Chapter 4
Production and nutritive value of perennial grasses under drought and defoliation

Introduction

Pasture production and nutritive value have been commonly studied to assess a plant's response to moisture and defoliation stresses.

Production attributes include yield, tiller numbers, basal area and plant persistence (Fairey 1985; Norris 1982; Norris and Thomas 1982a, 1982b; Culvenor 1993b, 1994). Dry matter (DM) production and growth rates are affected by frequency and height of cutting (Davis 1960; Hill 1989; Hill and Watson 1989) and water deficit (Perrier *et al.* 1961; Baker and Jung 1968; Norris 1982). Severe defoliation generally results in reduced DM yields and herbage availability (Langlands and Bennett 1973; Hill 1989; Hill and Watson 1989). Short–term water deficits generally result in a reduction in both yield and nutritive value, while long–term water deficits can result in plant death, especially in species with poor adaptation (Arcioni *et al.* 1985). These effects are modified by the timing and severity of the stress and plant growth stage (eg. Culvenor 1993a, 1994). In general, pastures that are heavily grazed have fewer plants and reduced ground cover (Schuster 1964; Langlands and Bennett 1973). Ground cover, through high plant basal area protects the soil against erosion.

Nutritive value varies with season, leaf–stem ratios, leaf senescence, species and lignification (McClymont 1969; Bittman *et al.* 1988; Frank *et al.* 1996). The nutritive value of a forage can be defined as its capacity to satisfy the potential requirements of an animal. Laboratory estimation of *in vitro* dry matter digestibility also acts as a surrogate for voluntary intake as there is a strong positive relationship between the two factors in most forages (Black 1987). The effects of drought on nutritive value are inconclusive (Woodman *et al.* 1931; Gifford and Jensen 1967; Harris and Lazenby 1974; Garwood *et al.* 1979; Misra and Singh 1982; Bittman *et al.* 1988). The effect of drought on plant species involves more that one process and varies with drought severity, however, factors which reduce leaf area

generally reduce DM yield and nutritive value (Frank *et al.* 1996). The severity of the drought may influence the response, for example, moderate drought reduces the rate of seasonal decline in plant digestibility (Bittman *et al.* 1988) while severe drought resulted in reductions in crude protein and nutritive value (Woodman *et al.* 1931).

Defoliation and drought responses have been extensively studied on a range of pasture grass species. However, the interactive effects of defoliation and drought have received little attention. It is important to quantify the response of valuable pasture species to defoliation during drought so that management strategies can be developed to enhance the persistence and production of these species in a pasture. The aim of this chapter is to examine the effects of defoliation intensity during droughts of different severity on the dry matter yield, basal area, the ability to maintain green foliage and the nutritive value of four introduced and two Australian native perennial grass species.

Materials and methods

Trial design and treatments

Details of the trial site preparation establishment, design, rain–out shelter and treatments are described in detail in Chapter 3. The particular methods relating to this chapter are described below.

Measurements

Dry matter yield

Plots were defoliated using a lawn mower and catcher when the ceiling biomass (900 and 2000 kg DM/ha for the severe and moderate defoliation treatments respectively) was reached. Eye estimation was used to determine the total plot biomass and hence defoliation time. Samples from the catcher were dried in an oven at 55°C and weighed. DM yields were recorded and samples retained for nutritive value assessment. DM yields were also recorded during the 6–month recovery period (ie. the period following each drought season).

From mid–November, the phenological growth stage of each plot was recorded prior to cutting. The scoring system was:

1 Vegetative

2 Stem elongation

3 Flowering

Basal area

Crown length and width (cm) of the c entral nine plants in each plot were measured at ground level every three months from December 1994 for both SS and SA. The crown dimensions were used to calculate crown area using Equation 4.1. Basal area was calculated as the sum of the central nine plants (per 0.36 m^2)

$$\text{Crown area} = \pi\left(\frac{length + width}{2}\right)^2 \qquad 4.1$$

Green foliage index

The ability of a plant to maintain green foliage was used as a measure of its tolerance to moisture and defoliation stress. The 'greenness' of the central nine plants in each plot was assessed monthly for both 6–month seasons using the following scoring system:

5 Greater than 80% of the plants' foliage green

4 Between 50%–80% of the foliage green

3 Between 10%–50% of the foliage green

2 A trace (less than 10%) of the foliage green

1 No green foliage (presumed either dead or dormant)

Following the 50 mm watering at the end of season, the plots were assessed fortnightly for six weeks to assess recovery.

Plant nutritive value

Yield samples from the start, middle and end of each season were ground in a Cyclotec sample mill through a 1 mm sieve. The ground samples were scanned using Near Infrared Reflectance (NIR) spectroscopy (NIRSystems 6500 Monochromator using NSAS™ software). A subset of 101 tiller samples was analysed for *in vitro* dry matter digestibility (DMD) using Alexander and McGowan's (1961) modified method of Tilley *et al.* (1960). A subset of 92 samples was analysed for nitrogen (Crooke and Simpson 1971). The laboratory values were used to produce calibration

Table 4.1: Comparison of *in–vitro* dry matter digestibility determined using near infra–red spectroscopy against the laboratory values for perennial grass species. Different samples were used to prepare the calibration and the validation calibration.

	Digestibility	Nitrogen
n	52	46
R^2	0.85	0.96
SEP (calibration)*	2.76	0.18
SEP (validation)*	3.11	0.17

* SEP = standard error of performance

Statistical analyses

Data were analysed within an experimental season as a split–split plot using the statistical package Genstat 5 (Genstat 5 Committee 1987). Analyses were conducted on individual assessments and over time. As each assessment in time was dependent (due to repeated measures), the degrees of freedom in the time stratum were reduced using the Greenhouse–Geisser factor (Greenhouse and Geisser 1959). The degrees of freedom were only adjusted where there were three or more assessments during the season. Residual and normality plots were examined for data normality. If the data were not normally distributed, they were transformed using a square root or logit transformation. Contrasts were used to compare differences between moisture treatments.

DM yields were accumulated to produce 3–monthly seasonal DM yields and total yields for each experimental season (6 months). The DM yield of each drought treatment was also calculated as a fraction of the yield of the non–stress moisture treatment to test the effect of the drought intensity.

The scored data for green foliage index were converted to the median percent–green (%–green) value and averaged over the nine plants assessed per plot. The means were divided into experimental period and recovery period, and analysed separately.

Some of the data presented in this chapter, and those following, have been back transformed for presentation. As l.s.d.s cannot be back transformed they are not presented on these figures, however, the level of significance is indicated in the caption for each figure. Complete analyses of variance are presented in Appendix 3.

Results

Dry matter yield

Yields of all species were greater in the SS than in the SA season. The majority of the yield was accumulated during the first three months while the effects of the treatments were most evident in the latter half of both experimental seasons. The ranking of the introduced species was consistent in both seasons with tall fescue having the highest total yield (main effect, $P<0.001$). The ranking of the natives varied with season, with weeping grass having the smallest yield in SS due to only limited growth in spring (Table 4.2).

Table 4.2: Dry matter herbage yield (g/m^2) of perennial grass species during the Spring–Summer and Summer–Autumn seasons. Data are averaged over moisture regime and defoliation intensity. Spring–Summer dry matter yield totals are back transformed. Like letters indicate no significant difference within a column ($P<0.01$).

	Spring–Summer			Summer–Autumn		
Species	Spring	Summer	Total	Summer	Autumn	Total
Tall fescue	417.6 a	115.8 a	507.2 a	260.6 a	54.8 a	315.4 a
Perennial ryegrass	345.3 b	91.4 a	408.0 b	144.8 b	33.2 b	178.0 bc
Cocksfoot	320.9 b	104.6 a	398.0 b	171.8 b	36.0 b	207.8 b
Phalaris	299.0 b	97.7 a	378.3 b	147.9 b	39.3 b	187.2 bc
Wallaby grass	324.1 b	102.9 a	413.7 b	145.3 b	18.8 c	164.1 c
Weeping grass	140.5 c	113.5 a	218.2 c	174.2 b	21.3 c	195.6 bc

There were moisture–defoliation–species interactions ($P<0.01$) in the latter three months of both experimental seasons (Figure 4.1). Species responses were similar in both seasons. In the non–stress moisture treatment, growth was stimulated by severe defoliation resulting in greater DM yields than under moderate defoliation. Wallaby grass, the exception, was not affected by defoliation intensity in either season. Both drought intensities reduced DM yield by a similar proportion. There was no effect of defoliation severity on yield during the severe drought (except for tall fescue).

When yield was analysed as a proportion of the non–stress moisture treatment, there was no significant effect of drought intensity in either experimental season. The yield proportion of all species fell as the SS and SA seasons progressed (data not shown).

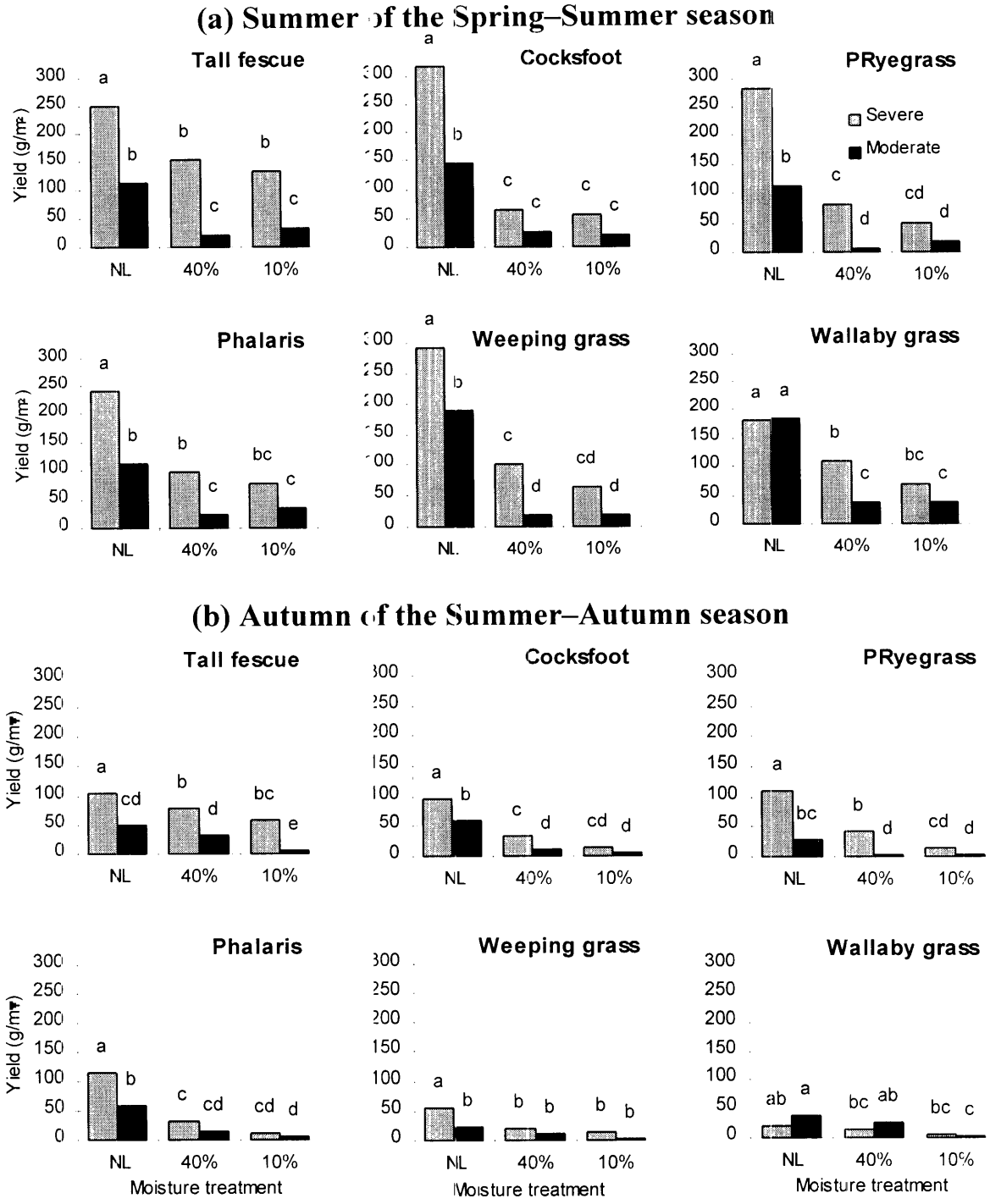

Figure 4.1: Effect of moisture and defoliation on the dry matter yield of six species for (a) summer of the Spring–Summer season and (b) autumn of the Summer–Autumn season. Severe: severe defoliation, Moderate: moderate defoliation. NL: Non–stress, 40%: 40% drought and 10%: 10% drought. Like letters indicate no significant difference within a species ($P < 0.01$).

The cumulative DM yield over the 6–month recovery period following the completion of the 1994–95 seasons was greater following the SA season than the SS season. There were differences in species yields over both recovery periods (main effect, $P<0.001$) with the native grasses having the lowest yields (data not shown). There was no carry over effect of defoliation intensity on herbage yield. There was also no continued effect of moisture regime following the SS season. However, following the 10% SA drought treatment the yield of wallaby grass continued to be reduced ($P<0.001$)(data not shown).

The growth stage varied with all treatments applied. In the SS season, drought appeared to have the greatest affect on growth stage, while in SA, defoliation intensity appeared to have the greatest affect.

In the SS season, the drought treatment plants generally remained vegetative throughout most of the assessment period (Figure 4.2) while the non–stress moisture treatment plots produced flowering stems throughout both spring and summer. The non–stress moisture plants of cocksfoot responded differently to the other species by remaining vegetative throughout the season. The drought effected plants (10% and 40% drought) of wallaby grass flowered until January before being vegetative for the remainder of the season.

In SA, severe defoliation generally resulted in the plants remaining vegetative (Figure 4.3). The moderately defoliated plants tended to produce flowering stems from mid–January, with flowering continuing until the end of the season. Wallaby grass was least affected by defoliation intensity. The tall fescue, phalaris and weeping grass plants in the non–stress moisture treatments were also unaffected by defoliation intensity.

Basal area

The species were ranked in similar order throughout both SS and SA (Table 4.3). In general the basal area of all species increased during the summer months of both seasons ($P<0.01$), however, there was little change in basal area during autumn.

The summer increase in basal area was reduced by severe defoliation in SS ($P<0.05$), while there was no significant effect of defoliation intensity during the SA season (data not shown).

Drought during the summer months reduced the increase in basal area of tall fescue, phalaris and weeping grass in SS ($P<0.05$) and phalaris in SA ($P<0.05$) compared to that observed in the non–stress moisture treatments. There was no effect of moisture regime on basal area during autumn of the SA season. The contrast between the two drought treatments in the SS season was not

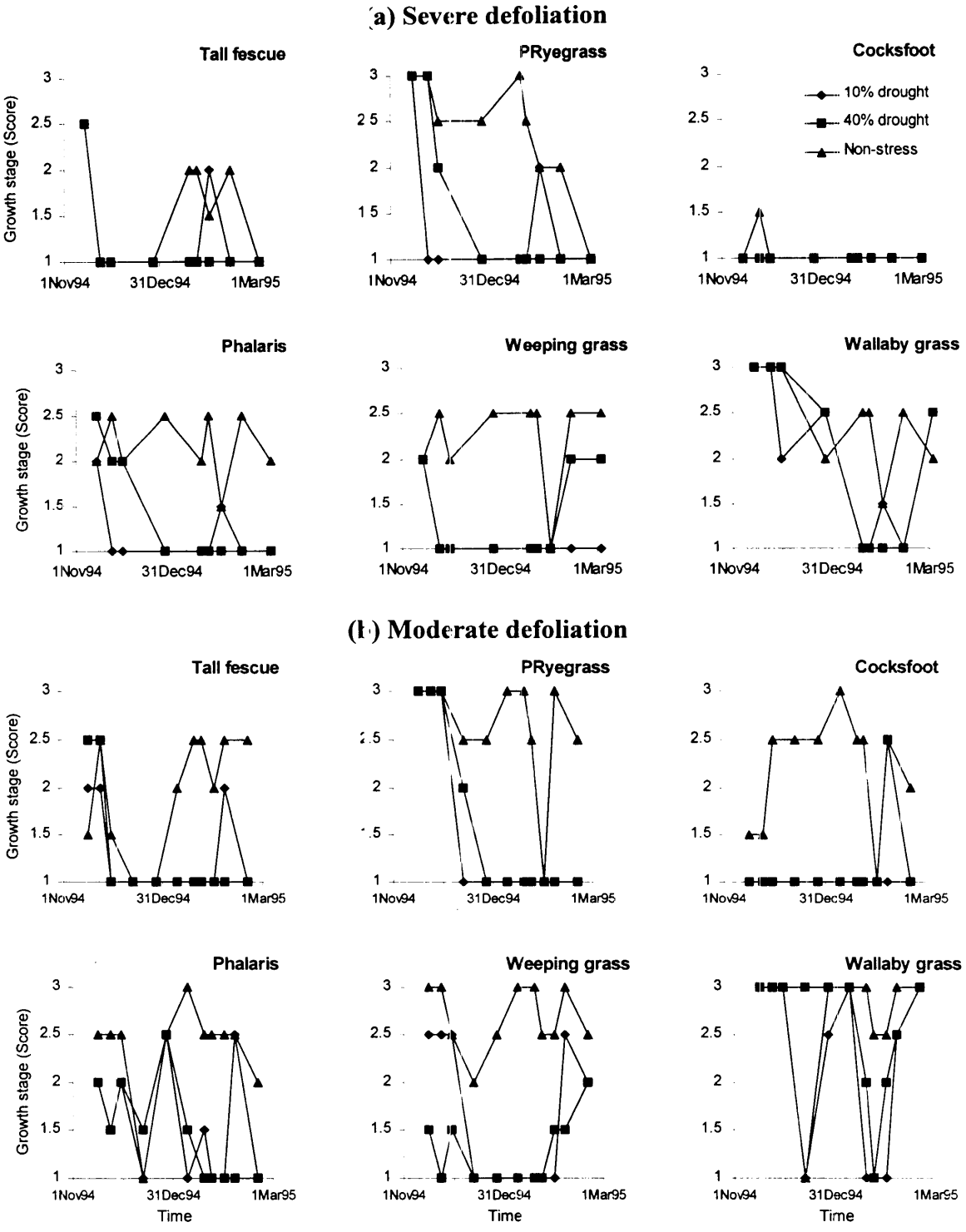

Figure 4.2: Growth stage, as a score, of six perennial grass species at three moisture regimes during the Spring–Summer season. Moisture treatments are 10% drought, 40% drought and non–stress. The scores are 1: vegetative, 2: stem elongation and 3: flowering.

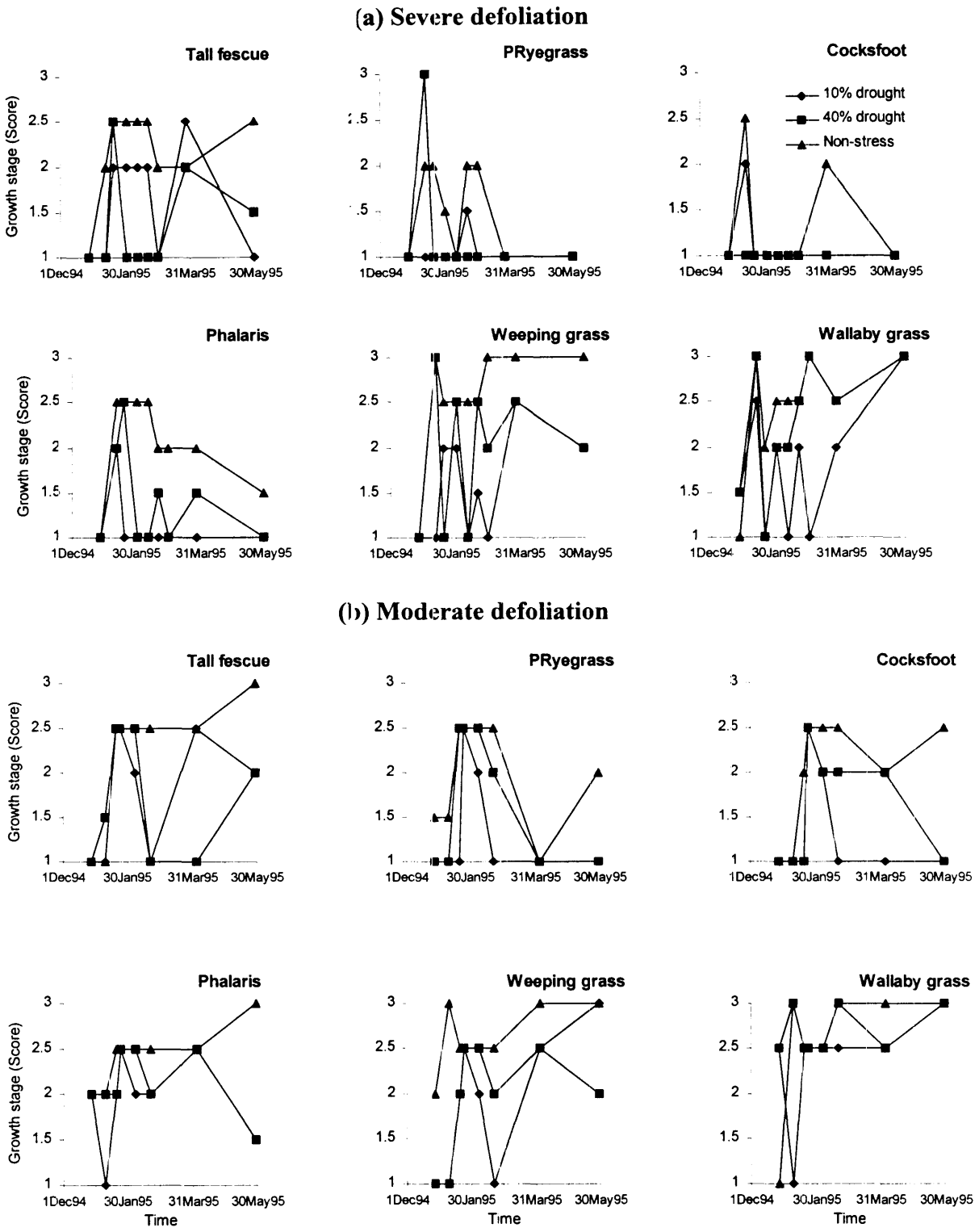

Figure 4.3: Growth stage, as a score, of six perennial grass species at three moisture regimes during the Summer-Autumn season. Moisture treatments are 10% drought, 40% drought and non–stress The scores are 1: vegetative, 2: stem elongation and 3: flowering.

Table 4.3: Percentage basal area of six perennial grass species at each assessment during the Spring–Summer season and the Summer–Autumn season (P<0.001). Data are averaged over moisture regime and defoliation intensity. Like letters indicate no significant difference within an assessment (P<0.01).

Species	Spring–Summer		Summer–Autumn		
	December 1994	March 1995	December 1994	March 1995	May 1995
Phalaris	29.93 a	35.51 a	34.85 a	38.70 a	38.09 a
Tall fescue	17.17 b	22.28 b	22.81 b	29.91 b	28.97 b
Weeping grass	15.88 bc	19.65 b	19.14 c	21.62 c	21.38 c
Perennial ryegrass	12.56 cd	15.12 c	15.12 d	17.97 cd	18.47 cd
Cocksfoot	14.87 cd	14.09 c	14.24 d	14.51 de	14.48 de
Wallaby grass	11.17 d	12.03 c	10.67 e	11.05 e	9.88 e

significant, while the contrasts of each drought treatment with the non–stress moisture treatment were significant (P<0.05). In SA the response in basal area to 10% drought and non–stress moisture treatment were significantly different (P<0.05), while the 40% drought was intermediate and not significantly different to either.

Green foliage index

All species maintained their greenness for the first two months of SS; weeping grass, tall fescue and wallaby grass remained green for the first three months (main effect, P<0.01). The values for %–green fell to their lowest values for most species in January–February. Weeping grass, wallaby grass and phalaris, remained green for much of the remainder of the season (P<0.001). In SA, the species were generally divided into two groups; the high %–green group consisting of tall fescue and weeping grass (main effect, P<0.001) and the low group consisting of the other species.

There were moisture interactions at individual assessments during both seasons as well as a number of interactions with time. There were significant 3–way interactions in both SS and SA; moisture–defoliation–time (P<0.01) in SS, defoliation–species–time (P<0.001) in SA and moisture–species–time (P<0.001) in both seasons.

The moisture–defoliation–time interaction (P<0.01) for SS is presented in Figure 4.4. During SS, there was no decline in plant greenness in the moderately defoliated non–stress moisture plots, while the %–green of the severely defoliated non–stress plots declined from December. The response in plant greenness was similar for the two moisture treatments, irrespective of the defoliation regime. Contrasts of the moisture treatments indicated that the differences between the

10% and 40% drought treatments were not significant, while both were different from the non–stress moisture treatment (P<0.01).

The moisture–defoliation–time interaction was not significant during the recovery period. Similarly, there was no defoliation-time interaction during the recovery period. However, the contrast of the two drought treatments was not significant, while the contrast of each drought treatment with the non–stress moisture treatment was significant (P<0.001).

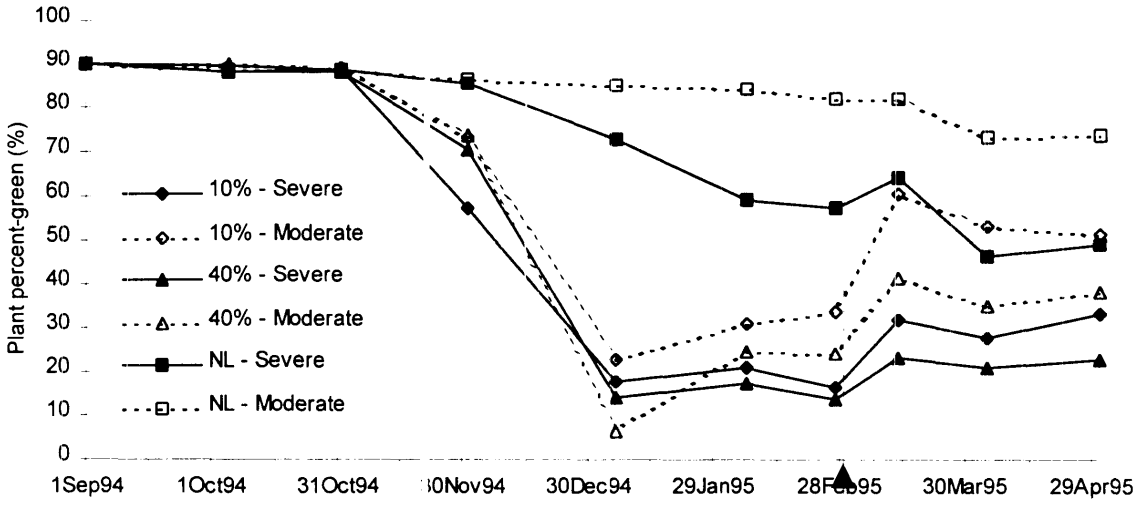

Figure 4.4: The temporal response of plant greenness at different intensities of drought and defoliation during the Spring–Summer season (P<0.01). The dotted line and arrow indicate the end of the experimental season and the application of 50 mm water for the recovery period. The data have been back transformed and are averaged over species. 10%: 10% drought; 40%: 40% drought, NL: non–stress moisture, Severe: severe defoliation, Moderate: moderate defoliation.

The moisture–species–time interaction for both seasons is shown in Figure 4.5. In SS, contrasts of pairs of moisture treatments indicated that the drought moisture treatments responded similarly, while both were different to the non–stress moisture treatment (P<0.001). In SA, contrasts of each pair of moisture treatments were significant (P<0.001) indicating that each of the moisture treatments responded differently through time.

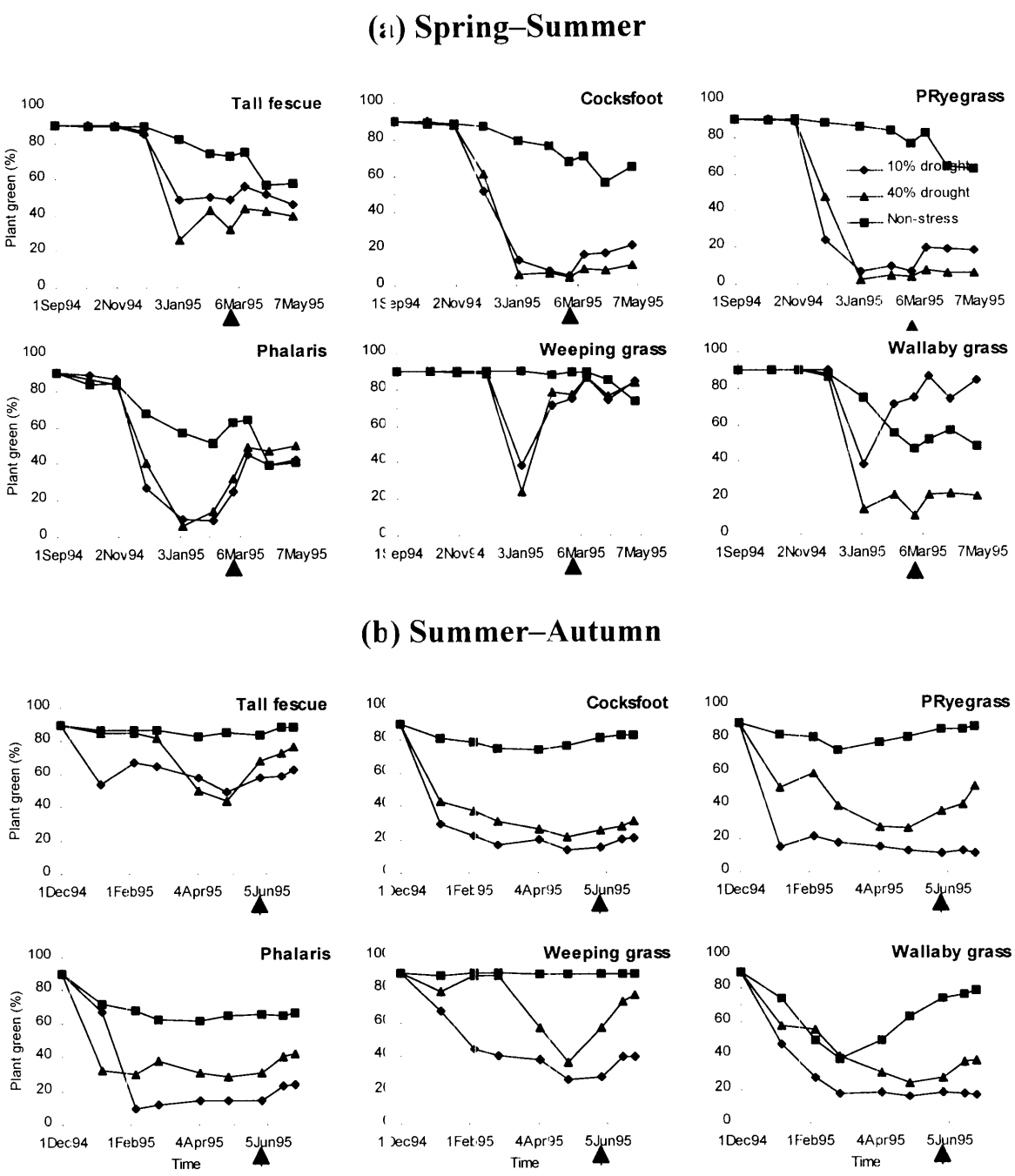

Figure 4.5: The temporal response of plant greenness of six perennial grass species at three moisture regimes during the (a) Spring–Summer and (b) Summer–Autumn seasons ($P<0.001$). The dotted line and arrow indicate the end of the experimental season and the application of 50 mm water to start the recovery period. 10%: 10% drought, 40%: 40% drought and NL: non–stress moisture. The data have been back transformed and are averaged over defoliation regime.

Figure 4.5 indicates the large species variation in response to the moisture treatments in both seasons. In SS, weeping grass maintained high green scores in all moisture treatments for the majority of the experimental season and recovery period, falling only at the January assessment. Plant greenness fell substantially in the non–stress plants of phalaris and wallaby grass compared to the other species. Cocksfoot and perennial ryegrass had similar responses in SS. In SA, cocksfoot and perennial ryegrass again had similar responses, as did tall fescue and weeping grass. In general, the majority of the changes in plant greenness occurred during a two month period early in each season, with little change during the remainder.

During the recovery period, there was a significant moisture–species–time interaction in both seasons ($P<0.05$). Following SS, contrasts of the moisture treatments indicate that the response of the 10% drought plants was different to the non–stress plants ($P<0.005$), while the 40% drought plants were intermediate ($P>0.05$). Following SA, contrasts of the moisture treatments indicated that the recovery response in %–green of the 10% drought and non–stress moisture plants were similar, while the recovery of the 40% drought plants was greater than both ($P<0.05$).

The temporal response of each species to defoliation intensity during the SA season is presented in Figure 4.6. The moderately defoliated plants were significantly greener than those severely defoliated in cocksfoot, phalaris and wallaby grass after one month of treatments ($P<0.001$). However, the difference in %–green between defoliation intensities continued until March in wallaby grass only. Cocksfoot and phalaris responded similarly, as did weeping grass and tall fescue ($P<0.001$).

Plant nutritive value

Nitrogen concentration

The species varied in N concentration with the natives and phalaris having the highest concentrations (main effect, $P<0.001$) at the start of the SS season (Figure 4.7). The N concentration in tall fescue, cocksfoot and perennial ryegrass did not change during the spring period, but increased during summer. N concentration fell during spring in wallaby grass and phalaris before increasing again in summer. Weeping grass responded differently with no change in N during the season.

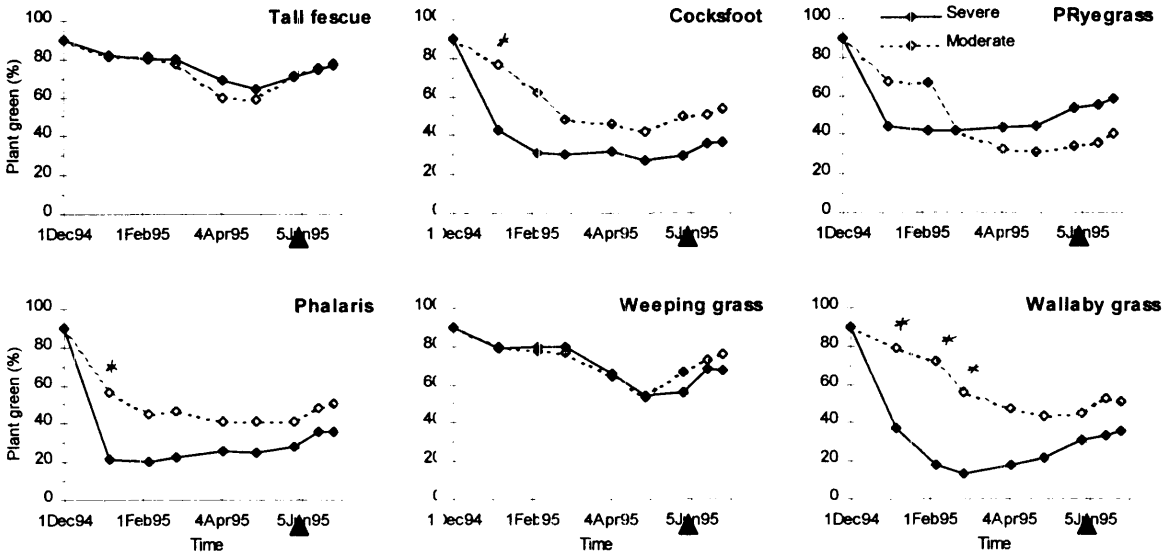

Figure 4.6: The temporal response of plant greenness of six perennial grass species at different defoliation intensities during the Summer–Autumn season (P<0.001). The dotted line and arrow indicate the end of the experimental season and the application of 50 mm of water. Severe: severe defoliation and Moderate: moderate defoliation. The data have been back transformed and are averaged over moisture regime. Significant differences are denoted by *.

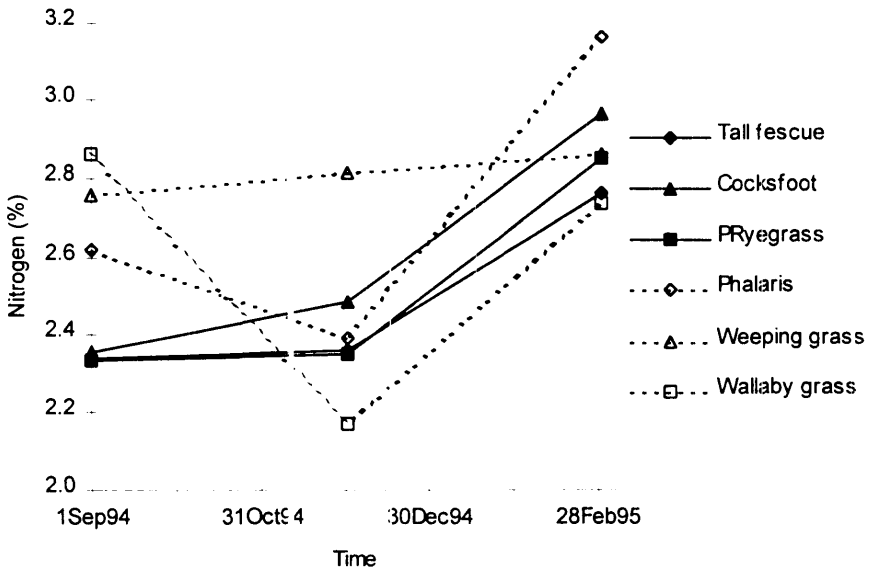

Figure 4.7: Nitrogen concentration in six perennial grass species during the Spring–Summer season (P<0.001). Data are averaged over moisture and defoliation intensities and are back transformed.

During the SS season, the response of plant N concentration varied with defoliation intensity and moisture regime over time (P<0.001) and is presented in Figure 4.8. Nitrogen concentration changed in all treatments, except the moderate defoliation–40% drought plants. During the spring months N concentration of the drought effected plants at both defoliation intensities generally declined (Figure 4.8). During summer however, N increased in all treatments except the moderate defoliation–non–stress and 40% drought plants in which there was no significant change. After six months of treatments, plants in all treatments had similar levels of N, with the exception of the 10% drought–severely defoliated plants which had significantly lower levels (P<0.001).

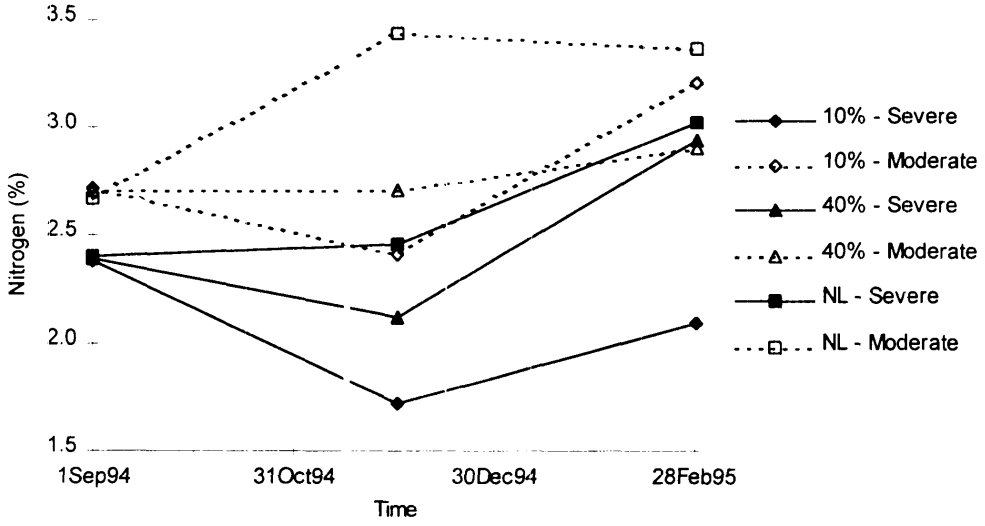

Figure 4.8: The response in plant nitrogen concentration at three moisture regimes and two defoliation intensities during the Spring–Summer season (P<0.001). Data are averaged over six grass species and are back transformed. 10%: 10% drought, 40%: 40% drought and NL: non-stress moisture, Severe: severe defoliation, Moderate: moderate defoliation.

Plant N concentrations declined during the SA season at all moisture regimes tested (P<0.001). N concentrations of the drought effected plants fell during the entire season, the 10% drought effected plants at a greater rate (P<0.001). After six months of treatments, the 10% drought effected plants had the lowest N concentration. The N concentration of the non–stress plants rose during spring but fell during summer to be similar to the 40% drought effected plants (data not shown).

There was a significant defoliation–species–time interaction during the SA season (P<0.05). The N concentration declined in the moderately defoliated plants of all species (Figure 4.9), while there was no change in the severely defoliated plants throughout the season, except tall fescue and

weeping grass. N concentrations in weeping grass declined during the SA season under both defoliation intensities.

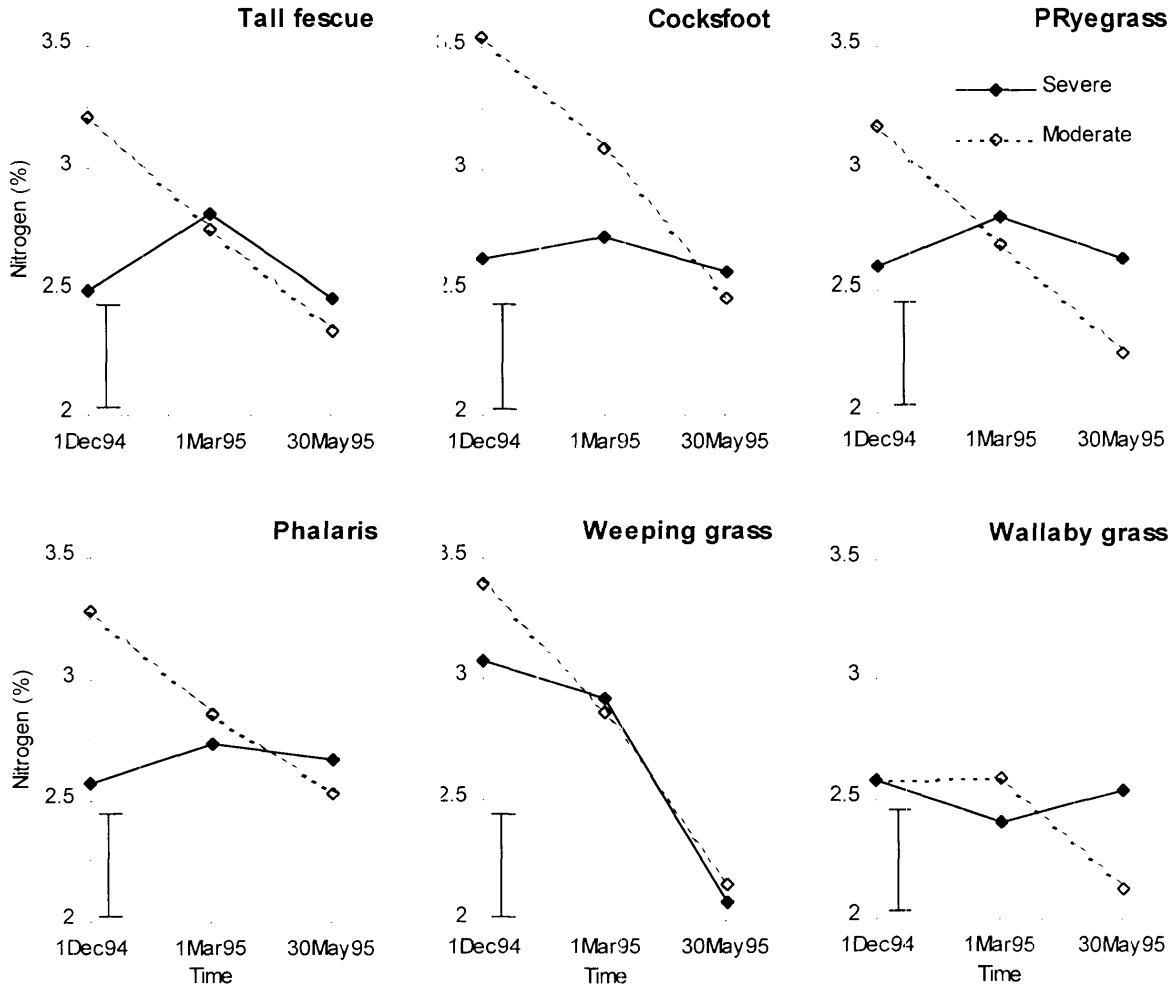

Figure 4.9: Nitrogen concentration in six perennial grass species at two defoliation intensities during the Summer–Autumn season (P<0.05). Data are averaged over moisture regime. Severe: severe defoliation, Moderate: moderate defoliation. The 5% l.s.d. for within a species–defoliation treatment is shown on each figure.

Dry matter digestibility

The temporal response of species DMD to defoliation intensity varied during the SS season (P<0.05) and is presented in Figure 4.10. There was a significant decline in DMD during spring and a significant increase during summer in the severely defoliated plants of all species except tall fescue and weeping grass. In tall fescue the increase was not significant while in weeping grass

there was no significant change in DMD during the season. At the final assessment, the moderately defoliated plants had significantly greater DMD than the severely defoliated tall fescue, perennial ryegrass and weeping grass, while the opposite occurred in phalaris (P<0.05).

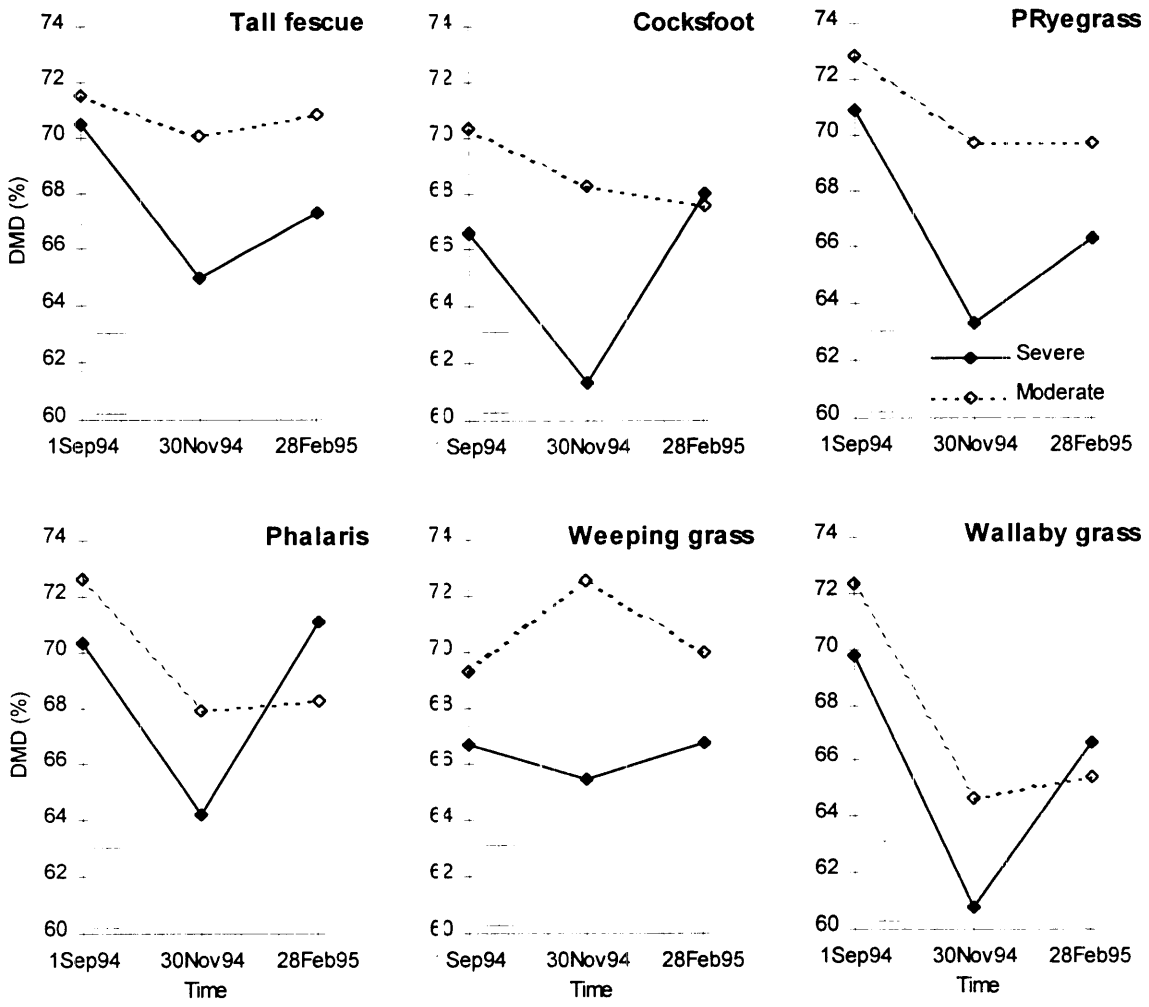

Figure 4.10: Dry matter digestibility (DMD) of six perennial grass species at two defoliation intensities during the Spring–Summer season (P<0.05). The data are averaged over moisture regime. The 5% l.s.d. for within a species treatment is shown on each figure.

The DMD interaction of moisture–defoliation–time was significant (P<0.001) in the SS season. The response of the treatments (data not shown) was similar to N concentration (see Figure 4.8).

The temporal response of DMD of the grass species during the SA season is presented in Figure 4.11. In general, plant DMD increased during summer then remained at similar levels during

autumn (P<0.001). Exceptions were cocksfoot and perennial ryegrass which had no change in DMD during the season, and weeping grass which had a significant decline in DMD during autumn.

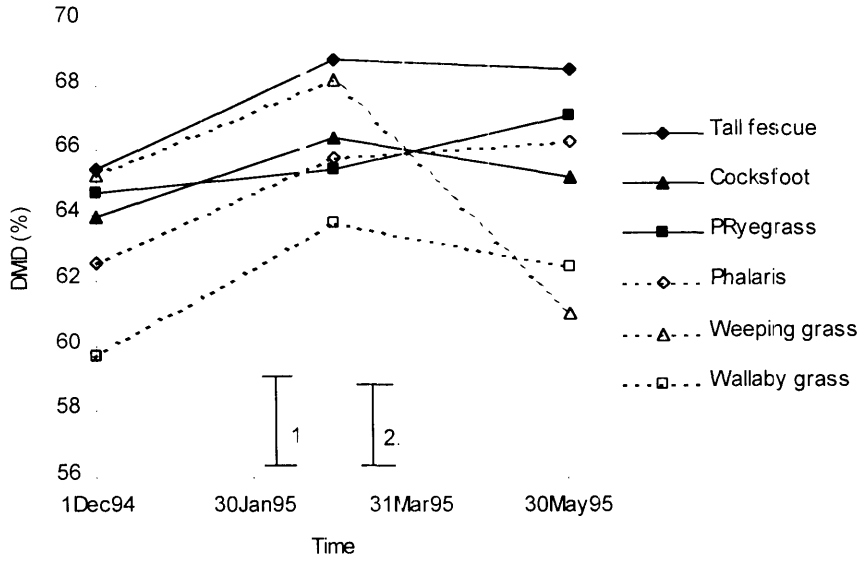

Figure 4.11: The temporal response of dry matter digestibility (DMD) of six perennial grass species during the Summer–Autumn season (P<0.001). Data are averaged over moisture and defoliation treatments. The 1% l.s.d.s for within a species treatment (1) and within a time treatment (2) are shown.

During summer of the SA season, there was a significant moisture–time interaction (P<0.001). The DMD of the non–stress and 40% drought effected plants increased while DMD of the 10% drought effected plants decreased (P<0.001). During autumn, DMD declined in the 40% drought effected plants while the other plants maintained DMD. DMD was significantly different under each of the three moisture regimes (P<0.001) after six months of treatments (data not shown).

The DMD of plants at the two defoliation intensities responded differently during the SA season (P<0.001). DMD of the severely defoliated plants increased during summer then remained constant, while the moderately defoliated plants remained constant during summer then declined in autumn (data not shown). Plant DMD at the two defoliation intensities were significantly different at the start and end of the season (P<0.001).

Discussion

Dry matter yield

Whether drought began in spring or summer, it was only when the plants were stressed (after three months) that DM herbage yield interactions between defoliation and drought severity occurred. Severe defoliation resulted in greater yields while drought, irrespective of intensity, reduced yield. Moderate or lenient defoliation is commonly reported as resulting in greater DM yields and pasture availability than severe or harsh defoliation (eg: Biddiscombe *et al.* 1956; Langlands and Bennett 1973; Hill 1989; Hill and Watson 1989), however, there have been exceptions (eg: Albertson *et al.* 1953). Possible reasons for the greater DM yields from the severe defoliation plots include:

1. Cutting stimulated regrowth (Albertson *et al.* 1953).

2. The experiment was conducted on spaced plants not swards, allowing room for horizontal tiller development, hence some avoidance of defoliation by a number of those tillers.

3. A lawn mower does not defoliate in the same manner as animals. Lawn mowers cut grass at a set height, do not remove the leaves from the lower edges of the plants which tended to grow horizontally, nor do mowers preferentially graze green leaf over dead.

4. Moderate defoliation leaves a greater stubble, which is older and less photosynthetically efficient and possibly shades the newer green leaves, thereby reducing the rate of regrowth and tiller initiation (Smetham 1973).

Although severe defoliation stimulated growth and yields were higher, continued severe defoliation for a longer period of time may have had detrimental effects to yield and plant persistence as reported by Albertson *et al.* (1953).

DM yields were reduced by drought regardless of the defoliation intensity imposed. Severe drought generally reduced plant growth and yield to the extent that defoliation intensity had no effect. Growth rates have been reported to fall during periods of little rainfall (Denison and Perry 1990) and under increased defoliation frequency (Fulkerson and Slack 1995).

Once drought was relieved and 'average' rainfall conditions returned, there was no carry over effect of either defoliation intensity or moisture regime on yield following the SS period. Following the SA period, there was no effect of defoliation, however the yield of wallaby grass was still reduced in the 10% drought plots. Weeda and Goold (1990) found that the effects of

summer grazing management were confined to summer, while hard grazing over a 3–month spring period reduced spring production with the effects extending into summer and possibly beyond.

Seasonal growth rates are highest in spring, while autumn growth rates are generally less than or equal to those in summer (Denison and Perry 1990). This explains the greater SS season yields. Species ranked by yield performance were in similar order to that reported by Robinson (1976). Tall fescue, the exception, yielded better in this experiment. Demeter tall fescue has been reported elsewhere to produce higher annual yields and have a longer growing season than both phalaris and perennial ryegrass (Hilder 1963b; Collins 1991). Wallaby grass yields less during autumn and winter compared to spring (Robinson 1976; Robinson and Archer 1988) which may explain the low ranking of wallaby grass during the SA season. Perennial ryegrass is considered to be sensitive to drought (Norris 1982), which is consistent with the results presented in this chapter.

Weeping grass had low production during spring, possibly due to the sward being immature. Most native grasses are slower to establish than introduced species (C. Jones pers. comm.). Also, the cultivar used in this study has been selected for amenity planting (eg. roadsides and parks) and has a low growing growth habit. Weeping grass is a species that tends to maximise its lateral spread before producing vertical growth (C. Jones pers. comm.). Species with a prostrate habit under grazing, such as phalaris and perennial ryegrass, tend to have higher residual leaf areas following defoliation (Simpson and Culvenor 1987; Culvenor 1993b). A high residual leaf area has been suggested as important for recovery (Culvenor 1993b), however species can adapt to regular cutting, improving their degree of tolerance to grazing (Simpson and Culvenor 1987).

It is often recommended that pastures are spelled or grazed less frequently during spring (eg. Fulkerson and Michell 1987; Culvenor 1994; Fitzgerald and Lodge 1997). The results in this chapter support this recommendation and further suggest that spelling is more vital during dry periods, whether they are severe (eg. 10% drought) or moderate (eg. 40% drought). Severe defoliation during the SA period results in plants remaining vegetative, while moderate defoliation allows species to flower during autumn irrespective of the rainfall.

The changes in phenology (growth stages) of the species were different (Figure 4.2 and Figure 4.3). For example, in this study, wallaby grass tended to flower almost continuously. This supports observations by G. Lodge (pers. comm.). Cocksfoot tended to remain vegetative, especially in the droughted and/or severely defoliated plots. Grazing management trials conducted in the southern temperate high rainfall zone of eastern Australia also found that cocksfoot tended to remained vegetative for most of the year (A. Avery, pers. comm.). However, similar trials in the northern

temperate high rainfall zone did flower (C. Harris, pers. comm.). The differences were possibly due to the summer rainfall pattern.

Culvenor (1993a) tested the sensitivity of phalaris to grazing at different stages of development during spring. He found that sensitivity to grazing increased with synchronous tiller development and that regeneration growth was reduced when plants were cut during reproductive development. In another study, Culvenor (1994) found that cutting at the early boot growth stage resulted in increased plant death. Plant death will be discussed in Chapter 7.

Basal area

Basal area increased during summer, however the rate of increase was reduced by severe defoliation. Summer drought also reduced crown extension, especially in phalaris, possibly due to summer dormancy. Basal area was not affected by defoliation intensity during the SA season. Basal area is a more stable measure of persistence than plant density, as plants tend to spread and fragment with maturity (Hutchinson 1970; Foss 1983; Culvenor and Oram 1996). Basal area has been reported to be generally higher under moderate defoliation regimes (Biddiscombe *et al.* 1956; Langlands and Bennett 1973).

The maintenance of ground cover is crucial to the sustainability of pastures. Managing pastures to maintain ground cover helps ensure that there is sufficient subsoil moisture for dormant stem and bud survival (McWilliam and Kramer 1968). Bud dormancy of phalaris has been reported to be lower in plants where stems have been removed by cutting or grazing and can result in tiller death (Culvenor 1994).

Green foliage index

Foliage greenness is not commonly assessed, hence there are few reports in the literature. However, foliage greenness is an indirect indicator of plant nutritive value. Green leaf and stem have better nutritive value than dead leaf and stem. Drought would be expected to reduce the greenness of foliage in most plant species, the rate varying with drought severity and duration. Perennial ryegrass is generally considered to be more susceptible to drought than cocksfoot and tall fescue (Amin and Thomas 1996). Limited water resulted in a more rapid decline in plant greenness in SA than SS. This was possibly due to higher summer temperatures and evaporation rates. However, the %–green fell to lower levels in SS (Figure 4.5). Foliage greenness fell to similar levels irrespective of the drought intensity. Weeping grass maintained its greenness until it reached what appeared to be a moisture threshold then senesced rapidly. However, during the SS season, it responded quickly to significant rainfall by producing green leaves. The increase in %–

green in a number of species in February of the SS season was largely a result of significant rainfall events in both drought treatments during the latter weeks of January.

Recovery in plant greenness following drought varied. Following SS, foliage greenness was not maintained, while following the SA season it was, suggesting either that the plants were more adversely affected by the SS season, or that the autumn recovery conditions were harsher than the winter recovery conditions. Following the SA season, the 40% drought plants had improved greenness while the 10% drought effected plants improved only marginally. Recovery following drought in either season was not sufficient in cocksfoot, perennial ryegrass and wallaby grass to return the plants to the same greenness as those which were not stressed. Reductions in autumn regeneration have been reported in phalaris possibly due to dormancy being interrupted then followed by cutting at the early stem elongation and early boot stages (Culvenor 1994).

Plant nutritive value

Criteria for quantifying the nutritive value of pastures include digestibility, ease of comminution, nonstructural carbohydrate levels and crude protein levels (Wheeler and Corbett 1989). Plant factors which affect the nutritive value are plant growth stage, leaf–stem ratio, soil fertility and fertiliser application, soil water levels during growth, nutritional value of the dead grass and plant species (McClymont 1969). Plant nutritive value generally produced similar trends whether measured as DMD or N, confirming the generally positive relationship between the two criteria.

The seasonal pattern of plant nutritive value tends to be consistent amongst grasses, with DMD being high in early spring, declining gradually during summer before rising again in late autumn (Woodman *et al.* 1931, 1932; Watanabe *et al.* 1996). Young vegetative pasture growth normally has high digestibility, however as the pasture matures, digestibility declines (McClymont 1965; Smetham 1973). Differences however can also exist between species at the same growth stage (Minson *et al.* 1960, 1964). Albertson *et al.* (1953) found that close cutting resulted in green, succulent, high protein regrowth compared to lightly cut or uncut grasses. The results in this chapter indicate that SS drought can interrupt the pattern of plant nutritive value with severe treatments (drought combined with defoliation) reducing the plant nutritive value compared to the more moderate treatments (Figure 4.8). These results also confirm that such nutritive value response can vary widely between species (eg. Figure 4.9 and Figure 4.10).

Early work suggested that Australian native grass species had low productivity, were unresponsive to fertilisers, were unable to utilise soil moisture reserves and had poorer nutritive value production compared to the introduced species (Donald 1975). However, weeping grass and wallaby grass have been found to have valuable agronomic features (Robinson and Archer 1988)

with nutritive value similar to the introduced species (Lodge 1983; Archer and Robinson 1988; Simpson 1992; Jones 1995). The results reported in this chapter support these findings during the early assessments in both seasons, however, at the later season assessments, the natives had low digestibility; wallaby grass in the SS and both species in SA. The poorer nutritive value of wallaby grass could be due to its high stem component (Robinson and Archer 1988) and its long flowering season. The response in nutritive value of weeping grass was different to the other species (eg. Figure 4.7 and Figure 4.10), however the mechanism involved is not understood. Archer and Robinson (1988) concluded that the nutritive value of native pastures and their potential for animal production varies with species composition, season and the presence of white clover.

Bittman *et al.* (1988) reported that drought increased the rate of seasonal decline in total N content. This is supported by the SA season results. The decline may be due to reduced uptake of soil N and increased translocation of N into the roots (Bittman *et al.* 1988). Drought has also been reported to slow the decline in DMD, by slowing the rate of increase in acid detergent fiber and lignin (Bittman *et al.* 1988). Nevertheless, DMD was always lower in the drought treatments in this study.

Conclusions

1. The greatest variation in the traits assessed was in the responses of individual species, irrespective of their 'native' or 'introduced' status. There were also important interactions between species and the treatments.

2. Yield interactions between defoliation intensity and moisture regime occurred after several months when the plants had begun to be stressed. The severe defoliation treatment stimulated growth, resulting in greater yields than those moderately defoliated. Yields were reduced by drought, irrespective of drought intensity, however under severe drought, defoliation intensity generally had no effect. Greater yields were produced during the SS season compared to the SA season.

3. Drought resulted in reduced phenological plant development during the SS season, while severe defoliation reduced development during SA.

4. Basal area increased during summer, however the rate of increase was reduced by severe defoliation and by drought.

5. The green foliage index was reduced by drought at a faster rate in the SA season than the SS season, possibly due to the higher temperatures and evaporation rates in summer. Species

green index was lower in the SS than SA season. In SS, severe defoliation reduced the green foliage index in the non–stress plants only. Once the green index fell due to drought, defoliation intensity did not have an effect. Drought had a greater effect on green index than defoliation intensity.

6. Following alleviation of the moisture stress between treatments the green foliage index of cocksfoot, perennial ryegrass and wallaby grass did not recover to the level of the non–stress moisture treatment plants in either season. Similarly, the greenness of phalaris did not recover in SA.

7. The nutritive value of the introduced species was similar while the natives responded differently, with wallaby grass tending to have lower nutritive value attributes than the other species. SS drought may interrupt the pattern of plant nutritive value with severe treatments of drought and defoliation reducing the nutritive value compared to the more moderate treatment combinations.

Chapter 5
Carbohydrate reserves of perennial grasses defoliated during drought

Introduction

Plant reserves, both carbohydrate and protein, are considered important for regrowth following defoliation. Although nitrogenous compounds were suggested as a plant reserve by Garber *et al.* (1927, cited in White 1973), their importance has only become widely recognised in the last decade (eg. Ourry *et al.* 1989; Ourry *et al.* 1990; Ourry *et al.* 1993) and is still somewhat controversial (White 1973; Simpson and Culvenor 1987; Volenec *et al.* 1996).

Plant reserves fluctuate diurnally and seasonally (Troughton 1957; White 1973; Pollock and Jones 1979; Simpson and Bonnett 1993) and vary in type (Smouter and Simpson 1989). The perennial pasture grasses most commonly sown on the Northern Tablelands of New South Wales are cool–season, temperate species, which predominantly store fructans as carbohydrate reserves (Schnyder and Nelson 1987; Smouter and Simpson 1989). Although the seasonal variation varies with species (White 1973), there are three stages in the annual cycle of fructan metabolism, ie. minimal fructan storage (during periods of active growth), followed by accumulation and then breakdown (Pollock and Jones 1979). Carbohydrate accumulation is favoured by conditions that stimulate carbon fixation or suppress growth, for example, long photoperiods, infrequent cutting or grazing, soil moisture stress, low temperatures and low nitrogen fertiliser. Accumulation is reduced when growth is stimulated or carbon fixation is low; for example, high fertiliser status, frequent defoliation, short photoperiods or high temperatures (Troughton 1957; Brown and Blaser 1965; Blaser *et al.* 1966; Pollock 1986). The major storage organs of carbohydrate reserves are the stem bases and rhizomes (Sullivan and Sprague 1943; Steen and Larsson 1986), however, smaller proportions of reserves are also stored in the roots (Sullivan and Sprague 1943; Troughton 1957; Steen and Larsson 1986).

Following defoliation, there is a short cycle of carbohydrate depletion and replenishment (Sprague and Sullivan 1950; Orodho and Trlica 1990), the pattern conforming to a U–shaped curve (Sullivan and Sprague 1943, 1949). Regrowth after cutting depends upon the mobilisation of reserves from the remaining parts of the plant (Troughton 1957; White 1973) and storage organs

(Smetham 1973). The effect of defoliation on a plant depends upon the proportion and parts which have been removed, the frequency of defoliation, the plant growth rate, the amount of reserves stored and the environment after defoliation (Sullivan and Sprague 1949; Sprague and Sullivan 1950; Davidson and Milthorpe 1966a, 1966b; Gonzalez *et al.* 1989; Ourry *et al.* 1989; Orodho and Trlica 1990; Fulkerson 1994; Fulkerson *et al.* 1994; Fulkerson and Slack 1995). Plants that are frequently defoliated have little opportunity to rebuild reserves, therefore have slower regrowth rates and lower yields (Smetham 1973). The more stubble remaining following defoliation, the greater the photosynthetic area and the faster the recovery. When sufficient photosynthetic area has been produced to sustain itself, carbohydrates are replaced in the storage organs (Troughton 1957; White 1973).

Early work on the effect of drought on plant carbohydrate reserves, reviewed by Troughton (1957) was contradictory, possibly due to differences in drought severity, duration or seasonal timing. However, it is now well established that growth is more sensitive to water stress than net photosynthesis (eg. Hsiao 1973; Frank *et al.* 1996) and that, at intermediate levels of water stress, carbohydrates accumulate in plant tissues (Blaser *et al.* 1966; Brown and Blaser 1970; Dina and Klikoff 1973; Busso *et al.* 1990; Volaire 1994). Hydrolysis of fructans has also been reported during drought (Virgona and Barlow 1991; Volaire 1994) and has been suggested as a mechanism to aid plant survival of cocksfoot during drought (Volaire 1994).

Busso *et al.* (1990) assessed the interaction of drought and defoliation on non–structural carbohydrates of crested wheatgrass and bluebunch wheatgrass. Although plant carbohydrates accumulated during drought compared to the natural rainfall or irrigated treatments, they found that the effect of cutting intensity and timing was inconsistent.

The effects of defoliation on plant carbohydrates are well documented, although, the effects of drought and drought intensity on reserves are less well documented. There is little literature on the effect of defoliation during drought on the reserves of important cool–season perennial grass species. The aim of this chapter is to investigate the effects of defoliation intensity during droughts of different intensity on the carbohydrate reserves of six perennial pasture grasses.

Materials and methods

Trial design and treatments

Details of the trial site preparation, establishment, design, rain–out shelter and treatments are described in Chapter 3. Methods with specific application to this chapter are described below.

Sample collection and analyses

Vegetative plant tillers were collected for analysis from the central nine plants in each plot. From December 1994 to March 1995 the plant tillers were collected monthly, however due to their small size, the tillers were bulked to form 2-monthly samples (December/January and February/March). From April 1995, tillers were collected every two months.

Tiller bases are the major site of fructan accumulation (Sullivan and Sprague 1943), hence entire tillers, from the root junction at the crown without roots, were collected. Tillers were collected around midday after a harvest, but on the same day. While in the field, the tillers were stored in a cooled insulated container to minimise respiration. The samples were heated in a microwave oven for 1–2 min (depending on sample size), to prevent enzyme degradation, then dried in an oven at 50°C. The dried tiller samples were ground in a small sample mill through a 1 mm sieve.

The ground samples were analysed to determine total water soluble carbohydrate (WSC) and fructan content. WSC was determined using a total hydrolysable reducing sugars method (Smith 1969). Fructan content was determined using a Fructan assay procedure (Megazyme®).

WSC determination. WSC were extracted from 500 mg of dry ground sample in 20 mL 0.2% benzoic acid. The samples were shaken for 1 hour then filtered using Whatman 42 filter papers.

The WSC determination process was conducted with an auto–analyser. The method (including flow rates) is as follows. The sample extracts (1.0 mL/min) were hydrolysed in 85% 1N hydrochloric acid (2.5 mL/min) through a 90°C coil. After hydrolysis, the sample solution was passed through a dialyser with alkaline potassium ferricyanide (2.5 mL/min). The resultant solution was heated again in a 90°C coil with the invert sugar reducing the yellow ferricyanide to colourless ferricyanide. Absorbence was read with a spectrophotometer at 420 nm against sucrose as the standard. A decrease in colour was directly proportional to the amount of sugar present.

A comparison of duplicate analyses conducted by another laboratory indicated that the Armidale procedure had underestimated the WSC. However, the relationship between the values of the two laboratories was consistent. Statistical analysis using Systat® indicated that the slope of the line representing the relationship between the analyses conducted in the two laboratories was significantly greater than 1 ($P<0.01$). The y–intercept was not significantly different from zero (Equation 5.1). As the data would be expected to pass through the origin, there was no justification to include the y–intercept. All data were adjusted using Equation 5.2 (se's are given in brackets, '**' denotes $P<0.001$, and 'ns', not significant).

Y=	1.1021X (0.05[illegible]5) **	+ 0.6372 (0.8236) n.s	R^2=0.973	5.1
Y=	1.13[illegible]4X (0.02[illegible]9) **		R^2=0.996	5.2

Fructan determination. Fructans were extracted from 100 mg of dried ground sample in three steps. Firstly, 8 mL 80% ethanol was added to each sample, heated in a water bath at 80°C for 60 min then centrifuged and the liquid extract removed. Distilled water (8 mL) was added to the sample, incubated for 60 min at 60°C and centrifuged. The liquid extract was again removed and added to the ethanol fraction. A further 8 mL of water was added to the original sample and the procedure repeated for a second time to ensure all fructans were removed. Of the 24 mL of liquid extract, 5 mL was freeze dried to remove the water and ethanol.

After freeze drying, the powdered extract was dissolved in 0.5 mL distilled water and centrifuged. Aliquots (0.2 mL) of the extract were dispensed into glass test–tubes. Diluted Sucrase/β–Amylase/Pullulanase/Maltase enzyme (0.2 mL) was added to each tube and incubated at 40°C for 30 min. Alkaline borohydride solution (0.2 mL) was added to each tube, shaken then incubated at 40°C for a further 30 min to complete the reduction of reducing sugars to sugar alcohols. Acetic acid (100 mM; 0.5 mL) was added to each tube and shaken on a vortex. A vigorous effervescence was observed.

Aliquots (0.2 mL) of the above solution were dispensed into new test tubes in quadruplicate. The fructanase enzyme (0.1 mL) was added to two of the quadruplicates, and sodium acetate buffer to the remaining two test tubes (the tubes with buffer added were used to correct for colour in the sample). The contents were stirred on a vortex stirrer. The tubes were incubated at 40°C for 60 min to effect hydrolysis of the fructans to fructose and glucose. PAHBAH reducing sugar assay reagent (5 mL) was added to all tubes, including four fructose standards and two reagent blanks. The tubes, covered with lids to prevent evaporation, were incubated in a boiling water bath for exactly 6 min, then immediately cooled in cold water (10–15°C) for approximately 5 min. Absorbence was measured on a spectrophotometer at 410 nm against the reagent blank. The absorbence of the buffer added samples was subtracted from the absorbence of the fructanase added samples.

Near infra–red reflectance spectroscopy

The ground samples were scanned using Near Infra–red Reflectance (NIR) Spectroscopy (NIRSystems 6500 Monochromator using NSAS™ software) over the range 400–2500 nm. The laboratory determined carbohydrate values were used to calibrate the NIR for WSC and fructan concentration and validate the calibration equations. The comparison of laboratory and NIR determined values are shown in Table 5.1.

Table 5.1: Comparison of water soluble carbohydrate and fructan data determined using near infra–red spectroscopy against the chemical laboratory values for perennial grass species.

	WSC	Fructan
n	41	48
R^2	0.99	0.89
SEP (calibration)*	0.50	1.34
SEP (prediction)*	2.07	1.61

* SEP = standard error of performance

Statistical analyses

WSC and fructan concentration data were analysed within an experimental season as a split–split plot using the statistical package Genstat 5 (Genstat 5 Committee 1987). To monitor hydrolysis of fructan during the experiment, the Fructan–WSC ratio was calculated and also analysed. Analyses were conducted on individual assessments and over time. As each assessment in time was dependent on the previous assessment, the degrees of freedom in the time stratum (where there were three or more assessments) were reduced using the Greenhouse–Geisser factor (Greenhouse and Geisser 1959). Where necessary, the data were transformed using a logit transformation after examining residual and normality plots. Contrasts were used to compare differences between moisture treatments.

Results

Water soluble carbohydrate concentrations

WSC concentration declined during summer of the SS season in all species (main effect, $P<0.001$), with the exception of weeping grass, which did not change (data not shown). Tall fescue and perennial ryegrass had the highest WSC concentration in December/January ($P<0.001$). At the February/March assessment, tall fescue had the greatest WSC concentration and cocksfoot and wallaby grass the lowest ($P<0.001$).

The WSC concentration of all species increased during the SA season (main effect, P<0.001), with tall fescue, perennial ryegrass, weeping grass and wallaby grass reaching a maximum in April before declining (data not shown). Cocksfoot and phalaris increased significantly until April, while perennial ryegrass did not increase until after the February/March assessment, reaching a maximum in June. The range in WSC increased throughout the season, being maximum in June. During the SA season, tall fescue and perennial ryegrass consistently had the highest WSC levels and wallaby grass the lowest.

At the December/January assessment, WSC concentration was greater in the SS plants than the SA season plants. By the February/March assessment, however the WSC concentration of individual species was similar in both experimental seasons. After six months of treatments, the WSC concentration of the species was greater in the SA season, except wallaby grass (data not shown).

Severe defoliation reduced the spring accumulation of WSC in tall fescue, cocksfoot and weeping grass in the SS season (P<0.05). The difference between the defoliation treatments persisted in tall fescue (P<0.05)(data not shown).

The drought treatment plants had higher levels of WSC than the non–stress plants after the spring treatments (main effect, P<0.05). During the summer period plant WSC levels fell in both drought treatments in cocksfoot and perennial ryegrass, in the 10% drought in tall fescue and the 40% drought in wallaby grass (P<0.01)(Table 5.2). There was no change in the WSC concentration in the other treatments. At the end of the SS season, perennial ryegrass, phalaris, weeping grass and wallaby grass were affected by moisture regime (P<0.001), with the moisture treatments generally ranked 10% drought>non–stress>40% drought.

Although there were no moisture–defoliation interactions at either SS season assessment, the interaction with time was significant (P<0.01). During the summer period, WSC of the drought treatments fell (Figure 5.1), resulting in all treatments having similar WSC concentrations at the end of the season.

During the SA season, defoliation intensity affected the WSC concentration at all assessments (main effect, P<0.05). The temporal response of defoliation intensity (Figure 5.2) was also significant (P<0.01). The concentration of WSC reserves of all species increased over time in both defoliation treatments (P<0.01), the moderate defoliation treatment plants reaching maximum concentrations in April and the severe defoliation plants at the end of the season. The moderate defoliation treatment plants had greater WSC concentrations than the severely defoliated plants until the final assessment (P<0.05), when the reverse occurred (P<0.01).

Although there was no moisture effect in WSC concentration at any one assessment during the SA season, the temporal response was significant (P<0.01). Plant WSC concentration increased between assessments, reaching a maximum in April in all moisture regimes. The WSC in the 40% drought effected plants declined during the final two months of the season (data not shown).

Table 5.2: Water soluble carbohydrate (WSC) concentration (mg/g) of six perennial grass species during summer of the Spring–Summer experimental season at three moisture regimes. Differences between moisture treatments within a species for each assessment are indicated with letters (P<0.01). Significant changes (P<0.01) during the summer period (between assessments) are indicated in the column 'Time'. The percentage change (%Δ) between each assessment is also shown.

Species	Moisture treatment	Time of assessment			
		December/January+	February/March+	Time++	%Δ
Tall fescue	10% drought	244.3 a	148.4 a	Sig	39
	40% drought	198.2 b	161.6 a	NS	18
	Non–stress	161.7 b	176.0 a	NS	-9
Perennial ryegrass	10% drought	223.9 a	153.9 a	Sig	31
	40% drought	201.9 ab	116.3 b	Sig	42
	Non–stress	171.6 b	133.9 ab	NS	22
Cocksfoot	10% drought	168.2 a	90.4 a	Sig	46
	40% drought	144.4 ab	91.6 a	Sig	37
	Non–stress	110.0 b	113.9 a	NS	-4
Weeping grass	10% drought	148 a	144.7 a	NS	2
	40% drought	123.4 ab	117.6 ab	NS	4.7
	Non–stress	85.2 b	114.2 b	Sig	-34
Phalaris	10% drought	130.2 a	114.7 ab	NS	12
	40% drought	144.5 a	121.7 a	NS	16
	Non–stress	121.9 a	92.5 b	NS	24
Wallaby grass	10% drought	138.8 a	107.7 a	NS	22
	40% drought	127.7 a	71.7 b	Sig	44
	Non–stress	70.7 b	90.3 ab	NS	-28

+ Like letters indicate no significant difference within a species
++ Sig indicates a significant change between assessments, NS indicates no significant change

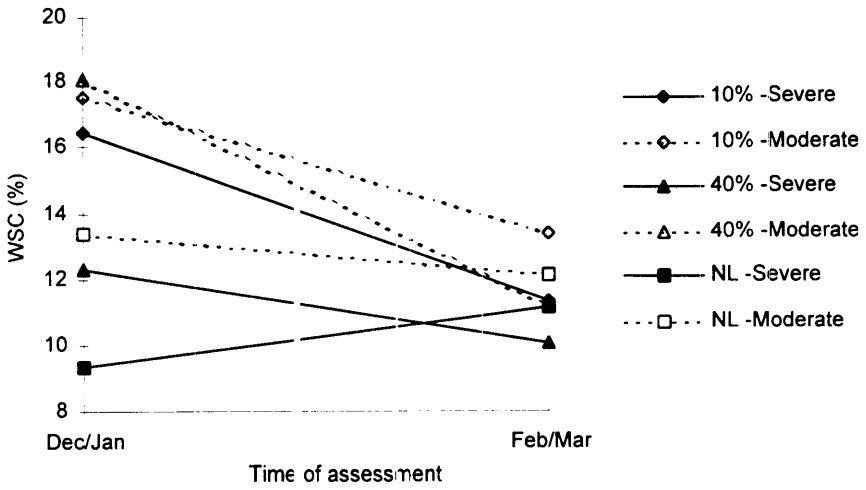

Figure 5.1: Plant water solub e carbohydrate (WSC) concentrations at three moisture regimes and two defoliation intensities during the Spring–Summer season ($P<0.01$). Data are averaged over speci:s and are back transformed. 10%: 10% drought, 40%: 40% drought and NL: non–stress, Severe: severe defoliation, Moderate: Moderate defoliation.

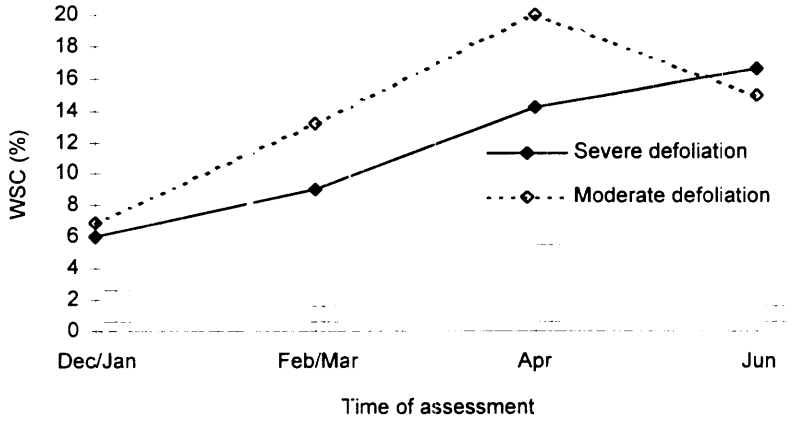

Figure 5.2: The temporal response of plant water soluble carbohydrate (WSC) concentration at severe and moderate defoliation intensities during the Summer–Autumn season ($P<0.01$). Data are averaged over species and moisture treatments. 5% l.s.d.s are shown for individual assessments only.

There were significant defoliation–species ($P<0.05$) and moisture–species ($P<0.05$) interactions in April. The moderately defoliated plants had greater levels of WSC in all species ($P<0.05$), except tall fescue, which had no effect. The moisture–species interaction averaged over time was also significant, affecting cocksfoot and perennial ryegrass ($P<0.01$). The 10% drought plants had significantly less WSC than the non-stress plants in cocksfoot, and less than the non–stress and 40% drought plants in perennial ryegrass (data not shown).

Fructan concentration

The response of fructan concentration was similar to WSC, except in the native species, which contained little fructan. Tall fescue had the highest fructan concentrations throughout both seasons (main effect, $P<0.001$), while the natives had the lowest. The fructan concentration fell during summer of the SS season ($P<0.001$) In contrast, fructan concentration increased during SA with the greatest increases occurring in the final two months in the introduced species ($P<0.001$). There were no changes in the fructan concentration of the native grasses.

At the December/January assessment, the concentration of fructans was greater in the SS season plants than those in the SA season. However, the SA season plants had higher fructan concentrations from February/March (data not shown). After six months of treatments, fructan concentration was greater in the SA season introduced grasses.

There were defoliation–species ($P<0.05$) and moisture–species interactions ($P<0.05$) at both SS assessments. The defoliation–species temporal response was not significant, however when averaged over time, there was less accumulation of fructan ($P<0.001$) in the severely defoliated plants of tall fescue and cocksfoot (data not shown).

Spring drought, irrespective of the intensity, resulted in the accumulation of fructans in the four introduced species when compared to the non–stress moisture plants ($P<0.01$). During the summer period, there was a significant decline in fructan concentration ($P<0.001$) in the drought effected plants of the introduced species, so that by the end of the SS season, only cocksfoot and perennial ryegrass were affected by moisture regime ($P<0.05$). The treatments were generally ranked non–stress>10% drought>40% drought (Table 5.3). There was no change in the fructan concentration in the native species.

Table 5.3: Fructan concentration (mg/g) of six perennial grass species during summer of the Spring–Summer experimental season at three moisture regimes. Differences between moisture treatments within a species for each assessment are indicated with letters (P<0.05). Significant changes (P<0.001) during the summer period (between assessments) are indicated in the column 'Time'. Percent change (%Δ) between each assessment is also shown.

		Time of assessment			
Species	Moisture treatment	December/January+	February/March+	Time++	%Δ
Tall fescue	10% drought	92.5 a	49.3 a	Sig	47
	40% drought	82.7 a	56.4 a	Sig	32
	Non–stress	45.5 b	54.9 a	NS	-21
Perennial ryegrass	10% drought	74.5 a	24.9 a	Sig	67
	40% drought	50.4 b	9.7 b	Sig	81
	Non–stress	31.0 b	29.3 a	NS	5
Cocksfoot	10% drought	55.8 a	14.3 ab	Sig	74
	40% drought	42.1 ab	8.4 b	Sig	80
	Non–stress	24.2 b	21.4 a	NS	12
Weeping grass	10% drought	36.6 a	1.5 a	Sig	96
	40% drought	32.4 a	2.2 b	Sig	93
	Non–stress	9.4 b	4.1 a	NS	56
Phalaris	10% drought	6.4 a	5.5 a	NS	14
	40% drought	9.0 a	0.0 a	NS	100
	Non–stress	13.0 a	2.7 a	NS	79
Wallaby grass	10% drought	11.2 a	2.0 a	NS	82
	40% drought	11.3 a	0 a	NS	82
	Non–stress	2.8 a	2.4 a	NS	14

+ Like letters indicate no significant difference within a species
++ Sig indicates a significant change between assessments, NS indicates no significant change

Although there was no moisture–defoliation interaction at either SS assessment, their interaction through time was significant (P<0.01). Fructan concentration declined in all drought treatment combinations during the summer period with the severely defoliated 40% drought plants declining least. There was no change in the fructan concentration in the non–stress moisture plants, irrespective of the defoliation intensity (data not shown).

The temporal response in fructan concentration of the three moisture treatments varied during the season (P<0.001). Fructan concentration of the 40% and non–stress moisture plants responded similarly during the SA season, not increasing until after the February/March assessment (P<0.001). The 10% drought plants did not increase in fructan concentration until after April. The 10% drought plants had lower fructan levels than the other treatments in April, while the non–stress plants had the highest level in June (data not shown).

In general, the concentrations of fructan increased during the SA season, with the greatest increases occurring during the final two months. The fructan concentration in phalaris plants did not increase until after April, while there were no changes in the fructan concentration of the native grasses.

Moderately defoliated plants of tall fescue, cocksfoot and perennial ryegrass had greater fructan concentrations (P<0.05) at the two mid–season assessments in the SA season. There was no effect of defoliation intensity at the final assessment (data not shown).

Proportion of fructan in total water soluble carbohydrate

Tall fescue had the greatest fructan–WSC ratio of the species in both the SS and SA seasons, while the natives had the lowest (main effect, P<0.001). The fructan–WSC ratios declined during the SS season in all species, except tall fescue and wallaby grass (P<0.01). During the SA season, there was no change in the ratio of tall fescue, weeping grass and wallaby grass while cocksfoot and perennial ryegrass increased during the final two months of the season (P<0.01). The ratio declined in phalaris early in the season, then increased again in the final months of the season (data not shown).

During the period December to March, tall fescue and phalaris had higher fructan–WSC ratios in the SA season than the SS season, however the opposite occurred in the native species. Cocksfoot and perennial ryegrass had similar reserves in both seasons (data not shown). After six months of treatments, there was a higher ratio in the SA plants, with the exception of weeping grass.

There was no effect of defoliation intensity on the ratio during the SS season, however there was a

defoliation–moisture–species interact on at the December/January assessment ($P<0.01$) affecting the introduced species and weeping grass (data not shown).

The fructan–WSC ratios of individual species were affected by moisture regime at the SS December/January assessment ($P<0.001$). The SS season temporal response was also significant ($P<0.01$) and is presented in Figure 5.3. The fructan–WSC ratios of the introduced species increased during spring drought (main effect. $P<0.001$). In contrast, the ratios fell in the drought effected plants of weeping grass ($P<0.001$), while wallaby grass was unaffected by spring drought. During summer of the SS season, there was no change in the ratio for tall fescue or wallaby grass. The fructan concentration of the drought treatments declined significantly during the summer months in the remaining introduced species ($P<0.01$). In contrast, the ratio fell in the non–stress plants of weeping grass during the summer period while there was no change in the drought effected plants.

There was a species–moisture interaction only at the initial assessment ($P<0.01$) during the SA season. However, the fructan–WSC ratio temporal response was significant ($P<0.05$) affecting only the introduced species (Figure 5.3). The ratio fell in all treatments during the summer and early autumn months before increasing again during the final months in all introduced species ($P<0.05$). Exceptions were tall fescue and the 40% drought and non–stress moisture plants of perennial ryegrass which did not change during the first four months of the SA season.

Discussion

Whilst it was necessary to combine the monthly samples to produce the December/January and February/March samples, it is possible that some of the temporal variation may have been masked. This needs to be considered when interpreting the results from this study.

The levels of carbohydrate reserves detected in plants varies with species (Smouter and Simpson 1989), season (Pollock and Jones 1979), environment and management. The species used in this experiment varied in their capacity to store WSC and fructans. Tall fescue had the highest concentrations of both WSC and fructan, while the natives tended to have the least. Although the introduced species used in this study store fructans (Smouter and Simpson 1989; Schnyder and Nelson 1987), the two native species have been reported to store the majority of their carbohydrate as sugars other than fructan, sucrose and starch (Smouter and Simpson 1989).

Defoliation intensity did not have either a large or a persistent effect on carbohydrate reserves in perennial grasses during the SS season. However, it was important for carbohydrate accumulation

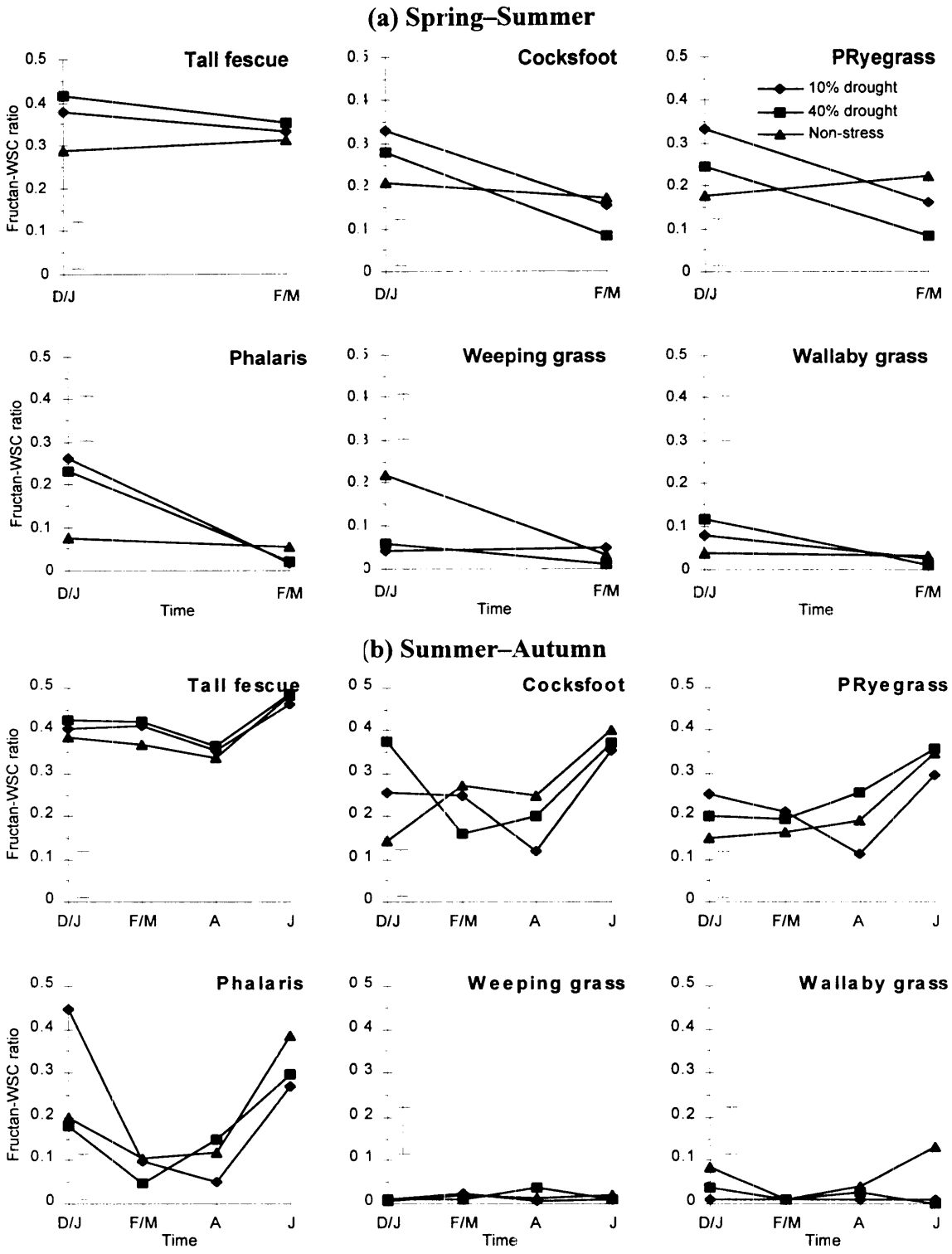

Figure 5.3: The fructan–total water soluble carbohydrate ratio (Fructan–WSC ratio) of six perennial grass species at three moisture regimes, 10% drought, 40% drought and non–stress during the (a) Spring–Summer ($P<0.01$) and (b) Summer–Autumn seasons ($P<0.05$). Times of assessments are D/J: December/January, F/M: February/March, A: April and J: June. 1% and 5% l.s.d.s (within a moisture–species treatment) are presented on the Spring–Summer and Summer–Autumn figures respectively.

during the SA season. Severe defol ation reduced carbohydrate reserves compared to moderate defoliation during spring in some sp ecies, however the carbohydrate reserves of tall fescue were reduced by severe defoliation throu;hout the SS season. Severe defoliation reduced the rate of carbohydrate accumulation during th SA season. These results suggest that maintaining a residual biomass of 1500 kg DM/ha during S/ ensures good carbohydrate accumulation.

Carbohydrates have been reported to accumulate during summer drought (Horst and Nelson 1979; Volaire 1994), however the results n this study suggest that carbohydrate reserves accumulate during a spring drought with the effe t of drought varying during summer.

If summer drought is preceded by a spring drought, these results suggest that the carbohydrate reserves, accumulated during sprin;, are u ilised during the summer months (Table 5.2). As growth was reduced in the drought plots com pared to the non–stress plots, this could suggest that the carbon gained by photosynthesis in the drought effected plants was less than the carbon lost through respiration (due to hot summ er days).

When summer drought was preceded by a spring with favourable rainfall and management, carbohydrate reserves generally incr ased at similar rates regardless of the moisture regime except for cocksfoot and perennial ryegrass. Drough reduced WSC accumulation in cocksfoot while only severe drought reduced WSC accur ulation in perennial ryegrass. The greatest differences and interactions between treatments occu red in April, suggesting that April is the time when plants are most susceptible to drought.

Carbohydrate hydrolysation has bee reported to occur during periods of active growth (Pollock and Jones 1979) and during drought (Virgona and Barlow 1991; Volaire 1994). However, severe drought (3 months of no rain) has been reported to retard fructan decline (Volaire 1994). The results presented in this chapter indi ated that carbohydrate accumulation occurred during spring, whilst the concentrations of both WS C and fr ctan fell during summer.

Virgona and Barlow (1991) found that drought did not affect the rate of non–structural carbohydrate decline of wheat durin; grain filling, but did affect the composition of carbohydrate reserves; the high molecular weight fructans being depolymerised to simple sugars. Spollen and Nelson (1994) found that WSC inc eased during drought, however it was unclear whether the decrease in fructan content was due to decreased synthesis, increased depolymerisation, or both. The results in this chapter indicated a decline in the fructan–WSC ratio, ie. a greater proportion of fructan was utilised than WSC. This suggests that hydrolysis of fructans may have occurred in the drought effected plants during sumn er. Carbohydrate utilisation occurred in equal proportions in tall fescue and wallaby grass. Weepi ng grass responded differently to all other species having a

higher proportion of fructan in the non–stress plants following spring drought. While the fructan levels did not change during summer, the WSC concentration continued to increase suggesting that simple sugars, or sugars other than fructans were accumulated.

During SA, defoliation intensity had a substantial effect on fructan accumulation and fructan–WSC ratio while drought had little effect. The rate of accumulation of fructan was slower than WSC in the severely defoliated plants, indicating that the conversion of sugars to fructans may have been inhibited compared to the moderately defoliated plants. From April however, fructan accumulation occurred in all introduced species as the level of fructan continued to increase while the WSC levels did not.

Carbohydrate accumulation during drought has also been suggested to enhance compensatory growth or rapid regrowth once the stress has been relieved (Horst and Nelson 1979; Busso *et al.* 1990; Volaire 1994). Busso *et al.* (1990) assessed the carbohydrate reserves of crested wheatgrass and bluebunch wheatgrass plants under different drought and defoliation stresses. Their results suggested that the plants which were exposed to prolonged drought (12 months), or drought with defoliation, may have rapid initial regrowth once the drought and defoliation stresses have been removed, due to the build up of carbohydrate reserves during stress.

The increase in WSC concentration and the decline in fructan–WSC ratio in phalaris between December/January and February/March suggests that some hydrolysis of fructan occurred with a large accumulation of simple sugars during this period. Fructan accumulation or the conversion of WSC to fructan in the introduced species increased from April to June, possibly in preparation for winter.

Mid–autumn appears to be an important period in reserve accumulation of sown perennial pasture species. In Armidale, autumn is a distinct season with cooler evenings, the first frosts and shortening days. These environmental changes combined with the pasture management regime could result in differences in the accumulation and utilisation rates of reserves.

Severe depletion of WSC reserves has been reported as the main cause of thinning of unadapted populations of cocksfoot (Volaire 1994) and ryegrass (Arcioni *et al.* 1985). Intense cutting has resulted in reduced production and increased plant mortality, with the quantity and composition of carbohydrate reserves also affected (McKell *et al.* 1966). Tiller death has been reported in plants with low carbohydrate levels, with suggestions that they were sacrificed due to insufficient reserves for regrowth (Alberda 1966).

Is the persistence of perennial grasses defoliated during drought related to plant carbohydrate levels? Plant persistence is a complex trait (Wilkins 1991; Cunningham *et al.* 1994), therefore it is likely to be related to more than plant carbohydrate levels. Persistence will be investigated in more detail in Chapter 7.

Conclusions

1. Defoliation intensity had a substantial effect on plant carbohydrate reserves during the SA season, and little effect during the SS season. Severe defoliation inhibited carbohydrate reserve accumulation.

2. Drought and drought severity had a greater effect on plant carbohydrate reserves in the SS season than the SA season.

3. Reserves accumulated during spring drought and were utilised, or depleted, during summer drought. However, after six months of SS drought, the severely drought effected plants had a higher concentration of reserves than the moderately drought effected plants, suggesting that the rainfall in a moderate drought may be sufficient for some growth but the defoliation regimes removed the new growth before reserves could be replenished.

4. Carbohydrate levels declined during summer when preceded by a spring drought, and rose during summer and autumn when preceded by spring with favourable rainfall.

5. Fructans appear to have been hydrolysed during the SS season summer drought, while fructan accumulation appears to have been inhibited by severe defoliation during the SA season.