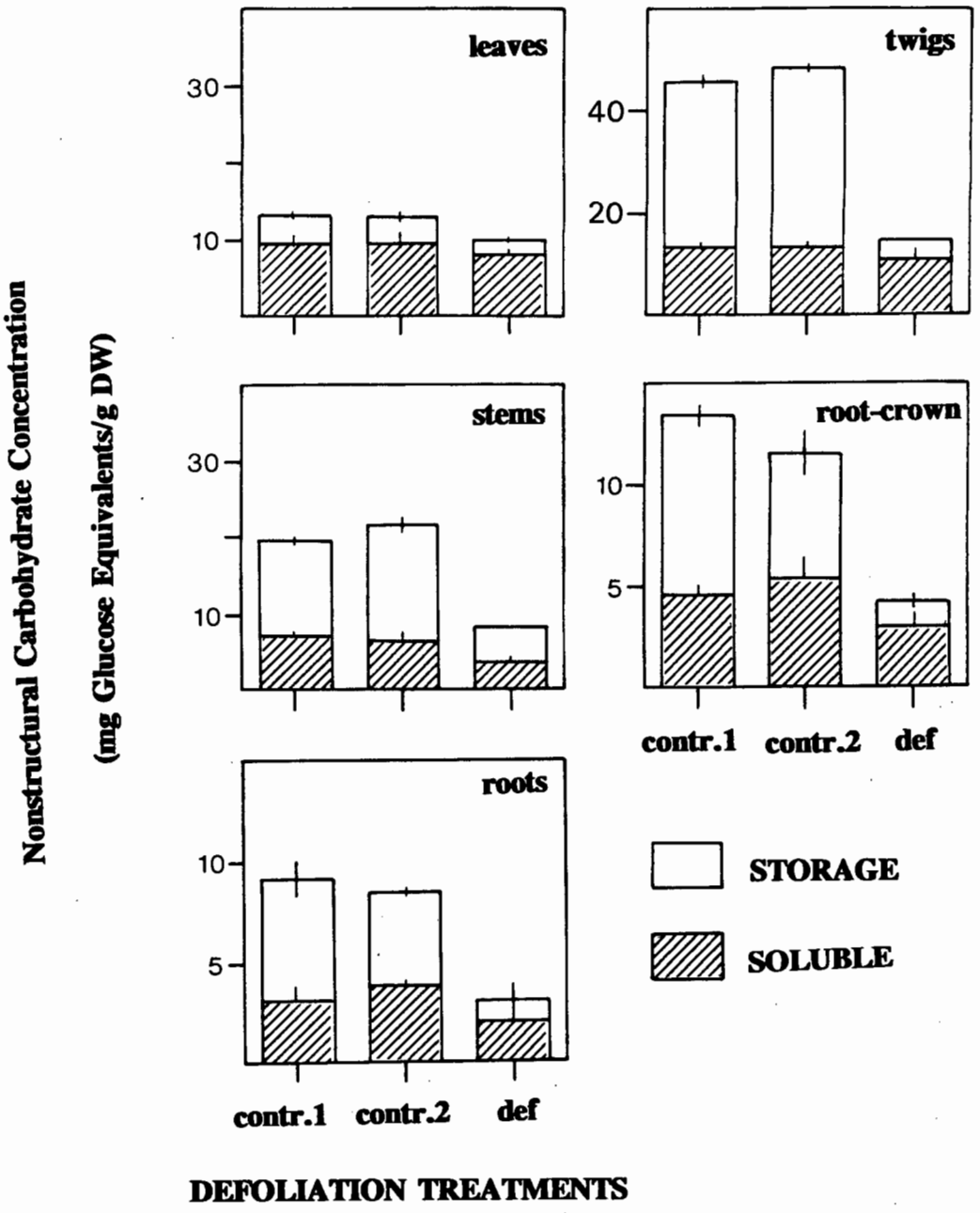

Fig. 6.4: Concentrations of sucrose and hexoses (soluble fraction), and of starch and fructans (storage fraction) in plant parts of undefoliated ***Pteronia pallens*** plants measured on the day of defoliation (contr.1) and again after 3 weeks (contr.2), and of 40% defoliated plants measured 3 weeks following defoliation (def). Each point represents the mean of 3 observations. Vertical bars represent $\pm$ SEM.

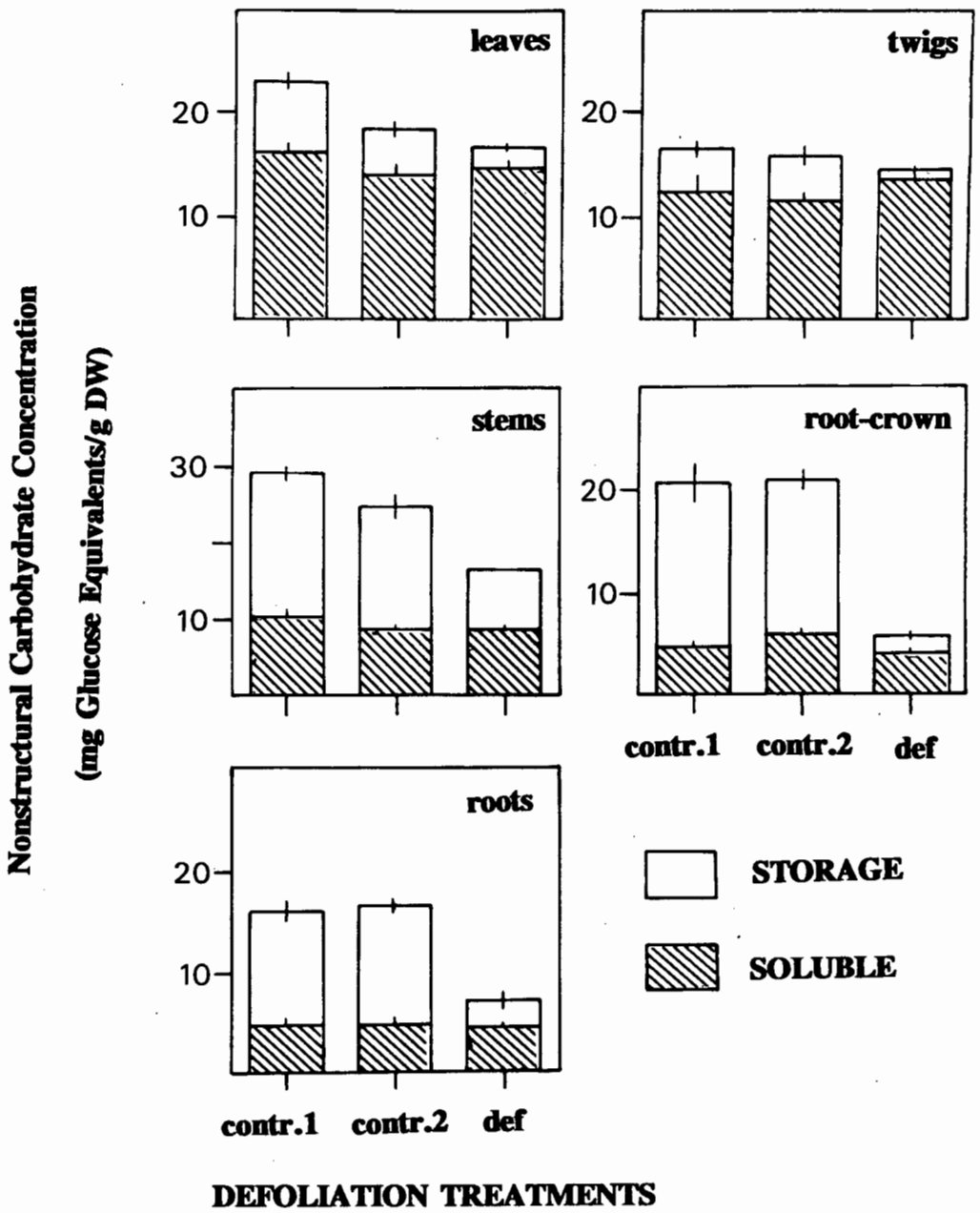

Fig. 6.5: Concentrations of sucrose and hexoses (soluble fraction), and of starch and fructans (storage fraction) in plant parts of undefoliated ***Ruschia spinosa*** plants measured on the day of defoliation (contr.1) and again after 3 weeks (contr.2), and of 40% defoliated plants measured 3 weeks following defoliation (def). Each point represents the mean of 3 observations. Vertical bars represent $\pm$ SEM.

TABLE 6.2: Two-way analysis of variance of total nonstructural carbohydrate concentrations of *Osteospermnum sinuatum*, *Pteronia pallens* and *Ruschia spinosa*. Data for the soluble fraction (SOLUB) and the storage (STOR) fraction were tested seperately. The two factors being simultaneously tested are defoliation treatment and plant part. Values are F-values which are the main effects and the interaction of the two factors. Degrees of freedom for the defoliation treatment factor are 2, 44 and for the plant part are 4, 44. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

		DEFOLIATION	PLANT PART	INTERACTION
O. sinuatum	SOLUB	3.191 ^{NS}	370.4 ***	2.306 *
	STOR	73.45 ***	350.7 ***	24.67 ***
P. pallens	SOLUB	6.36 **	39.42 ***	0.252 ^{NS}
	STOR	205.5 ***	264.5 ***	53.38 ***
R. spinosa	SOLUB	0.710 ^{NS}	77.89 ***	1.07 ^{NS}
	STOR	105.7 ***	62.80 ***	7.097 ***

6.3.4 Distribution of ^{14}C-assimilates

A small proportion of ^{14}C-assimilates (3 - 6%) was allocated to the soluble and storage carbon fractions of undefoliated plants of all three species, while by far the greatest percentage (> 88%) was allocated to the structural carbon fraction (Table 6.3 - 6.5). In terms of spatial partitioning within the plant, most ^{14}C-assimilates were allocated to the leaves (> 60%), followed by the twigs, stems and roots, and lastly the root-crown which incorporated the lowest levels (< 1%) of radioactive carbon 24h after $^{14}CO_2$ labelling. Defoliation did not have a notable effect on ^{14}C partitioning to the soluble, storage or structural carbon fractions of *O. sinuatum* and *R. spinosa* (Table 6.3 & 6.5). In contrast, defoliation resulted in an increase in partitioning to the soluble and storage carbon fractions of the *P. pallens* plants, and an overall decrease in the allocation to the structural carbon fraction (Table 6.4). A general increase in ^{14}C partitioning in response to defoliation was observed in the stems of *O. sinuatum*, in the twigs and stems of *P. pallens*, and in roots of *R. spinosa* (Table 6.4 - 6.5).

The percentage ^{14}C-label in the structural carbon fraction of the leaves decreased significantly ($P < 0.001$) following defoliation of *O. sinuatum* and *P. pallens* (Table 6.6 & 6.7). Significant increases in ^{14}C partitioning, following defoliation of *O. sinuatum*, were only observed in the storage and structural fractions of the stems and no significant differences were observed between carbon fractions of the controls and of the defoliated plants in the twigs, root-crown and roots. The percentage ^{14}C recovered in all carbon fractions (soluble, storage and structural) more than doubled in the twigs and stems of *P. pallens* following defoliation ($P < 0.001$, Table 6.7). Similarly, increases in ^{14}C partitioning in the soluble fraction of leaves of this species and in the soluble and storage fractions of the roots ($P < 0.001$, Table 6.7) were also observed. In *R. spinosa,* significant decreases ($P < 0.001$) were observed in the structural fraction of twigs and in the soluble fraction of stems and root-crown. The only significant increase in percentage ^{14}C partitioning noted for this species was in the structural carbon fraction of the roots.

TABLE 6.3: Allocation of ^{14}C to soluble, storage and structural carbon fractions and the total ^{14}C partitioning (of all fractions) among plant parts in defoliated and undefoliated ***Osteospermum sinuatum*** plants harvested 24 hrs after $^{14}CO_2$ labelling. Data are expressed as the percentage of the total ^{14}C recovered in the plant at the time of harvest. Each value represents the mean of three measurements $\pm$ SEM.

		CONTROL	DEFOLIATED
CARBON FRACTIONS	SOLUBLE	5.27 $\pm$ 2.33	7.33 $\pm$ 1.19
	STORAGE	6.33 $\pm$ 1.65	8.20 $\pm$ 1.15
	STRUCTURAL	88.34 $\pm$ 3.89	84.21 $\pm$ 2.88
PLANT PARTS	LEAVES	64.37 $\pm$ 4.49	53.05 $\pm$ 2.93
	TWIGS	15.66 $\pm$ 4.27	9.46 $\pm$ 1.69
	STEMS	9.42 $\pm$ 1.31	24.59 $\pm$ 1.57
	ROOT-CROWN	0.93 $\pm$ 0.12	0.98 $\pm$ 0.10
	ROOTS	9.58 $\pm$ 1.77	11.66 $\pm$ 2.48

TABLE 6.4: Allocation of ^{14}C to soluble, storage and structural carbon fractions and the total ^{14}C partitioning (of all fractions) among plant parts in defoliated and undefoliated ***Pteronia pallens*** plants harvested 24 hrs after $^{14}CO_2$ labelling. Data are expressed as the percentage of the total ^{14}C recovered in the plant at the time of harvest. Each value represents the mean of three measurements $\pm$ SEM.

		CONTROL	DEFOLIATED
CARBON FRACTIONS	SOLUBLE	3.67 $\pm$ 0.67	11.98 $\pm$ 1.36
	STORAGE	5.92 $\pm$ 0.81	10.37 $\pm$ 1.15
	STRUCTURAL	90.41 $\pm$ 1.39	77.11 $\pm$ 5.77
PLANT PARTS	LEAVES	80.05 $\pm$ 1.90	64.49 $\pm$ 2.51
	TWIGS	3.80 $\pm$ 0.17	13.2 $\pm$ 1.56
	STEMS	3.25 $\pm$ 0.73	9.67 $\pm$ 1.77
	ROOT-CROWN	0.48 $\pm$ 0.03	0.85 $\pm$ 0.22
	ROOTS	11.78 $\pm$ 4.13	11.26 $\pm$ 2.38

TABLE 6.5: Allocation of ^{14}C to soluble, storage and structural carbon fractions and the total ^{14}C partitioning (of all fractions) among plant parts in defoliated and undefoliated ***Ruschia spinosa*** plants harvested 24 hrs after $^{14}CO_2$ labelling. Data are expressed as the percentage of the total ^{14}C recovered in the plant at the time of harvest. Each value represents the mean of three measurements $\pm$ SEM.

		CONTROL	DEFOLIATED
CARBON FRACTIONS	SOLUBLE	4.67 $\pm$ 1.32	3.43 $\pm$ 1.63
	STORAGE	5.68 $\pm$ 0.58	4.97 $\pm$ 0.92
	STRUCTURAL	89.39 $\pm$ 0.81	92.43 $\pm$ 2.34
PLANT PARTS	LEAVES	90.39 $\pm$ 3.00	91.18 $\pm$ 2.03
	TWIGS	5.72 $\pm$ 0.23	2.42 $\pm$ 0.84
	STEMS	1.41 $\pm$ 0.31	2.24 $\pm$ 0.76
	ROOT-CROWN	0.86 $\pm$ 0.23	0.38 $\pm$ 0.18
	ROOTS	1.28 $\pm$ 0.21	4.39 $\pm$ 0.18

TABLE 6.6: Allocation of ^{14}C to soluble, storage and structural carbon fractions in five tissue categories of defoliated and undefoliated ***Osteospermum sinuatum*** plants harvested 24 hrs after $^{14}CO_2$ labelling. Data are expressed as the percentage of the total ^{14}C recovered in the plant at the time of harvest. Each value represents the mean of three measurements $\pm$ SEM. Dissimilar letters designated to the values for the defoliation and control treatments show significant differences at $P < 0.001$ using Tukey's multiple range test.

		CONTROL	DEFOLIATED
LEAVES	SOLUBLE	2.31 $\pm$ 0.49 **a**	3.68 $\pm$ 0.89 **a**
	STORAGE	3.16 $\pm$ 0.69 **a**	3.45 $\pm$ 0.58 **a**
	STRUCTURAL	58.9 $\pm$ 4.44 **a**	45.9 $\pm$ 1.67 **b**
TWIGS	SOLUBLE	0.81 $\pm$ 0.33 **a**	1.04 $\pm$ 0.24 **a**
	STORAGE	1.05 $\pm$ 0.40 **a**	0.84 $\pm$ 0.15 **a**
	STRUCTURAL	13.8 $\pm$ 3.96 **a**	7.57 $\pm$ 1.45 **a**
STEMS	SOLUBLE	0.69 $\pm$ 0.14 **a**	1.04 $\pm$ 0.25 **a**
	STORAGE	0.85 $\pm$ 0.20 **a**	2.05 $\pm$ 0.29 **b**
	STRUCTURAL	7.89 $\pm$ 1.35 **a**	21.5 $\pm$ 4.03 **b**
ROOT-CROWN	SOLUBLE	0.26 $\pm$ 0.06 **a**	0.30 $\pm$ 0.08 **a**
	STORAGE	0.13 $\pm$ 0.05 **a**	0.18 $\pm$ 0.08 **a**
	STRUCTURAL	0.55 $\pm$ 0.10 **a**	0.51 $\pm$ 0.28 **a**
ROOTS	SOLUBLE	1.20 $\pm$ 0.58 **a**	1.27 $\pm$ 0.54 **a**
	STORAGE	1.12 $\pm$ 0.56 **a**	1.68 $\pm$ 0.31 **a**
	STRUCTURAL	7.20 $\pm$ 2.59 **a**	8.72 $\pm$ 1.70 **a**

TABLE 6.7: Allocation of ^{14}C to soluble, storage and structural carbon fractions in five tissue categories of defoliated and undefoliated ***Pteronia pallens*** plants harvested 24 hrs after $^{14}CO_2$ labelling. Data are expressed as the percentage of the total ^{14}C recovered in the plant at the time of harvest. Each value represents the mean of three measurements $\pm$ SEM. Dissimilar letters designated to the values for the defoliation and control treatments show significant differences at $P < 0.001$ using Tukey's multiple range test.

		CONTROL	DEFOLIATED
LEAVES	SOLUBLE	1.14 $\pm$ 0.44 **a**	2.73 $\pm$ 0.38 **b**
	STORAGE	3.29 $\pm$ 0.42 **a**	3.52 $\pm$ 0.85 **a**
	STRUCTURAL	76.3 $\pm$ 2.03 **a**	58.2 $\pm$ 7.21 **b**
TWIGS	SOLUBLE	0.52 $\pm$ 0.09 **a**	3.59 $\pm$ 0.95 **b**
	STORAGE	1.14 $\pm$ 0.29 **a**	2.47 $\pm$ 0.76 **b**
	STRUCTURAL	2.14 $\pm$ 0.08 **a**	7.29 $\pm$ 1.07 **b**
STEMS	SOLUBLE	0.77 $\pm$ 0.15 **a**	2.65 $\pm$ 0.28 **b**
	STORAGE	0.58 $\pm$ 0.16 **a**	2.28 $\pm$ 0.35 **b**
	STRUCTURAL	1.90 $\pm$ 0.53 **a**	4.67 $\pm$ 1.24 **b**
ROOT-CROWN	SOLUBLE	0.25 $\pm$ 0.06 **a**	0.39 $\pm$ 0.13 **a**
	STORAGE	0.07 $\pm$ 0.02 **a**	0.23 $\pm$ 0.07 **a**
	STRUCTURAL	0.16 $\pm$ 0.05 **a**	0.23 $\pm$ 0.03 **a**
ROOTS	SOLUBLE	1.02 $\pm$ 0.05 **a**	2.61 $\pm$ 0.57 **b**
	STORAGE	0.86 $\pm$ 0.16 **a**	1.86 $\pm$ 0.13 **b**
	STRUCTURAL	9.91 $\pm$ 2.48 **a**	6.77 $\pm$ 1.85 **a**

TABLE 6.8: Allocation of ^{14}C to soluble, storage and structural carbon fractions in five tissue categories of defoliated and undefoliated ***Ruschia spinosa*** plants harvested 24 hrs after $^{14}CO_2$ labelling. Data are expressed as the percentage of the total ^{14}C recovered in the plant at the time of harvest. Each value represents the mean of three measurements $\pm$ SEM. Dissimilar letters designated to the values for the defoliation and control treatments show significant differences at $P < 0.001$ using Tukey's multiple range test.

		CONTROL	DEFOLIATED
LEAVES	SOLUBLE	2.95 $\pm$ 0.79 **a**	2.06 $\pm$ 1.02 **a**
	STORAGE	4.67 $\pm$ 0.31 **a**	4.00 $\pm$ 0.69 **a**
	STRUCTURAL	82.8 $\pm$ 2.77 **a**	85.1 $\pm$ 3.19 **a**
TWIGS	SOLUBLE	0.59 $\pm$ 0.05 **a**	0.78 $\pm$ 0.31 **a**
	STORAGE	0.46 $\pm$ 0.15 **a**	0.46 $\pm$ 0.20 **a**
	STRUCTURAL	4.67 $\pm$ 0.40 **a**	1.19 $\pm$ 0.37 **b**
STEMS	SOLUBLE	0.42 $\pm$ 0.06 **a**	0.18 $\pm$ 0.07 **b**
	STORAGE	0.24 $\pm$ 0.06 **a**	0.24 $\pm$ 0.12 **a**
	STRUCTURAL	0.76 $\pm$ 0.11 **a**	1.82 $\pm$ 0.68 **a**
ROOT-CROWN	SOLUBLE	0.38 $\pm$ 0.08 **a**	0.07 $\pm$ 0.02 **b**
	STORAGE	0.19 $\pm$ 0.03 **a**	0.10 $\pm$ 0.04 **a**
	STRUCTURAL	0.27 $\pm$ 0.10 **a**	0.21 $\pm$ 0.09 **a**
ROOTS	SOLUBLE	0.33 $\pm$ 0.04 **a**	0.34 $\pm$ 0.02 **a**
	STORAGE	0.11 $\pm$ 0.02 **a**	0.17 $\pm$ 0.03 **a**
	STRUCTURAL	0.84 $\pm$ 0.08 **a**	3.78 $\pm$ 0.77 **b**

6.4 DISCUSSION

Growth ratios of *P. pallens* and *R. spinosa* plants growing in their natural environment (Chapter 2) were lower than the growth ratios found for the plant individuals used in this laboratory study. This indicates that under irrigation these plants grew more leaf and twig biomass than in the field. The particularly high growth ratio of *P. pallens* was probably a function of plant age, with young *P. pallens* shrubs having relatively less woody stem tissue, which would increase growth ratio values. Plant age and irrigation did not influence the relative leaf and twig biomass of *O. sinuatum* plants since the growth ratios of the young plants grown under irrigation in this study had comparable growth ratio values to the plants studied in their natural environment (Chapter 2). No regrowth was recorded in any of the defoliated plants over the 3-week study period, even though plants were irrigated. This lack of regrowth is consistent with the results of the field study which showed no regrowth of moderately defoliated plants in the first 2 to 4 weeks following defoliation (Chapter 2).

Partial defoliation had no effect on photosynthetic rates of the three karoo species, at least not to the extent reported for some grass species where almost two-fold increases in photosynthetic rates were recorded 2 to 4 days following moderate defoliation (Wallace *et al.*, 1984). Defoliated karoo shrubs, with their lower photosynthetic area, would therefore have a lower total plant carbon gain than that of undefoliated shrubs. The reduction in storage carbohydrate concentrations in defoliated plants, reported in Chapters 4 and 5 as well as in the present study, therefore suggests that carbon was required in amounts greater than could be supplied by the photosynthetic processes of the reduced leaf biomass.

It appears that photosynthetic stimulation following defoliation is restricted to species native to resource-abundant environments (McNaughton, 1983). These species have probably evolved physiological mechanisms to compete favourably in this highly competitive environment and compensatory photosynthesis following herbivory is but one of these physiological mechanisms. In contrast, compensatory photosynthesis has not been reported for karoo shrubs which indicates that these plants have not evolved the same physiological responses (as some grasses) to leaf removal.

Changes in carbohydrate concentrations following defoliation reflect the responses identified for these species in a study undertaken in their natural habitat (Chapter 5) i.e. a lowering of TNC concentrations in all plant parts, apart from the leaves. The decrease in storage carbohydrates, in particular, confirms that starch and fructans (high molecular weight compounds) were mobilized from carbohydrate storage sites following defoliation.
The greatest proportion of ^{14}C-asimilates was recovered in the leaves of karoo shrubs 24 h after labelling, which is in contrast with the patterns observed for many grass species where from 1 - 4% of total assimilated ^{14}C was found in ^{14}C fed leaves after 24h (Borland & Farrar, 1989; Danckwerts & Gordon, 1989). The bulk of ^{14}C allocated to leaves of karoo shrubs was in the structural carbon fraction which included nitrogenous compounds. This is not an unexpected finding since slow growing plants such as the karoo shrubs are thought to allocate more to structural components while fast growing plants such as grasses accumulate more organic N-compounds (Poorter & Bergkotte, 1992).

Defoliation resulted in the redistribution of assimilates in all plant species. In defoliated *O. sinuatum* and *P. pallens* plants the redistribution patterns appear to be related to the commencement of regrowth. Since apical meristems were removed with defoliation, the only regrowth that could take place was from meristems situated on the stems and twigs. The relative increase in partitioning of ^{14}C-assimilates to structural carbon in the stems of *O. sinuatum*, and in the twigs and stems of *P. pallens* indicates that cell differentiation was taking place in the stem secondary meristems, which designate the start of regrowth. Defoliated *O. sinuatum* and *P. pallens* plants also allocated relatively more photosynthate to soluble and storage carbohydrates of the twigs and stems than the control plants. Over an extended period, continued partitioning of these compounds to carbohydrate storage sites could lead to the elevation in pool sizes of labile carbohydrates to levels greater than those of the controls. These translocation patterns are therefore consistent with the responses of these species in terms of temporal changes in carbohydrate concentrations following defoliation, which showed the elevation of TNC concentrations to levels greater than those of the controls during certain times of the year (Chapters 4 & 5).

Defoliation of *R. spinosa* lowered the incorporation of photosynthates into the soluble carbohydrate fraction of stems. The continual decrease in photosynthate allocation to this

site of carbohydrate storage of defoliated *R. spinosa* plants, coupled with the continued utilization of storage carbohydrates from stems following defoliation (Chapter 5) could lead to very low TNC levels in this organ over the long term. This confirms results showing reduced TNC levels in the stems of this species for intervals of 26 weeks or more following defoliation (Chapter 4). Defoliated *R. spinosa* plants also allocated relatively more ^{14}C to the structural carbon of the roots than was the case in undefoliated plants. No clear explanation can be advanced for this translocation pattern since plants usually respond to defoliation by reduced photosynhate incorporation into the roots (Ryle & Powell, 1975; Bassman & Dickmann, 1985).

Karoo shrubs respond to defoliation by modifying normal photosynthate allocation patterns. The shift in assimilate partitioning from roots in order to benefit new leaf production, as was noted for grass species and poplar trees (Ryle & Powell, 1975; Bassman & Dickmann, 1985), was not observed for the karoo shrubs. ^{14}C photosynthate distribution patterns of these shrubs support results (Chapter 4) which showed defoliation to cause an elevation of TNC levels in storage sites of the non-succulent species, and a decrease in TNC levels of the carbohydrate stores of the succulent species.

CHAPTER 7

RESERVE CARBOHYDRATE MOBILIZATION FOLLOWING DEFOLIATION

7.1 INTRODUCTION

The contribution of stored nonstructural carbohydrates to regrowth of plants following defoliation appears to vary among plant species. Certain studies suggest that nonstructural carbohydrates are the principal carbon source for regrowth (White, 1973) while other studies have failed to demonstrate a direct relationship between nonstructural carbohydrate reserves and regrowth following defoliation (May, 1960; Davidson & Milthorpe, 1966b; Caldwell *et al.*, 1981). Richards & Caldwell (1985) suggested that (i) large contributions of photosynthates to regrowth, (ii) meristemetic limitations and (iii) unsuitable analytical procedures are possible explanations for the lack of correlation between regrowth following defoliation and reserve carbohydrate stores. In order to determine a more direct relationship between regrowth and reserve carbohydrates, Richards & Caldwell (1985) have compared the production of new leaf tissue and the reduction in carbohydrate reserves in the absence of photosynthesis (etiolated regrowth) in two *Agropyron* species. The authors assumed that the maximum potential contribution of stored carbohydrates to regrowth under normal conditions (in the light) is represented by regrowth in the dark. It was concluded that an average of 95% of the carbon in regrowth tissue of the two *Agropyron* species was gained from photosynthesis.

This Chapter reports on a study which employed the etiolated regrowth technique (Richards & Caldwell, 1985) to examine the relationship between utilization of stored nonstructural carbohydrates and regrowth following defoliation of *Osteospermum sinuatum*. Regrowth as well as changes in total nonstructural carbohydrate (TNC) pool sizes were compared between

defoliated plants growing in the light, and defoliated plants growing in the dark. Regrowth in the dark, although an artificial situation, was adopted in this study to assess the relative importance of photosynthates versus stored carbohydrates in regrowth following defoliation.

7.2 MATERIALS AND METHODS

7.2.1 Plant Material and Regrowth Experiments

Seeds of *Osteospermum sinuatum* were sown in March 1990 in soil collected from the Tierberg study site. Seedlings were transplanted individually into 9-litre pots one month after germination. Plants were grown in a greenhouse and each plant was irrigated with 500 ml distilled water on a weekly basis. On 10 September 1991, four of the 18-month old seedlings were harvested and divided into the following plant tissue categories: leaves, twigs, stem and below-ground plant parts. For this experiment the root-crown and roots were combined into a single "below-ground" category since the root-crowns of the 18-month old plants were particularly small and could not be easily distinguished from the roots. Plant parts were immersed in liquid nitrogen followed by drying at 70°C for 72 h, and dry weights determined. On the same date, 10 Septèmber 1991, 24 plant individuals were defoliated by removing 80% of the leaf and twig (diameter < 1 mm) biomass, thereby simulating sheep grazing. The position of the remaining leaves on plants were clearly marked so that subsequent regrowth could be accurately monitored. Half the plants were transferred to a dark environmentally controlled growth room and the other half transfered to one with an incident photosynthetic photon flux density of 600 μmol $m^{-2}s^{-1}$. The conditions in the growth rooms were identical in all other respects i.e. a photoperiod setting of 12 h (25°C/50% RH) day and 12 h (18°C/65% RH) night cycles. At 2-week intervals following the initial defoliation, four plants each were removed from the dark and the light environmental growth rooms. Plants were divided into the same plant organ categories outlined above, killed in liquid nitrogen, dried at 70°C for 72 h, and dry weights determined. At each harvest new leaves were separated from the remaining leaves and considered as an separate plant tissue category.

7.2.2 Carbohydrate Analyses

Dried plant material was ground in a Wiley Mill to pass a 40-mesh screen. Subsamples were then used to determine total nonstructural carbohydrate (TNC) concentration (Chapter 3, Smith, 1981) in each plant part. TNC content in the various plant parts were expressed as TNC pools which were calculated by the multiplication of plant part TNC concentration with plant part dry weight. Data were analyzed using a three-factor analysis of variance technique (Zar, 1974) to assess the effects of light regime (dark or light regrowth), time (weeks) since defoliation and the plant part factor on TNC pools. This technique was also employed to establish whether the interaction between factors had a significant effect on TNC concentrations.

7.3 RESULTS

7.3.1 Plant Regrowth

There was a gradual increase in the production of new leaves of plants grown in the light over the monitoring period, with most of the growth occurring between 0 and 4 weeks, and almost no regrowth during the final 4 to 6 weeks (Fig. 7.1). At the end of the 6-week period, leaf biomass of the defoliated plants in the light was almost half that of the undefoliated plants. All the new leaf tissue of the plants grown in the dark was produced within the first two weeks following defoliation (Fig. 7.1). Between 4 and 6 weeks following defoliation, no additional leaves were produced and the new leaves already formed appeared necrotic. At 6 weeks following defoliation almost all the new leaves produced at 0 to 2 weeks had fallen off. Although no abscission of the remaining leaves of the plants grown in the dark were observed, there were visual signs of a decline in leaf vigour.

7.3.2 Nonstructural Carbohydrates

TNC pool sizes of leaves produced between 0 and 2 weeks following defoliation in the light were relatively low with levels of approximately 3 mg/new leaf category recorded (Fig. 7.2). TNC content in these tissues increased up to 15 mg/new leaves at 4 weeks following defoliation and remained unchanged up to 6 weeks following defoliation. In contrast, TNC pool sizes of new leaves produced in the dark were very low (0.7 mg/new leaves) and

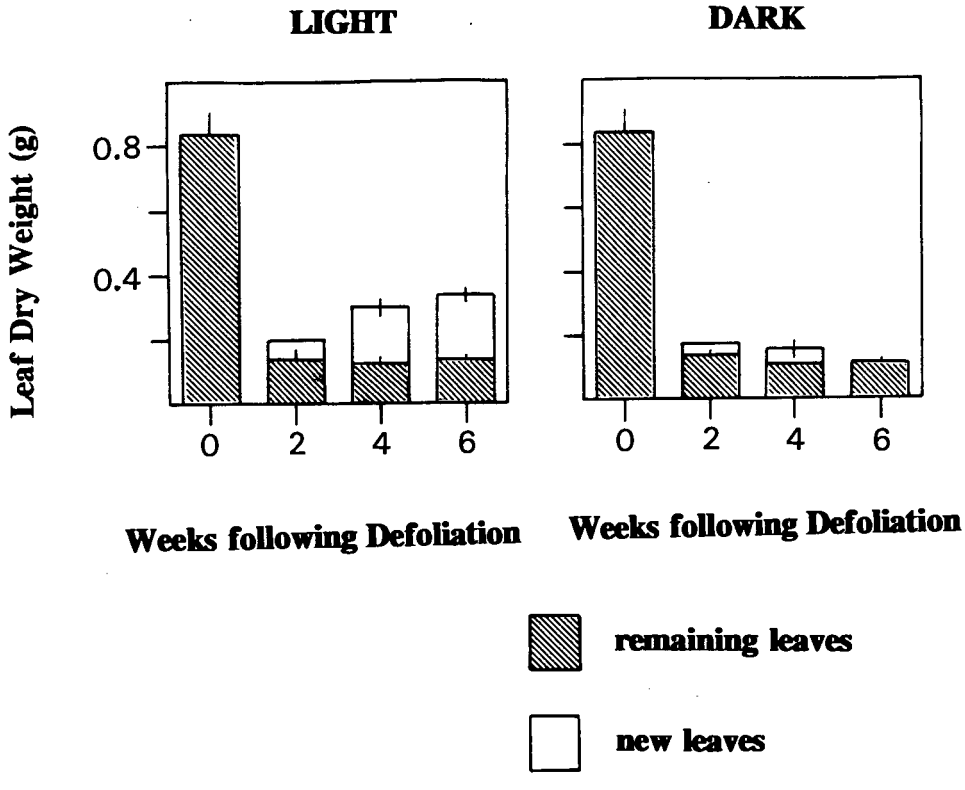

Fig. 7.1: Leaf biomass prior to defoliation (0 weeks), and biomass of remaining leaves and new leaves measured at 2-weekly intervals following 80% defoliation of *Osteospermum sinuatum* plants grown in the light and in the dark.

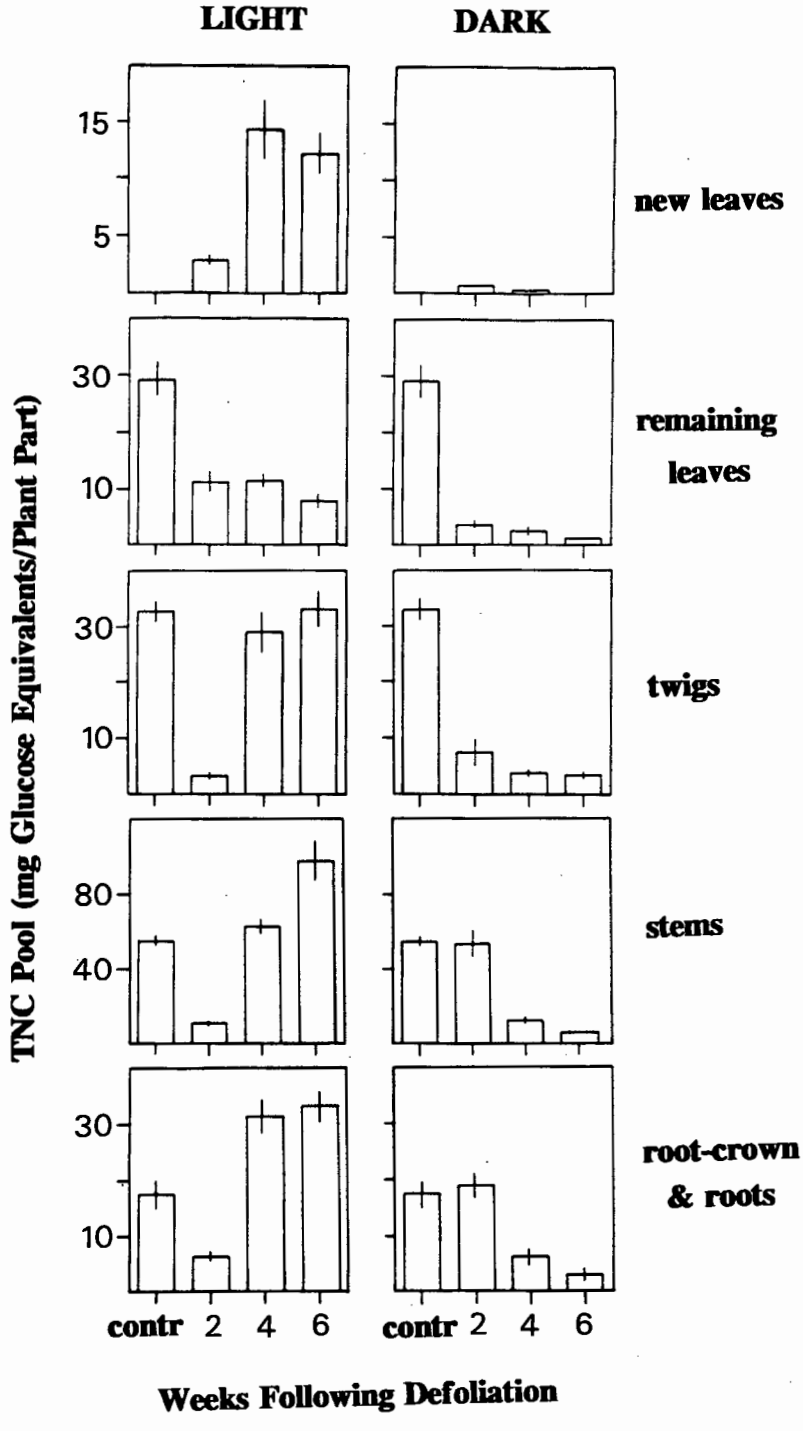

Fig. 7.2: Total nonstructural carbohydrate pools of plant parts of *Osteospermum sinuatum* plants prior to defoliation (contr), and of 80% defoliated plants grown in the light and in the dark, measured at 2-weekly intervals following defoliation.

TABLE 7.1: Three-way analysis of variance of total nonstructural carbohydrate pools of *Osteospermum sinuatum*. The three factors being simultaneously tested were growth environment (light or dark, A), time since defoliation (B) and plant part (C). Values appearing in the table are F-values for the main effect of each factor as well as intercations between factors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	F-VALUES	DF
MAIN EFFECTS		
GROWTH ENVIRONMENT (A)	34.61 ***	1
TIME (B)	9.29 ***	3
PLANT PART (C)	45.01 ***	4
INTERACTIONS		
A x B	24.91 ***	3
A X C	2.86 NS	4
B x C	2.49 **	12

decreased even further at 4 weeks following defoliation (Fig. 7.2). At 6 weeks following defoliation all leaves produced in the dark had fallen off (Fig. 7.1).

TNC pool sizes of the remaining leaves of the light grown plants decreased at 2 weeks following defoliation and remained the same over the rest of the monitoring period (Fig. 7.2). TNC concentrations of the remaining leaves, however, remained unchanged during this period (Table 7.2). In all other plant tissues there were immediate decreases in TNC pool sizes and TNC concentrations at 2 weeks following defoliation, and at 4 weeks following defoliation complete refilling of these nonstructural carbohydrate stores. At 6 weeks following defoliation no further increases in TNC pool sizes were observed apart from in the stems where a further increase of 30 mg/stems were measured from 4 to 6 weeks following defoliation (Fig. 7.2).

In the remaining leaves and in the twigs of defoliated plants grown in the dark, reductions in TNC pool sizes (Fig.7.2) and TNC concentrations (Table 7.2) were recorded at 2 weeks following defoliation, and these levels decreased even further up to 6 weeks following defoliation (Fig. 7.2). Stem and below-ground plant part (root-crown & root) TNC pool sizes (Fig. 7.2) and TNC concentrations (Table 7.2), however, decreased only between 2 and 4 weeks following defoliation and declined to lower levels at 6 weeks following defoliation (Fig. 7.2; Table 7.2).

TNC pool sizes differed significantly ($P < 0.001$) (i) between plants grown in the light and plants grown in the dark, (ii) at the intervals following defoliation (2, 4 and 6 weeks) and (iii) among the various plant parts (Table 7.1). There appears to be an interaction of (i) growth condition (light or dark) and time since defoliation ($P < 0.001$) and between (ii) time since defoliation and plant part in determining TNC pool sizes ($P < 0.05$, Table 7.1). However, the differences between TNC pool sizes of defoliated plants grown in the light and in the dark, were not dependent on plant part (no interaction between factors, Table 7.1).

Plant TNC pool sizes decreased by 70% at 2 weeks following defoliation when plants were grown in the light (Table 7.3). At 4 weeks following defoliation plant TNC pool sizes were of the same order as that of the controls and at 6 weeks following defoliation increased by

Table 7.2: Total nonstructural carbohydrate concentrations (mg Glucose Equivalents/g DW) of plant parts of ***Osteospermum sinuatum*** plants prior to defoliation (control), and of 80% defoliated plants grown in the light and in the dark, measured at 2-weekly intervals following defoliation. Values are mean ± SEM. ***# rc & rts*** = root-crown and roots; ***rem lvs*** = remaining leaves.

		CONTROL (PRIOR TO DEFOLIATION)	
	leaves	34.99 ± 3.30	
	twigs	69.69 ± 2.42	
	stems	52.41 ± 4.05	
	rc & rts	42.53 ± 7.22	
		80% DEFOLIATED	
		LIGHT	**DARK**
2 WEEKS	***new lvs***	30.86 ± 2.38	18.58 ± 2.44
	rem lvs	35.93 ± 3.88	26.83 ± 2.44
	twigs	6.90 ± 1.20	17.35 ± 3.95
	stems	10.32 ± 0.62	46.83 ± 6.52
	rc & rts	12.16 ± 2.16	38.74 ± 4.16
4 WEEKS	***new lvs***	76.63 ± 1.90	7.46 ± 0.55
	rem lvs	38.66 ± 3.76	21.42 ± 1.21
	twigs	73.03 ± 4.31	7.83 ± 2.22
	stems	52.51 ± 3.28	15.03 ± 1.62
	rc & rts	46.80 ± 4.63	13.54 ± 1.07
6 WEEKS	***new lvs***	73.66 ± 2.79	0
	rem lvs	31.68 ± 2.67	12.44 ± 0.51
	twigs	80.97 ± 2.39	7.89 ± 0.17
	stems	85.98 ± 4.41	6.77 ± 0.61
	rc & rts	48.51 ± 3.16	7.52 ± 2.05

Table 7.3: Total nonstructural carbohydrate (TNC) content (mg Glucose Equivalents/plant) prior to defoliation (control), and of 80% defoliated ***Osteospermum sinuatum*** plants grown in the light and in the dark. TNC content of the defoliated plants was measured at two-weekly intervals. Values are mean ± SEM.

CONTROL	134.41 ± 7.128	
DEFOLIATED	**LIGHT**	**DARK**
2 WEEKS	31.507 ± 1.487	83.544 ± 9.155
4 WEEKS	134.643 ± 4.065	25.771 ± 1.305
6 WEEKS	172.309 ± 16.420	14.903 ± 1.861

28%. However, TNC pool sizes decreased steadily over time when defoliated plants were grown in the dark. At 6 weeks following defoliation TNC pool sizes of the defoliated dark grown plants were 11 % of the control plant levels (Table 7.3).

7.4 DISCUSSION

Changes in TNC pool sizes on a plant part basis cannot always be interpreted as the utilization of stored carbohydrates or as the refilling of carbohydrate stores for plant parts (leaves and twigs) which have changed in biomass in response to defoliation i.e. changes in TNC pool sizes should be interpreted both in terms of TNC concentration changes and plant part biomass changes. For example, the reduction in TNC pool sizes in the leaves of defoliated plants grown in the light is not indicative of mobilization of carbohydrate reserves from the leaves, but is a reflection of the decrease in leaf biomass due to defoliation. TNC concentrations of leaves remained unchanged following defoliation (Table 7.2) which indicate that carbohydrates of remaining leaves were not utilized following defoliation of *O. sinuatum* plants growing under optimum conditions. Similarly, the striking elevation in TNC pool sizes of new leaves from 2 to 4 weeks following defoliation of plants grown in the light

(almost 5 times higher) is due to a large increase in TNC concentration (Table 7.2) in these tissues as well as doubling of biomass levels of new leaves over this period (Fig. 7.1).

The greatest decrease in TNC pool size immediately following defoliation (2 weeks) was observed in the twigs of plants grown in the light, and it could be interpreted that this plant part is the most important carbohydrate storage site since most carbohydrates were mobilized from this plant part. However, the huge reduction in twig TNC pool size was a function of a decrease in TNC concentration (Table 7.2), suggesting twig reserve carbohydrate utilization, as well as a decrease in twig biomass as a result of defoliation. The elevation in twig TNC content at 4 and 6 weeks following defoliation should therefore be interpreted as overcompensation in terms of TNC accumulation since the twig biomass of the defoliated plants were much lower than that of the controls (defoliated plants never regrew completely over 6 weeks), and yet the TNC pool sizes were of a similar magnitude. Similar overcompensation responses in terms of nonstructural carbohydrate accumulation was also observed in stems and in below-ground plant parts at 4 and 6 weeks following defoliation of plants grown in the light.

The greatest increment in new leaf production in the light-grown plants occurred between 2 and 4 weeks, at a time when the TNC levels in the carbohydrate storage sites returned to levels comparable (or higher) to that of control plants (Table 7.3). The co-occurrence of maximal leaf growth and rapid refilling of reserves between 2 and 4 weeks suggests that carbon from stored carbohydrates was not exclusively utilized for the production of new leaves, but rather carbon from photosynthates produced by remaining and new leaves. If stored carbohydrates were primarily used for regrowth between 2 and 4 weeks, maximal production of new leaves should have been accompanied by the maximum reduction in TNC pool sizes in the storage sites, which was not the case. Further confirmation for this notion is represented by observations of extremely limited new leaf production in the dark, suggesting that photosynthesis was required for new growth of defoliated *O. sinuatum* plants. However, it cannot be overlooked that initial production of new leaves is dependent in part on carbon from carbohydrate reserves as was shown for plants grown in the light, where leaf production did occur from 0 to 2 weeks and reductions in TNC pools were observed in the storage organs at this time. Similarly, restricted production of etiolated growth did occur

in the dark and was accompanied by reductions in TNC pools of the remaining leaves and twigs, which suggests that initial leaf production is dependent on stored carbohydrates. New leaves produced in the dark had a limited life span which indicates that further development of new leaves is dependent on photosynthesis. These findings suggest that regrowth is dependent on stored carbohydrates for a limited period (0 - 4 weeks) and that this reserve carbon utilized is of a small magnitude relative to the carbon used from photosynthates for the establishment of new growth.

The relative difference between dark regrowth and light regrowth of *O. sinuatum* is in contrast with that of *Agropyron desertorum* and *Agropyron spicatum*, two semi-arid bunchgrasses (Richards & Caldwell, 1985). Dark regrowth ranged from 1% to 11% of light regrowth of defoliated *Agropyron* plants (Richards & Caldwell, 1985). In contrast, dark regrowth of *O. sinuatum* was 62% of light regrowth at 2 weeks following defoliation and 30% of light regrowth at 4 weeks after defoliation. Assuming that the amount of dark regrowth synthesised by a defoliated plant represents the maximum potential contribution of stored carbon to regrowth under normal (light) conditions (Richards & Caldwell, 1985), then 89 - 99% of the carbon in regrowth tissues of the two *Agropyron* species was derived from current photosynthates, while 40% of the regrowth of *O. sinuatum* (after 2 weeks) originated from current photosynthates. This marked difference in the contribution of current photosynthesis to regrowth between *Agropyron* species and *O. sinuatum* confirms the belief expressed earlier (Chapter 5) that shrubs are dependent on stored carbohydrates to a greater extent (than grasses) in order to produce new foliage following defoliation.

Reductions in TNC pool sizes were recorded in all non-photosynthetic plant parts, which indicates that these tissues can all be considered as primary storage sites. However, when plants were grown in the dark, decreases in leaf TNC pools and leaf TNC concentrations were also observed. The reduction in leaf TNC content under these conditions suggests that during severe stress such as drought, when photosynthesis is limiting, nonstructural carbohydrate reserves accumulated in the leaves may be utilized for plant maintenance respiration. Data for TNC utilization when plants were grown in the dark, further suggest that carbohydrates accumulated in the leaves and twigs are utilized before stem and below-ground storage carbohydrates, which append temporal and spatial components to the pattern

of reserve carbohydrate mobilization in defoliated *Osteospermum sinuatum* plants.

Defoliated *O. sinuatum* plants grown in the dark showed TNC allocation patterns widely different to those of defoliated plants grown in the light. This clearly indicated that photosynthesis of the remaining leaf biomass was quantitatively of significance for regrowth of this species although to a much lesser extent than that observed in a grass species (Richards & Caldwell, 1985). This study, however, has considered only a single karoo species grown under optimum conditions. Future research should consider other species from semi-arid ecosystems of different growth form and phenology (evergreens and succulents) growing in the field to quantitatively compare the amounts of carbon supplied to regrowth from storage and from photosynthesis. This would allow the determination of (i) the importance of morphological constraints (growth form) and environmental factors in determining the allocation of the carbon resource (photosynthesis or stored carbon) following defoliation and (ii) the efficiency with which various growth forms allocate carbon from these sources for the production of new photosynthetic tissue.

CHAPTER 8

SYNTHESIS

Grazing by livestock has profound detrimental effects on karoo vegetation structure and composition possibly leading to severe desertification in some areas (Acocks, 1955; Roux, 1980). Initial studies addressing the problem of desertification in the Karoo have taken the form of descriptions of changes in vegetation composition in response to different grazing systems (van der Walt, 1971; Skinner, 1976; Roux & Vorster, 1983; Vorster & Roux, 1983). Such grazing trials have led to current karoo management policies which are based almost entirely on plant community dynamics and which have disregarded species specific and guild responses to foliage removal. The alternative approach of investigating grazing effects on plant growth involves mechanistic physiological studies of whole plant responses to defoliation which have only been made possible due to recent advances in technology such as techniques to quantify gas exchange, water and nutrient use and radiocarbon tracer studies. It is now possible in ecophysiological studies to accurately quantify metabolic responses of plants to their environment and to integrate these responses with observed patterns of plant growth. Plant ecophysiology has increasingly became a more predictive science and has proved a valuable tool in ecosystem management particularly in areas such as pollution assessment and forestry development (Mooney *et al.*, 1987). Ecophysiological studies of the effects of foliage removal have similarly enabled grassland and pasture scientists to understand processes determining growth responses following defoliation and this has ultimately led to an improved insight of how to manage grassland ecosystems and pastures (Caldwell *et al.*, 1981; McNaughton *et al.*, 1983; Coughenour *et al.*, 1985; Caldwell & Richards, 1986; Cox *et al.*, 1988; Ryle *et al.*, 1989; Gold & Caldwell, 1990). Coughenour (1984), for example, has been able to use ecophysiological approaches to develop a simulation model to inter-relate the effects of defoliation with growth, photosynthesis, water use and light interception of east African graminoids.

The range of environmental conditions and the unpredictability of climatic events necessitate management strategies of a predictive nature for the karoo region. The distinct lack of

studies of karoo plant defoliation effects at the whole plant level, however, has been a major constraint in the development of predictive management policies. Empirical grazing trials fulfil a meaningful role in rangeland research in that they should serve as the definitive test of predicted plant responses in the community (Caldwell & Hodgkinson, 1986). Progress in the understanding of optimal plant utilization for livestock production can however only be made by close collaboration between ecophysiologists and ecosystem ecologists. Data from studies reported in this thesis indicate that even among the small sample of karoo shrubs studied markedly different responses in their physiological adaptation to the semi-arid environment are apparent, especially in terms of responses to foliage removal (section 8.2). The relationships observed between defoliation of karoo shrubs, plant carbon economy and plant regrowth are used to illustrate how vital whole plant studies are for the development of predictive mangement policies (section 8.4).

8.1 Carbohydrate Accumulation in Karoo Shrubs

Patterns of spatial variation in nonstructural carbohydrate accumulation were found to be similar for all three karoo shrubs studied (Chapter 3). Twigs and leaves generally contained the highest concentrations of total nonstructural carbohydrates (TNC) while the stems accumulated the largest quantities (carbohydrate pools) by virtue of having the greatest plant part biomass. The high above-ground TNC accumulation in these karoo shrubs is in conflict with reports in the literature which have suggested below-ground organs or plant parts close to the soil surface (stem bases of grasses) to be the principal location of carbohydrate stores in woody plants (Loescher *et al.* 1990) and grasses (White, 1973). Roots of *O. sinuatum*, however, showed exceptionally large increases in nonstructural carbohydrate concentrations during spring (Chapter 5). This feature is probably distinctive of the deciduous growth form which undergoes extensive changes in storage compound levels because total leaf loss and the complete replacement of the leaf biomass place great demands on stored carbohydrates.

Concentrations of nonstructural carbohydrates in undefoliated plants increased or decreased in plant parts with growth stages of the plant. Decreases in concentration were particularly evident with the initiation of growth (Chapter 5). The magnitude of seasonal fluctuations in

TNC levels differed among species and was interpreted in terms of vegetative growth patterns and their dependence on stored carbohydrates. The deciduous species, *Osteospermum sinuatum*, exhibited the greatest fluctuations in TNC concentrations in both aboveground and belowground tissues, but most markedly in the roots. Growth of this species was therefore more dependent on stored carbohydrates following the leafless stage. The seasonal changes in nonstructural carbohydrate levels were less marked in the *R. spinosa* because vegetative growth and its associated carbon costs in this species relied less on stored carbohydrates and was more dependent on current photosynthates.

Forms of carbohydrate accumulated in the different species were similar for phylogenetically related species, in that *Osteospermum sinuatum* and *Pteronia pallens* (Asteraceae) accumulated predominantly fructans while in *Ruschia spinosa* (Mesembryanthemaceae), fructans and starch contributed equally to the TNC component of all plant organs (Chapter 3). None of the species studied accumulated oligofructans (DP: 1 - 7). Fructans accumulated by karoo shrubs have a low degree of polymerization, probably somewhere between 7 and 26 (Chapter 3). Hypotheses concerning the selective advantage of fructan accumulation are generally associated with the osmotic characteristics of fructans. There is good evidence that fructans contribute to cold temperature and drought tolerance (Brocklebank & Hendry, 1989), adaptations which would be well suited to the karoo shrubs which experience both these environmental stresses during different times of the year. The question arises as to which attributes of fructans ensure that the predominantly fructan accumulators respond in an apparently superior fashion following defoliation in terms of regrowth (Chapter 2) and carbohydrate re-allocation (Chapters 5 & 6). It is likely that vacuolar fructan accumulation (Hendry, 1987), as opposed to long-term starch storage in amyloplasts (Lewis, 1984), facilitates efficient mobilization of fructans (or its degradation products) to sites within the plant where carbon is in demand. This difference in subcellular location between fructans and starch could explain the superior regrowth ability (Chapter 2) or the efficient replenishment of depleted carbohydrate reserves (Chapters 4 & 5) of fructan accumulating species, *O. sinuatum* and *P. pallens*, compared to the partial fructan accumulator, *R. spinosa*. It has indeed been proposed that vacuolar fructan accumulation may be an important contributing factor to the role of fructans in low temperature tolerance (Edelman & Jefford, 1968) and in the regulation of osmotic potential (Pontis & del Campillo,

1985).

8.2 Defoliation Effects on Carbon Relations of Karoo Shrubs

A primary aim of this study was to quantify the effects of foliage removal on the patterns of nonstructural carbohydrate use following defoliation. Levels of nonstructural carbohydrates in this study of karoo shrubs were expressed mostly in terms of concentrations. It has been suggested that a more exact appreciation of the involvement of reserve carbon in regrowth can be obtained by using carbohydrate pools as opposed to carbohydrate concentrations (Caldwell et al., 1981; Richards, 1986). However, storage compound pool sizes cannot be a reliable tool to use when working with plant individuals of uncertain age, such as in studies reported in this thesis, because plant age differences and the associated differences in terms of tissue biomass and physiological history would entail variations with respect to carbohydrate pools. When experiments were undertaken on plants individuals which had been grown from seed (Chapter 7), the size differences among plant individuals were minimal, and analysis of total qauntities (pools) and concentrations of carbydrates provided a more comprehensive insight of carbon movement within the plant in response to defoliation.

Although all plant parts analyzed accumulated significant quantities of nonstructural carbohydrates, the sites of carbohydrate storage were not equally important to the plant following defoliation. There is sufficient evidence to illustrate that TNC was used preferentially from the twigs and that stem and belowground stored carbohydrate reserves were utilized at later stages following defoliation (Chapters 5 & 7). There are also indications that leaf nonstructural carbohydrates can also be important sources of carbon when plants are under severe stress and photosynthesis is lacking for extended periods (Chapter 7).

The effects of different degrees of consistent and repeated removal of photosynthetic tissue (frequency of defoliation) on nonstructural carbohydrate concentrations of karoo shrubs varied greatly among species and with defoliation frequency (Chapter 4). Defoliation

resulted in the elevation, or had no effects on TNC concentrations of *O. sinuatum* (deciduous non-succulent species) tissues, even at very frequent multiple defoliations (6-week intervals) applied over a one-year period. Defoliation at heavy and at lenient frequencies resulted in a decrease in TNC concentrations in the principal storage sites (stems and roots) of *R. spinosa*, the evergreen succulent species. A one-year application of frequent defoliations (6-week interval) caused decreases, and the lenient (26-week interval) defoliation treatment resulted in the elevation of TNC concenrations in tissues of *P. pallens*, the evergreen non-succulent shrub. The three karoo shrubs were unable to compensate for defoliation by complete vegetative regrowth over a one year period. However, in two of the species overcompensation with respect to TNC storage was noted. This inability to completely replace leaf tissue was therefore due to factors other than the carbon resource, probably nutrient or meristematic limitations.

Measurement of changes in TNC concentrations at 2-weekly intervals following defoliation indicated that the length of time during which the defoliated plant was dependent on stored carbohydrates varied among species and with season (Chapter 5). A close association was also found between regrowth and the degree of utilization of carbohydrate reserves. During spring, when all species produced some degree of regrowth following defoliation, the continuous decline in TNC concentrations was observed over the 6-week monitoring periods. This indicates a dependency on stored carbohydrates for at least 6 weeks following defoliation. In *P. pallens*, for example, the complete depletion of stored carbohydrates over 6 weeks was recorded in some plant parts during spring and autumn, the only seasons when plant individuals regrew within 6 weeks following defoliation. Regrowth therefore places severe demands on carbohydrate stores. However, when no regrowth was recorded the period of utilization of carbohydrates was shortened considerably (< 2 weeks) and the refilling of reserves was noted within 6 weeks. In some cases the replenishment of carbohydrate stores was so extensive (*P. pallens*) that TNC concentrations greater than that of undefoliated plants were recorded within 2 weeks following defoliation (Chapter 5). The co-occurence of depletion of stored carbohydrates and regrowth indicates that grazed karoo shrubs are dependent (for regrowth) on stored carbon for considerable periods (2 - 6 weeks) following defoliation.

In the evergreen species, *R. spinosa* and *P. pallens*, the heavy defoliation intensity placed greater demands on carbohydrate stores than the lenient defoliation intensity (Chapter 5). However, in the deciduous species, *O. sinuatum*, moderate and heavy defoliation intensities resulted in a similar magnitude of TNC utilization which suggests that deciduous species are adapted physiologically to seasonal leaf loss and that a moderate defoliation is not appreciably different from a heavy defoliation in terms of carbon demands.

Defoliation had no significant effects on net photosynthetic rates of the remaining leaves up to 11 days following defoliation (Chapter 6). However, partitioning of photosynthetic products was significantly altered by defoliation (Chapter 6). Patterns of photosynthate partitioning in response to defoliation supported the observed changes in TNC allocation patterns. A relatively greater allocation of photosynthetic products to storage was observed in defoliated *O. sinuatum* and *P. pallens* plants which is consistent with the TNC allocation reponses of these species where elevations of levels of stored carbohydrates were often measured. In contrast, there was a reduction in allocation of photosynthates to storage in *R. spinosa* which confirms observations of reductions in TNC levels of this species up to 26 weeks following defoliation.

The production of new leaves following defoliation of *O. sinuatum* was intially dependent on stored carbohydrates. However, subsequent leaf growth and the refilling of partially depleted carbohydrate reserves were reliant on photosynthates produced by new and remaining leaves (Chapter 7). That regrowth and overcompensation in TNC storage occurred simultaneously under well watered conditions (Chapters 7) and not in the field (Chapters 4 & 5) suggests that water avialabilty is indeed a major limiting factor in the karoo ecosystem.

Overcompensation in TNC storage was only observed in *O. sinuatum* and *P. pallens* and this occurred prior to complete vegetative regrowth (Chapters 4, 5 & 7). A factor or factors other than the carbon resource probably was limiting regrowth. Low nutrient availability of semi-arid soils (Hadley & Szarek, 1981) or the shortage of meristems following defoliation (Richards & Caldwell, 1985) present potential limiting factors of plant regrowth following defoliation of karoo shrubs. Since photosynthesis was proceeding in the absence of regrowth, carbon based compounds were synthesized from photosynthates produced in excess of

immediate demand. The two non-succulent species allocated excess carbon to storage as nonstructural carbohydrates. *R. spinosa*, however, allocated the excess photosynhates to the production of secondary carbon defense compounds (Appendix 1). These physiological responses of karoo shrubs do not support apparency theories such as the optimal defense theory (Rhoades, 1979) which propose plant-herbivore coevolution and that plants have evolved chemical defenses in response to grazing pressure from herbivores. Firstly, levels of polyphenolics and tannins were found to be similar for all plant organs within a species (Appendix 1). Karoo shrubs therefore do not appear to maximize individual fitness to the organs of greater value to the plant. Secondly, changes in concentrations of these secondary defence compounds in response to defoliation were only found in *R. spinosa*. The absence of significant defoliation induced changes in polyphenol allocation and the lack of spatial variation in polyphenol levels are in contrast to active defense mechanisms which are implicit in apparency theories. It is proposed that replenishment of nonstructural carbohydrate stores and production of secondary defense compounds represent a dichotomy in the evolution of physiological responses of karoo shrubs to defoliation.

8.3 Shrub versus Grass Defoliation Responses

Leaf removal causes a wide array of plant responses. Despite the fact that the three karoo shrubs studied have distinctly dissimilar responses with respect to regrowth capacity, demands on stored nonstructural carbohydrates and allocation of photoassimilates, there are broad trends which characterize the responses of these shrubs and which are appreciably different from the responses of grasses.

Total aboveground grass production is commonly considered to be maximized at moderate grazing intensities (McNaughton, 1979). Evidence for this point of view, however, has been questioned (Belsky, 1986). Fast regrowth rates or compensatory regrowth responses following defoliation of grasses (McNaughton, 1979; McNaughton, 1983) was not observed for karoo shrubs (Chapter 2). Defoliated karoo shrubs did not reach undefoliated plant leaf biomass levels up to 26 weeks following severe clipping. It was proposed that low nutrient availability or the lack of secondary meristems are potential limiting factors of karoo shrub

regrowth following defoliation. In contrast to the compensatory regrowth responses of grasses, karoo shrubs allocate carbon either to secondary defense compounds (*R. spinosa*) or overcompensate in terms of replenishment of carbohydrate stores.

Severe defoliation of actively growing grasses resulted in the immediate reduction in nonstructural carbohydrates in the remaining stubble and in the roots for periods between 4 and 6 days (Davidson & Milthorpe, 1966b). However, in karoo shrubs, the period of decline in plant part TNC levels ranges from 2 weeks under well water conditions (Chapter 7) to periods exceeding 26 weeks (Chapter 4) when investigations were carried out in their natural environment. This indicates that karoo shrubs were dependent on stored carbohydrates for periods much longer than those quoted for grass species (Davidson & Milthorpe, 1966b; Gonzales *et al.*, 1989). This greater dependency of shrubs on stored carbohydrates is not unexpected in such a hostile environment as the Karoo.

Partial defoliation resulted in an almost two-fold increase in photosynthetic rates 2 to 4 days following defoliation of grasses (Wallace *et al.*, 1984) whereas no effect on photosynthetic rates of the three karoo shrubs were recorded (Chapter 6). In other grass species minimal elevations (Painter & Detling, 1981; Senock *et al.*, 1991) or decreases (Ryle & Powell, 1975) in photosynthetic rates were found. Photosynthate allocation of both grasses and karoo shrubs were altered in response to defoliation. Many grasses respond to defoliation by increasing the partitioning of photoassimilates to young leaves or regrowing tillers (Gifford & Marshall, 1973; Ryle & Powell, 1975). Within 24 hours the greatest proportion of ^{14}C-assimilates ($> 50\%$) was found in the fed leaves of karoo shrubs (Chapter 6) while only 1 to 4% was found in the fed leaves of grass species (Borland & Farrar, 1989; Danckwerts & Gordon, 1989). Grasses usually respond to defoliation by reduced allocation of photosynthates to the roots in order to benefit new leaf production (Ryle & Powell, 1975). This response was not observed in karoo shrubs and in one of the species (*R. spinosa*) an increase in photosynthate allocation to the roots was recorded (Chapter 6). A particularly marked difference in the relative contributions of current photosynthates and reserve carbon to regrowth was also noted between grass species (Richards & Caldwell, 1985) and the deciduous karoo shrub, *O. sinuatum* (Chapter 7). The contribution of carbon from storage exceeded photosynthetic carbon for only 2,5 days following defoliation when regrowth rates

of two *Agropyron* grass species were very high (Richards & Caldwell, 1985). However, when apical meristems were removed the contribution of photosynthetic carbon to regrowth was immediatly higher than that of storage carbon. In the karoo shrub, *O. sinuatum*, most of the regrowth was derived from stored carbon (60%) up to 2 weeks following defoliation. Regrowth of the defoliated karoo shub was therefore relatively more dependent (than grasses) on reserve carbon with respect to the duration of dependence, and quantities utilized to produce new leaves (Chapter 7).

8.4 Implications for a Karoo Management Policy

The karoo biome cannot be considered as a single uniform system in terms of its biology and physical features. Soils of the karoo region differ in morphological, physical and chemical properties and range from sand-clay loam soils to deep uniform coarse textured (sand) soils (Ellis & Lambrechts, 1986). Similarly, predictability of rainfall decreases from south to north and average annual rainfall decreases westwards from approximately 500mm in the east to less than 100mm in the north-western regions (Venter *et al.*, 1986). There is a high incidence of days with maximum temperatures exceeding 30°C in the northern regions and a high frequency of days with minimum temperatures below 0°C over the central Karoo (Venter *et al., 1986).* High temperatures result in high evaporative water losses, and low temperature limits plant growth and efficient utilization of available water. Similarly, plants growing in sandy soils would be subject to relatively lower soil water availability than plants in clay soils since water moves faster through a sandy soil profile. Karoo plants of the same species growing in different regions will therefore be subjected to different degrees of water stress because of regional variation in the physical environment (climate & soils), and will therefore have different regrowth patterns following defoliation. The close relationship between water avialibilty and plant regrowth capacity following defoliation has been illustrated for karoo shrubs (Chapter 2) and for rangeland grasses (Coughenour *et al.*, 1985b; Pande & Singh, 1985). The link between water availability and plant growth is probably mediated via the well-established relationship between water abundance and carbon gain (Mooney, 1972; Osmond, 1987). Furthermore, regrowth of defoliated karoo shrubs has been shown to be greatly dependent on stored carbon (Chapters 5 & 7). No single grazing system

for livestock production can therefore be advanced for the karoo region and management strategies will have to consider the regional changes in soil, temperature and rainfall features, factors which determine plant water availability and consequently regrowth potential.

Broad trends of plant growth form distribution is evident in the karoo (Hoffman & Cowling, 1987). Leaf succulent species dominate in the Succulent Karoo, tall deciduous shrubs are common in the northern subtropical fringes of the Karoo and sclerophyllous evergreen shrubs dominate in the southern relatively mesic regions (Hoffman & Cowling, 1986). Growth form distribution is probably determined by soil water availabilty which in turn is a function of soil properties, temperatures and seasonality and amount of rainfall. The increase in grass cover from west to east, for example, correlates with an overall increase in rainfall volume and the greater probability of summer rain (Acocks, 1953; Rutherford & Westfall, 1986) in the east. Since it has been shown that karoo shrub growth forms have distinct regrowth responses as well as physiological attributes which determine the different regrowth capacities, management policies for karoo plant communities will differ. For example, rests between grazing in the Succulent Karoo should be much longer than for those communities dominated by dwarf evergreen and deciduous shrubs which regrow faster than succulents following foliage removal.

Grazing management practices of the karoo region presently rely on field observations of the grazing effects on plant communities and are based to a very limited extent on knowledge of physiological responses to foliage removal. A minimum rest period of 120 days under a moderate grazing pressure (50%) is traditionally recommended for karoo rangelands (Hobson, 1989). The present study shows that periods of much longer than 26 weeks (182 days) are required even for the faster growing karoo shrubs (*O. sinuatum*) to re-establish foliage following intense grazing and that even longer periods will be required for slow growing succulent plants such as *R. spinosa* (Chapter 2). During years of severe moisture stress, which would limit photosynthetic carbon gain and nutrient release processes, resting periods should be prolonged since the timing and the amount of rainfall has been shown to strongly determine regrowth capacity following defoliation (Chapter 2).

The major apparent advantage of grazing systems which involve high stocking rates coupled

with short periods of grazing (non-selective grazing, NSG; short duration grazing, SDG) is that livestock are forced to consume both preferred and unpalatable species. This should theoretically eliminate the competitive advantage of the unpreferred species which is a likely phenomenon under selective grazing systems (Hoffman, 1988). However, karoo plants do not have the same ability to regrow following defoliation (Chapter 2), and plant species with high regrowth capacities such as grasses (section 8.3) would be favoured under NSG or SDG systems. Such grazings systems have indeed been developed and applied in grass dominated plant communities, such as the eastern Karoo (Acocks, 1966), and the sole measure of the efficiency of the grazing system has been the rate of grass establishment following grazing (Acocks, 1966). NSG or SDG management practices advocated appear to be successful grazing systems, but only where grasses are the dominant growth form in the community and re-establish quickly following defoliation. Because of the poor regrowth capacity of karoo shrubs (compared to grasses) these grazing practices would be harmful to shrub regrowth because the relatively short rest periods associated with these systems (eg. 40 - 50 days, SDG; Hoffman, 1988) would force livestock to feed on shrubs which have not fully recovered from prior grazing, leading to irreversible damage. In parts of the western Karoo dominated by shrubs and succulents, NSG or SDG would deleteriously affect the entire plant community leading to plant death and the onset of desertification processes.

The apparent benefits of high stocking rates have also been associated with the improvement of soil hydrological properties (trampling), enhanced nutrient cycling (urine and dung deposits) and the creation of germination sites (trampling) (Hoffman, 1988). However, there is no evidence for the stimulation of these processes and it has been shown, to the contrary, that trampling has a negative effect on soil hydrological processes and germination (Hoffman, 1988). The results of this thesis provide additional evidence why grazing systems which entail high stocking rates should be avoided in karoo rangelands. Trampling which is associated with high stocking rates could result in the destruction of secondary meristems and of crucial carbohydrate reserves in the twigs and stems (Chapter 3). Moreover, involving extensive foliage removal (eg. SDG) should be strongly discouraged since regrowth of karoo shrubs and the replenishment of reserves rely to a great extent on carbon gained from photosynthesis by the remaining leaf biomass. When photosynthetic plant carbon gain of the defoliated plant is curtailed by extensive foliage removal, and conditions such as drought

further reduces photosynthetic rates, extreme demands would be placed on stored carbohydrates which could lead to plant death if the stress situation (eg. drought) persists for extended periods.

8.5 Future Research Priorities

Karoo plants are subjected to a number of environmental stress factors, such as drought, extremes of temperatures and nutrient poor soils, all of which present suboptimal conditions for plant growth. Since the vegetation of the karoo ecosystem is used extensively for livestock production native plants also have to contend with grazing pressures. Most of the research on the physiological responses of plants to defoliation has focussed on the responses to leaf removal only (controlled environment conditions), or the responses to a suite of environmental stresses simultaneously experienced by the plant under field conditions. The latter studies are therefore only useful with respect to a specific set of field conditions under which the experiments were undertaken and the effects of single environmental factors cannot be rigidly assessed. These studies therefore have limited predictive value. Further studies should consider the physiological and growth responses (controlled environment conditions) of plants subjected to the combination of foliage removal and single stress factors such as water and nutrient availability. Such an approach could lead to the development of a predictive understanding of plant function under the range of environmental conditions characteristic of the ecosystem. Although recent trends in studies of plant physiological ecology are characterized by investigations of intergrated stress responses and not of single environmental factors (Chapin, 1991), the lack of fundamental physiological data precludes an understanding of karoo plant function which is vital for predictive karoo management models. Coughenour (1991), for example, recently developed a model of dwarf shrub and gramonoid defoliation reponses which included the effects of nitrogen and water and their interaction with foliage removal. The author concluded that primary defoliation responses such as nitrogen balance and secondary effects (eg. nitrogen balance in relation to photosynthesis) need to be considered in simulation models intended for application over extensive areas such as the karoo.

Future research should start to intergrate cellular aspects of plant physiology to explain plant growth, adaptation and distribution in such a way which is pragmatic for the management of the karoo ecosystem. For example, studies of the ecological significance of fructans may offer an insight of the association between a high regrowth capacity and fructan accumulation (Chapter 3). Fructans appear to be associated with cellular osmoregulation and adaptation to low temperatures and may well be related to drought tolerance (Hendry, 1987) which in turn would affect rates of regrowth folowing foliage removal. Studies investigating these potential roles of fructans in the karoo flora should be intergrated with whole plant growth responses by simultaneously determining distribution of fructan accumulating species, plant performance and competitive ability under different grazing systems. Investigations such as these may lead to the development of appropriate predictive tools (eg. fructan versus starch accumulating species) of plant responses to herbivory.

A factor not addressed in this study, that could also account for limited foliage regrowth following defoliation, is the absence of active meristemetic tissues in the defoliated plant. It has been shown, for example, that meristemetic limitations is an important factor determining regrowth capacities of two *Agropyron* species (Richards & Caldwell, 1985). All apical meristems were removed during clipping of heavily and moderately defoliated karoo shrubs to simulate grazing and to allow comparisons of plant reponses without imposing different meristemetic limitations. However, it was not known where the remaining secondary meristems were located, and if they were active in the defoliated karoo shrub. The possible absence or shortage of secondary twig and stem meristems (or basal meristems) could be an important limitation of complete vegetative regrowth (over 26 week periods) following defoliation. Resolving the question of location and number of active meristems following defoliation is vital for a more complete understanding of regrowth responses of karoo shrubs.

It has been shown that the timing of root growth following defoliation can be of crucial importance in understanding differences in foliage regrowth of two *Agropyron* species (Richards, 1986). The more grazing-tolerant species, *A. desertorum*, curtailed root growth which allowed for greater allocation of carbon to vegetative regrowth. In contrast, *A. spicatum*, continued root growth following defoliation which reduced the capacity for

aboveground regrowth. Unfortunately, root growth in response to defoliation could not be adequately assessed for karoo shrubs. However, *R. spinosa* partitioned a significant percentage of its current photosynthates to structural carbon in the roots at 3 weeks following defoliation (Chapter 6). This indicates that defoliation favoured root growth of this species and this allocation response could explain the limited foliage regrowth of this succulent species following defoliation. It is therefore apparent that knowledge of the effects of defoliation on root growth is an important consideration that should be addressed in order to entirely appreciate species specific foliage regrowth responses.

Very little progress has been made beyond the descriptive approach after 40 years of agricultural research in the Karoo. The ecophysiological studies reported in this thesis opened new avenues to understanding processes which determine growth responses following defoliation. With more data predictive plant defoliation response models could be developed to determine suitable management practices for the karoo region.

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APPENDIX 1

Regrowth and Tannin Production in Woody and Succulent Karoo Shrubs in Response to Experimental Defoliation.

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This paper has been submitted for publication. I have contributed 33% to the work involved with this study and I include it as an Appendix to the thesis.

signed.....F van der Heyden

SUMMARY

(1) Allocation of carbon to chemical defences has often been suggested to be a direct response to defoliation by herbivores. This study quantitatively compares total polyphenol and tannin production in response to experimental defoliation of three karoo shrubs in order to test this induced defence hypothesis.

(2) The three species studied responded to defoliation by either rapid regrowth or by increasing polyphenol production in the remaining tissues. These patterns do not bear any phylogenetic relationships but are weakly associated with the observed palatability status of each species.

(3) The highly palatable deciduous species, *Osteospermum sinuatum,* which is capable of rapid regrowth, showed no or very low levels of constitutive and defoliation induced total polyphenolics, condensed tannins and protein precipitating tannins.

(4) The evergreen sclerophyllous species, *Pteronia pallens*, showed a limited regrowth capacity and had intermediate levels of polyphenols while the evergreen succulent species, *Ruschia spinosa*, showed no regrowth over the study period. *R. spinosa* contained the highest constitutive and defoliation induced levels of polyphenolics, condensed tannins and protein precipitating tannins.

(5) Two of the species have the uncommon co-occurrence of more than one anti-herbivore defence feature. *Pteronia pallens* foliage contains both hepatotoxins and polyphenolic compounds while *Ruschia spinosa* has both structural (spines) and chemical defences.

(6) Responses of karoo shrubs to defoliation are interpreted as being the result of passive alterations in plant chemistry induced by environmental constraints rather than as any active defence response to herbivores.

INTRODUCTION

The ability of a plant to withstand grazing or browzing is a heritable trait acted upon by natural selection across both ecological and evolutionary timescales (Bryant et al. in press). Mechanisms underlying plant resistance to defoliation may include a rapid regrowth capacity (McNaughton 1983, Ruess et al 1983, Coughenour et al.1985) or the production of chemical defences which prevents further tissue loss to herbivores (Gulmon and Mooney 1986). Compensatory growth (enhanced growth in response to defoliation) is only possible under conditions where resources (light, water and nutrients) are abundant because in environments with limited resource availability plants are unable to acquire the materials necessary for replacement growth (Freeland & Janzen 1974, Bryant et al. 1983). Slow growing plants from resource constrained environments have been suggested to be well defended (Coley et al. 1985) with defence compounds being allocated to organs and tissues in direct proportion to the risk of loss and the value of the organ or tissue in maximizing individual fitness. Of the range of defence compounds recorded, carbon-rich polyphenolics (tannins) have most often been implicated as anti-herbivore defences despite being non-toxic at levels commonly found in plant material (Feeny 1970, Swain 1977, Cooper & Owen-Smith 1985, Glyphis & Puttick 1988, Bernays et al. 1989). Studies of carbon allocation patterns of plants have attempted to elucidate how different species balance the requirements of growth, reproduction and defence so as to maximise individual fitness (Optimal Defence Theory, Rhoades 1979, Bazazz et al. 1987, Gulmon and Mooney 1986). Defoliation studies of shrub species in the extensively utilized rangelands of the semi-arid karoo region of southern Africa have shown that even in this water constrained environment some plants (eg *Osteospermum sinuatum*) respond to defoliation by rapid regrowth (van der Heyden & Stock submitted). Other species have limited regrowth *(Pteronia pallens*) while another group (*Ruschia spinosa*), do not show any compensatory growth following defoliation. These considerable differences in growth responses and resource allocation patterns of shrubs from the karoo environment provide an opportunity to test hypotheses underlying suggested trade-offs between possible chemical defence production and regrowth in plants characteristic of a resource constrained environment (water). Changes in plant quality such as the amount and location of chemical defences have been suggested to be induced in direct response to defoliation by herbivores

(Ericsson et al. 1985, Williams and Meyers 1984, Clausen et al. 1989). Evidence for such induced responses is scarce (Karban and Myers 1989) and it has been shown that changes in plant chemistry need not have evolved in response to selection by herbivores but may be the consequence of metabolic disruptions in the nutritional balance of the plant (plant carbon/nutrient hypothesis Bryant et al. 1983, Coley 1983).

The implications of a potential trade-off between regrowth potential and patterns of polyphenolic production of different karoo shrub forms are investigated in this paper. In particular attention was focussed upon 1) the relationship between the constitutive levels of total polyphenolic compounds, condensed and hydrolysable tannins and the observed palatability of the species, 2) whether induction of polyphenolic synthesis after defoliation was associated with a reduced regrowth ability, 3) whether chemical and regrowth responses were similar and potentially generalizable across families and growth forms, and 4) whether allocation of phenolics to different organs of potentially high or low loss to herbivores differed.

METHODS & MATERIALS:

Study site and species description

This study was carried out at the Tierberg Karoo Research Centre (33° 10'S, 22° 17' E) on the southern edge of the Great Karoo. Average annual precipitation is 167 $\pm$ 7mm (n = 92 years) with rain events most frequent between February and May (approx 75mm). Droughts are common from late spring through summer (September to January). High temperatures occur during summer with maximum temperatures ranging between 29 - 36°C while low temperatures are common in winter (minimum temperatures of 3° - 5°C recorded) (Milton et al. in press).

Two Asteraceous shrub species, *Osteospermum sinuatum* [(DC.) T.Nor.] and *Pteronia pallens* (L.F) , and one succulent member of the Mesembryanthemaceae, *Ruschia spinosa [*(L.) H.E.K. Hartm. & Stuber] were selected for the study because of their differing degrees of palatability to domestic livestock (sheep). *Osteospermum sinuatum* is a highly palatable

dwarf deciduous shrub with orthophyllous, fleshy leaves while *Pteronia pallens* is a toxic evergreen shrub with sclerophyllous leaves (Prozesky et al. 1986). *R. spinosa* is a dwarf evergreen shrub with spinescent branches and succulent leaves which although often palatable is a species not preferred by sheep.

Field studies were undertaken during 1990 in the major growth period for the three species which is from autumn to early winter. Five similarly sized individuals of each species were subjected to one of each of the following three defoliation treatments: 1) Control - no leaf or twig material removed, 2) 40% defoliation of leaf and twig material, and 3) 80% defoliation of leaf and twig material. Defoliation was undertaken by clipping leaf and twig material to simulate browsing by livestock. Remaining leaves and twigs were spotted with paint immediately following cutting so that leaf and twig replacement could be quantified at the time of harvest.

Above ground plant parts were harvested 8 weeks after defoliation by severing the stems at ground level between 7.30 and 10.00 am while the air temperature was less than 8°C. Each plant was placed into a brown paper bag and packed into an insulated freezer box with dry ice (-78°C) prior to transportation to the laboratory. Within 48 hrs each plant was divided into 1) new leaf, 2) old leaf and 3) old stem categories and then slowly dried for 96 hrs at 55°C (recommended by Hagerman 1988), and dry weights determined. Each plant part was then ground through a 40 mesh sieve in a Wiley Mill prior to chemical analyses.

Growth ratios

A growth ratio was calculated from the following formula in order to compare the relative differences between species in terms of regrowth after defoliation (Van der Heyden and Stock submitted). Growth ratio = dry mass of new leaves and twigs/dry mass of old leaves, twigs and stem x 100.

Total polyphenols

Total polyphenolic concentrations of each plant category were quantified by the Prussian Blue assay (Price & Butler 1977) which has been reported to be less susceptible to protein interference than other total polyphenolic assays (eg Folin assay) (Hagerman & Butler 1989).

Extraction of phenolics from each plant category was undertaken by shaking 60 mg of ground plant material in 3 ml of methanol. This suspension was then vacuum filtered through Whatman No 1 filter paper and the extraction vessel and residue were rinsed with a further 3 ml of methanol. Fifty ml of distilled water were added to the 6 ml filtrate and polyphenol concentrations were determined within an hour after extraction. The Prussian Blue assay as detailed by Price and Butler (1977) was followed and samples were compared to a catechin (Sigma Chemical Co) standard. Sample readings are given as catechin equivalents (mg CEQ g^{-1} dry wt).

Condensed Tannins

Condensed tannins were determined by the Vanillin assay (Price et al. 1978) which has been suggested to be specific for flavanol units even in the presence of hydrolysable tannins or other polyphenolics (Hagerman & Butler 1989). Condensed tannins were extracted by shaking 200 mg of plant material with 10 ml of methanol for 20 mins. The suspension was vacuum filtered through Whatman No 1 filter paper and the filtrate analysed by the method of Price et al. (1978). Sample readings are expressed as catechin equivalents (mg CEQ g^{-1} dry wt).

Protein precipitating tannins

Protein precipitating tannins (index of possible biological activity) were quantified by the radial diffusion assay of Hagerman (1987). Plant tissue (400 mg) was shaken in 2 ml of 70% acetone for 1 hr. After standing for 15 mins a 20 ul aliquot was added to 4mm diameter wells punched into the agarose protein plates (Hagerman 1987). The plates were sealed with polythene film and incubated for 96 hrs. at 25°C. Diameters of protein precipitation rings around each well were measured and compared to ring formation of tannic acid standards (mg TAE g^{-1} dry wt.).

RESULTS

Growth ratio

Regrowth or the three species after defoliation showed that the woody shrubs *O. sinuatum* and *P. pallens* had a very different response to that exhibited by the succulent *R. spinosa*. Both the woody shrubs showed a considerable regrowth capacity (Figure 1) with growth ratios of defoliated plants the same as or even greater than the controls 8 weeks after defoliation. The succulent species, *R. spinosa*, did not regrow after the defoliation event and significant differences ($p < 0.05$) between defoliation intensities were found.

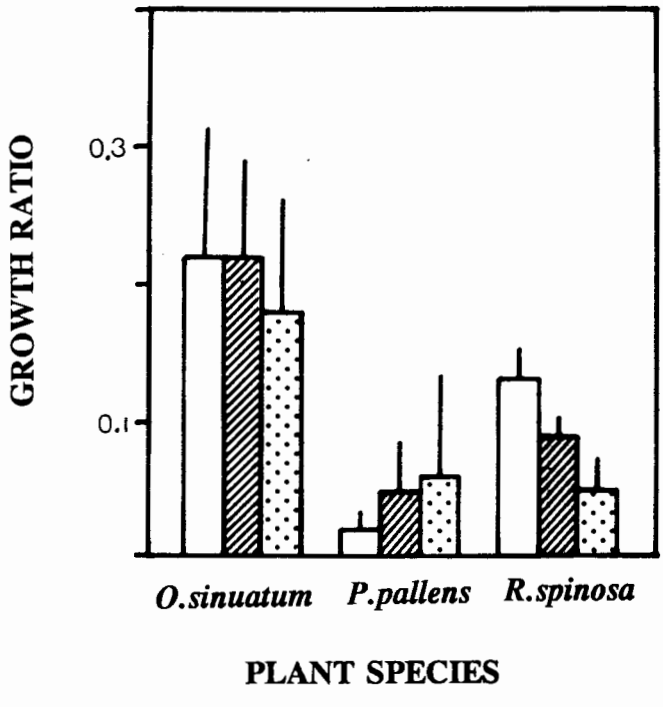

Figure 1: Growth ratios (dry mass of new leaves and twigs/dry mass of old leaves, twigs and stem x 100) of *O. sinuatum*, *P. pallens* and *R. spinosa* in response to defoliation treatments, ☐ control, ▨ 40%, ⊡ 80%.

Total polyphenolics

Total polyphenolic concentrations in equivalent organs varied significantly ($p < 0.05$) among species with *R.spinosa* generally having the highest concentrations and *O. sinuatum* the lowest (Figure 2). Differences in polyphenolic concentrations between organs of undefoliated plants were only significant ($p < 0.05$) in *P. pallens* where new and old leaf categories exhibited higher polyphenolic concentrations than the stems (Figure 2, Table 1). Defoliation effects were evident in *R. spinosa* and *P.pallens* but not in *O.sinuatum*. In defoliated *R.spinosa* plants, significant ($p < 0.05$) increases in polyphenolic concentrations of the old leaf and stem categories were found (Figure 2). Increasing the defoliation intensity from 40 to 80% did not show enhanced effects and thus minor defoliation intensities appear to trigger the increase in polyphenolic concentrations in these organs. *P. pallens* showed the reverse response to *R. spinosa* as polyphenolic concentrations in the older organ categories decreased in response to defoliation. Defoliation intensity also influenced polyphenolic compound production since plants on the 80% treatment showed the largest decreases (Figure 2) in concentrations of all the treatments.

The allocation of polyphenolics to the different organs of the three species showed the old stems to be the major reservoir with between 60 and 90% of total plant polyphenolics being found in this organ (Figure 3). Defoliation had little effect on allocation patterns in *P. pallens* while in *O. sinuatum* and *R. spinosa* the new leaf and old leaf categories become more important reservoirs of polyphenolics in the 40% defoliated plants At 80% defoliation intensities, the old stems are the main stores of polyphenolics probably as a result of the limited biomass of leaf material remaining on the plants.

Condensed tannins

Condensed tannins were only found in one of the three species, *R. spinosa* (Figure 4). Significant differences in condensed tannin concentrations ($p < 0.05$) between old stem and new and old leaf categories were found (Table 2). The very low concentrations of condensed tannins in the stems did not follow patterns of total polyphenolic concentrations (Figure 2) in which old stems had similar total polyphenolic concentrations to the leaves. The effects of defoliation were apparent in the old leaf categories of both the 40 and 80% treatments (Figure 4) as condensed tannin concentrations increased significantly ($p < 0.05$) relative to

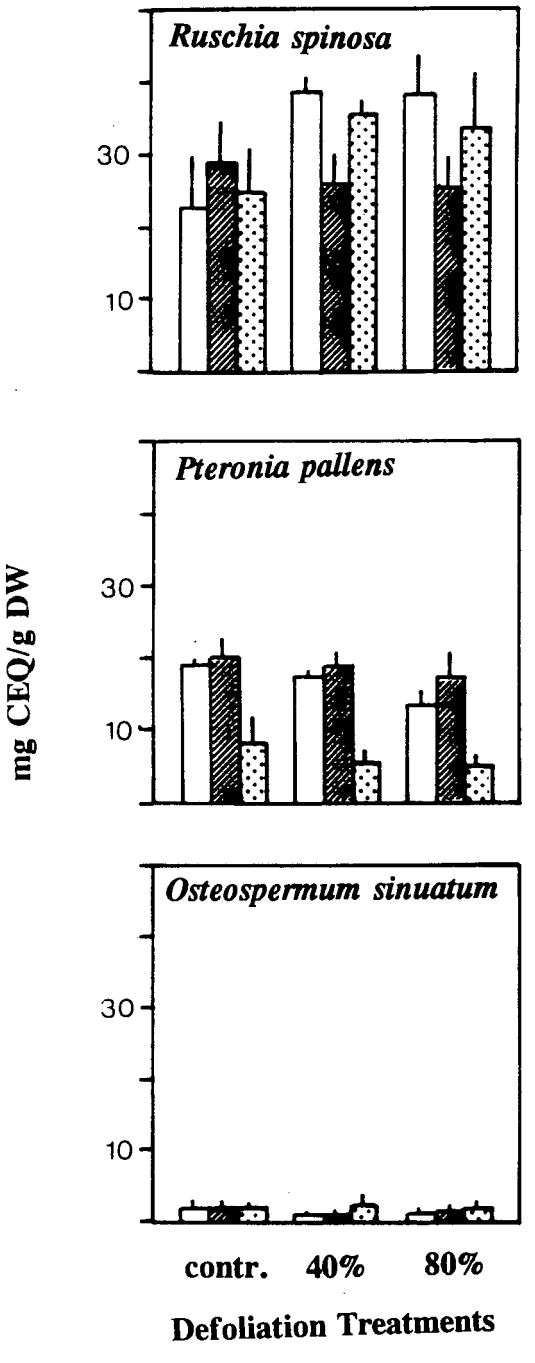

Figure 2: Total polyphenolic concentrations in each organ, ☐ old leaf, ▨ new leaf, ⁘ old stem, of *R. spinosa*, *P. pallens* and *O. sinuatum* in response to defoliation. Values are means ± SD.

TABLE 1: Variation by two-way analysis of variance of plant polyphenolic concentrations of different organs of *R. spinosa*, *P. pallens* and *O. sinuatum* in response to defoliation. Values are F values, * significant differences at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	R. spinosa	*P. pallens*	*O. sinuatum*
DEFOLIATION	6.07 **	7.52 **	1.35 NS
ORGANS	3.54 NS	86.84 ***	3.40 NS
INTERACTION	3.49 *	0.50 NS	0.91 NS

control plants. Concentrations of condensed tannins in the new leaves however did not change in response to defoliation (Figure 4).

Allocation patterns show the old and new leaves to be the major deposits of condensed tannins in the undefoliated plants (Figure 5). With increasing defoliation intensity the old stems become more important as reservoirs with a contribution of some 45% in the 80% defoliated plants (Figure 5) despite the actual concentrations being very low (Figure 4).

Protein precipitating tannins

Protein precipitating tannins were found only in the succulent shrub *R. spinosa* (Figure 6). Old leaves had significantly ($p<0.0.5$) higher concentrations than the new leaves in the two defoliation treatments (Figure 6 and Table 3) while concentrations in the old stems remain uncertain because they were not included in the assay. Patterns of change in concentrations of protein precipitating and condensed tannins were very similar which suggests that the component of the plant extracts responsible for causing the biologically relevant protein precipitation effect appears to be associated with the condensed tannin group.

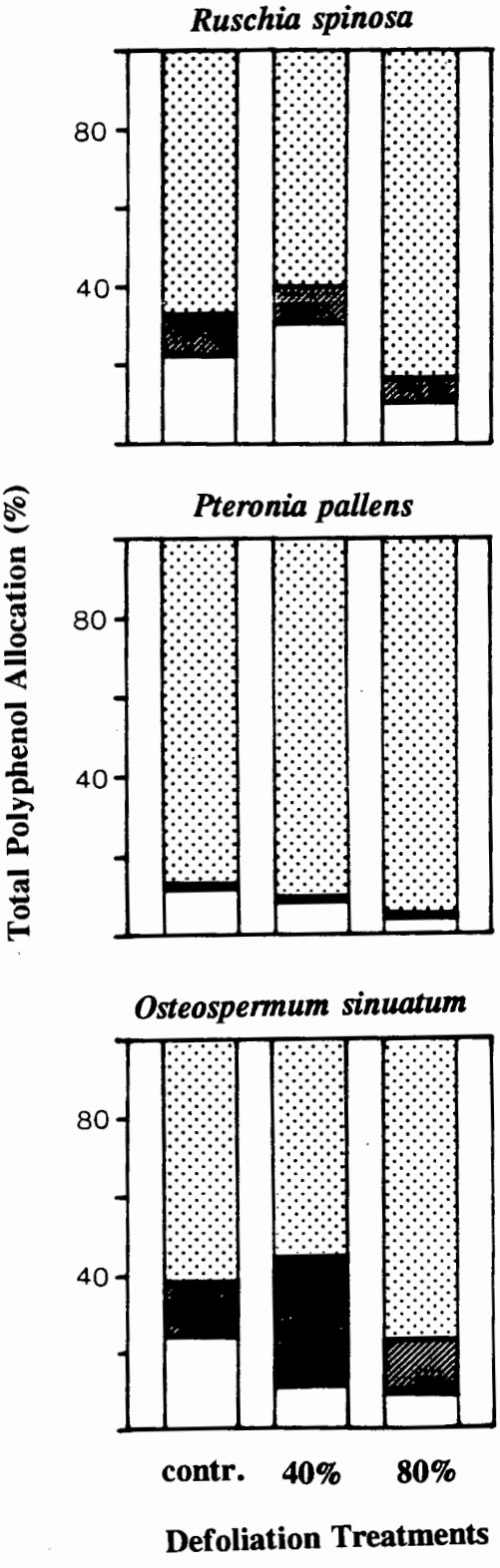

Figure 3: Allocation of total polyphenolics to the various organs, ☐ old leaf, ▨ new leaf, ⚄ old stem, of *R. spinosa*, *P. pallens*, and *O. sinuatum* in response to defoliation.

TABLE 2: Variation by two-way analysis of variance of condensed tannin concentrations of different organs of *R. spinosa* in response to defoliation. Values are F values, * significant differences at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	R. spinosa
DEFOLIATION	46.31 ***
ORGANS	4.20 *
INTERACTION	2.49 NS

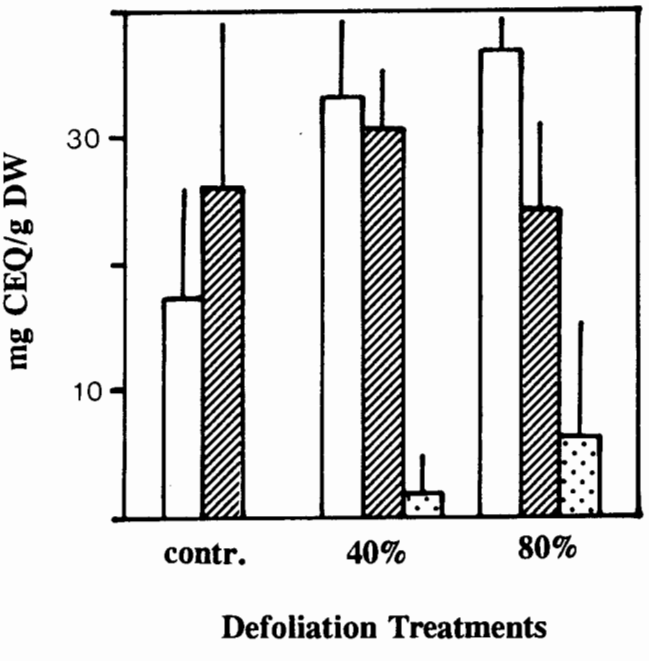

Figure 4: Condensed tannin concentrations in each organ, ☐ old leaf, ▨ new leaf, ⊡ old stem, of *R. spinosa* in response to defoliation. Values are means ± SD.

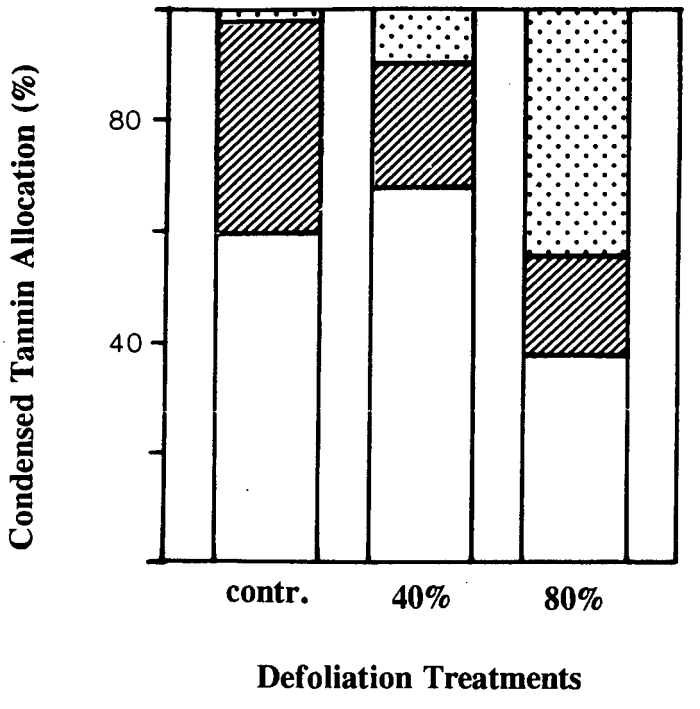

Figure 5: Allocation of condensed tannins to the various organs, ☐ old leaf, ▨ new leaf, ⊡ old stem, of *R. spinosa*, *P. pallens*, and *O. sinuatum* in response to defoliation.

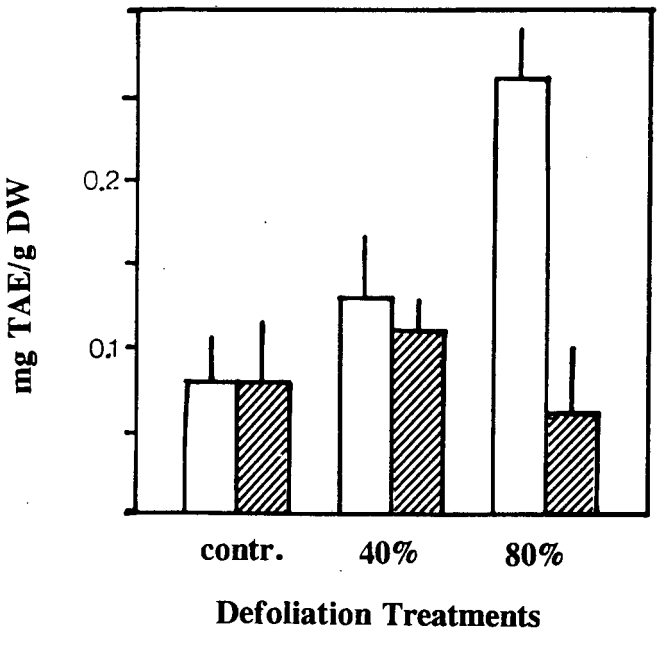

Figure 6: Protein precipitating tannin concentrations in each organ, ☐ old leaf, ▨ new leaf, ⊡ old stem, of *R. spinosa* in response to defoliation. Values are means ± SD.

TABLE 3: Variation by two-way analysis of variance of protein precipitation tannin concentrations of different organs of *R. spinosa* in response to defoliation. Values are F values, * significant differences at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	R. spinosa
DEFOLIATION	17.08 **
ORGANS	7.36 *
INTERACTION	13.04 **

DISCUSSION

The results of this study show that woody and succulent species of the arid Karoo respond to defoliation by either compensatory regrowth (see also van der Heyden & Stock submitted) or by increasing the polyphenolic content (putative compounds) of remaining tissues. A trade-off appears to exist between the conflicting requirements of regrowth and chemical production. Those species capable of rapid compensatory regrowth, such as *O. sinuatum*, have very low concentrations of total polyphenolics, condensed tannins and protein precipitating tannins. The other species, *P. pallens and R. spinosa*, with limited or no regrowth capacity, had considerably higher concentrations of polyphenolics with leaf concentrations of polyphenolics exceeding 1% and 2.5% (relative to catechin standard) in *P. pallens* and *R. spinosa* respectively.

Total polyphenol levels recorded in these karoo shrubs are at the low end of those reported for woody plants of the neighbouring fynbos (2.32%) and savanna (1-30%) biomes (Cooper & Owen-Smith 1985, Glyphis & Puttick 1988) and of unknown significance in reducing the palatability of the species. Cooper & Owen-Smith (1985) showed that foliage acceptance to mammalian herbivores and polyphenolic concentrations (even up to 30%) were not correlated in savanna shrubs and trees. The relative palatability of the study species does however follow polyphenolic concentrations with *O. sinuatum* being more highly sought after by livestock than *R. spinosa* with its higher polyphenolic concentrations (Milton, S.J. personal

communication). *P. pallens* although having intermediate levels of polyphenolics contains unidentified hepatotoxins (Prozesky et al. 1986) and thus the polyphenolics in this species are probably of little importance in deterring herbivory.

Induction of putative chemicals in response to defoliation events implies that defences are costly and are only produced when such an allocation will improve fitness (Rhoades 1979, Haukioja 1980). Mechanisms of resistance induction have been debated with evidence for coevolutionary models such as active- responses arising from experimental defoliations (Haukioja 1980; Clausen et al. 1989) being contrasted with passive-defence models invoking environmental constraints on plant chemistry (Bryant et al. 1983, Tuomi et al. 1984, Myers and Williams 1984, Coley 1988, Baas 1989). The results obtained in this study do not support an active-defence hypothesis as polyphenolic induction in response to defoliation was minimal or did not occur at all in two of the three study species (*P. pallens* and *O. sinuatum*). Defoliation responses of these karoo species show a close association with the predictions arising from passive-defence models. Differential responses to defoliation shown by the enhanced production of polyphenolics in slow growing evergreens versus their absence in faster growing deciduous species is consistent with the suggestion that passive alterations in the carbon/nutrient balance of a plant determines patterns of chemical allocation to defence. In plants such as *O. sinuatum*, which have evolved rapid regrowth potential in response to frequent leaf loss under hostile environmental conditions (regular drought events) there has been strong selection for carbon to be invested directly into growth. In slow growing evergreens the reverse condition is found where allocation to defence is higher because defoliation is more damaging than to a deciduous species as storage reserves of nutrients and carbon are often removed (Bryant et al. 1988). Thus a clear dichotomy exists between the disturbance-adapted deciduous species as compared to the evergreens with patterns of regrowth and chemical defence production being closely associated with the mechanism of water stress resistance. The deciduous stress avoiding species invested in regrowth while the water stress tolerators with evergreen sclerophyll or succulent leaves had higher constitutive and inducible chemical defences.

Evidence further mitigating against any active defence mechanisms operating in karoo shrubs relates to the lack of defoliation induced changes in patterns of polyphenolic allocation to the

various organs of the plant. According to Rhoades' (1979) Optimal Defence Theory organisms evolve and allocate defences in such a way as to maximize individual fitness with those tissues of greatest value and risk of loss being allocated most defences. In the karoo shrubs concentrations of polyphenolics and condensed tannins were very similar among all organs of each species and small changes in response to defoliation were only found in *R. spinosa.* These findings can be interpretted as either that all organs are of equal importance to the plant and therefore equally defended or that defoliation by herbivores has had little selective influence on the chemical composition of the plant. Van der Heyden and Stock (submitted) have shown that considerable differences exist between organs of karoo shrubs in that the major sites of carbohydrate storage are the twigs and stems of all species which places these reserves at a considerably higher risk to loss by herbivores than if they were stored below ground. Thus patterns of polyphenolic allocation to various organs shows no clear association with the possible value of each organ to plant fitness which supports the idea that herbivory has had little selective influence on plant chemistry particularly in *O. sinuatum* and *P. pallens* with their very low levels of polyphenolics.

Few plants invest simultaneously in more than one mechanism of deterring herbivory (Campbell 1986, Owen-Smith and Cooper 1987) and spinescence is seldom found in combination with chemical defences. Milton (1991) suggested from a survey of spinescence in the arid Karoo that this dfence mechanism has evolved frequently as a deterrent against herbivory and mechanical trampling in unrelated taxa characteristic of nutrient-rich moist sites. Spinescent taxa listed by Milton (1991) included members of the *Eberlandsia/Ruschia* (related genera undergoing revision) group of which one was found to have the highest levels of polyphenolics in our study *(R. spinosa).* Unusual as the combination of spinescence and polyphenolics may be, it would appear that in this species spinescence and polyphenols could both have been selected as defences against mamalian herbivores. This contrast in defence mechanisms between *R. spinosa* and the other two study species which have either a pronounced regrowth capacity or toxic properties demonstrates the spectrum of defences that can be adopted by related and unrelated taxa from the same habitat. Extrapolation across life forms or even phylogenetically related species is therefore difficult and for effective management of natural rangelands, such as those of the karoo, defence mechanisms of individual species need to be understood.

It may be concluded from the results of this study that defoliation responses of karoo shrubs are largely related to their mechanisms of water stress resistance. Disturbance-adapted deciduous species show a pronounced regrowth potential while slow growing evergreen stress tolerators increase the polyphenolic content of remaining tissues. Such induced responses have often been seen as having significance in the defence of a plant. Physiological responses of karoo plants to environmental constraints appear sufficient to explain patterns of constitutive and induced changes in plant chemistry without invoking active-defence explanations.

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