Chapter 5

Effect of grazing frequency in spring on perennial ryegrass plant survival over summer, and its relationship with various sward characteristics

5.1 Introduction

Results from Chapter 4 showed that survival of perennial ryegrass plants over summer can be substantially improved by appropriate defoliation management the previous winter and early spring. Defoliating at the 3-leaf stage of regrowth throughout winter and early spring has been shown to significantly increase the number of ryegrass plants surviving through summer by 33% (Chapter 4) and 140% (Fulkerson and Bryant 1994), compared to defoliation at the 1-leaf stage of regrowth. Minimising ryegrass plant death over summer is critical for persistence, as the bare areas left by death of ryegrass plants are colonised by tropical grasses. Improved survival of ryegrass was associated with a more extensive root system, and this confirms earlier studies by Fulkerson et al. (1993b). The mechanism of action of defoliation frequency on root growth and hence plant survival may be mediated through plant reserve substances, especially WSC (Evans 1972, see also Chapter 2, section 2.3.4). In this regard, Arcioni et al. (1980, 1985a, b, c) have shown an association between WSC and perennial ryegrass plant survival through summer in a Mediterranean environment; plants higher in WSC also had a more developed root system (Arcioni et al. 1980, 1985a, b). Similarly, very frequent defoliation (3 and 6 days) of perennial ryegrass has been shown to substantially deplete WSC in stubble, reduce DM yield and increase plant mortality in glasshouse studies (Fulkerson 1994). However, these studies have either been cut-plot experiments in the field or in glasshouse situations, and questions remain about the effects under grazing in the field.
The aim of this study was to confirm the effect of defoliation frequency on survival of perennial ryegrass over summer under grazing, and to monitor the changes in WSC, root integrity and tiller dynamics in an attempt to explain this response. This study comprised 2 parts: the first was concerned with altering grazing frequency in early spring in a first year pasture, the second monitoring perennial ryegrass plants over summer in a 2 and 3 year old pasture, noting differences in sward structure, tiller dynamics and root DM.

5.2 Materials and Methods

5.2.1 Site
The study was located on river flats near Casino on the north coast of New South Wales, and undertaken between September 1995 and May 1996. For details of climate and soil see Chapter 3, section 1. Rainfall over the experimental period was approximately 55% below the long-term average (132 years) during winter and early spring 1995, but about 50% above average from November 1995 to January 1996 (see Figure 3.2)

The pasture type examined was a mix of perennial ryegrass (cvv. Yatsyn and Roper) and white clover (cv. Haifa), sown on 24 March 1995 with 15 kg Yatsyn, 5 kg Roper and 4 kg Haifa/ha. This Chapter deals predominantly with the ryegrass component of the pasture, while the clover component is dealt with in Chapter 12.

5.2.2 Study 1. Effect of grazing frequency in spring

5.2.2.1 Experimental design. Two 20 x 30 m plots of a uniform area of 5 month old pasture were selected from 5 plots in a 5 ha block, on the basis of similar DM production above a 50 mm stubble height (1,115 and 1,179 kg DM/ha), and pasture composition in mid-August 1995. Plots were fenced separately, and grazed by Friesian heifers on 2 September, slashed to 50 mm stubble height and the following grazing interval treatments imposed:
Infrequent: Grazed twice at the 3-leaf stage.
Frequent: Grazed twice at the 1½-leaf stage, then 3 times at the 1-leaf stage.

At a mean Li of 11 days, this gave a total treatment period of 67 days. Duration of grazing on each plot was 8 to 10 hours, at a stocking intensity of 100 cows/ha, which gave a post-grazing pasture residue of about 800 kg DM/ha (41 mm on an Ellinbank rising plate meter (Earle and McGowan 1979)). After the treatment period, both plots were grazed at approximately the 3-leaf stage until March 1996. The use of an Ellinbank rising plate meter to measure pasture DM is shown in Plate 7.

Plate 7. Measuring pasture DM using an Ellinbank rising plate meter.

On 18 October 1995, 100 kg urea/ha was applied to both plots to remove a N deficiency observed in the grass component of the pasture at that time.
5.2.2.2 Measurements.

a. DM yield and pasture composition. Following each grazing over the 2 cycles of 3-leaf regrowth, the yield of pasture in each of six 1 m² exclosure cages/plot was harvested to the average post-grazing pasture height outside the cage, using hand shears. The cages were moved to a fresh area of pasture after each grazing. Plate 8 shows placement of the exclosure cages in a pasture, to protect pasture from being grazed.

Plate 8. Grazing exclosure cages in a perennial ryegrass/white clover pasture prior to grazing.

Samples were separated into ryegrass, clover, tropical grasses (on this site, primarily kikuyu, paspalum and barnyard grass (*Echinochloa crus-galli* (L.) Beauv.)) and weeds, then DM yield determined by drying samples at 80°C in a forced-draught oven for 24 hours. Further botanical composition of pasture was determined in January and March 1996 from 15 forage samples (100 mm x 150 mm) cut at random positions in each plot to a 50 mm stubble height using hand shears. Post-grazing pasture residues were
calculated between 6 September and 10 December 1995 using an Ellinbank rising plate meter based on a pooled calibration equation (Fulkerson and Slack 1993); these residual masses are shown in Table 5.1.

**Table 5.1.** Post-grazing pasture residue (kg DM/ha) estimated using an Ellinbank rising plate meter.

<table>
<thead>
<tr>
<th>Defoliation treatment</th>
<th>Grazing Number 1</th>
<th>Grazing Number 2</th>
<th>Grazing Number 3</th>
<th>Grazing Number 4</th>
<th>Grazing Number 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 leaf/tiller</td>
<td>983</td>
<td>777</td>
<td>861</td>
<td>820</td>
<td>863</td>
</tr>
<tr>
<td>3 leaves/tiller</td>
<td>-</td>
<td>917</td>
<td>-</td>
<td>-</td>
<td>1,000</td>
</tr>
</tbody>
</table>

The mean post-grazing residues of the infrequently-grazed plot were 98 kg DM/ha higher than the frequently-grazed plot.

**b. Plant density and number of plants pulled by stock (sod pulling).** Plant density was determined on 18 November 1995 and 6 March 1996 from the number of individual ryegrass and tropical grass plants per 0.09 m² quadrat, placed at random positions in 20 locations/plot. Following each grazing between September 1995 and February 1996, the number of individual ryegrass plants pulled from the soil by stock in each of five 1 m² fixed quadrates/plot were counted, then removed.

**c. Tiller development.** The number of ryegrass tillers in a 120 mm diameter soil plug were recorded from 15 plugs/plot, taken at random positions within the plots in October, November and December 1995 and March 1996. Tiller number and DM were obtained from 6 randomly selected plants/plot in November and December 1995 and February 1996. Thirty individual ryegrass tillers were marked with coloured wire loops along an identifiable transect within each plot on 2 October 1995, and monitored monthly until 2 January 1996. At each monitoring event, tillers were classed as being vegetative, reproductive or dead, and the initiation of daughter tillers was also noted.
**d. Root development.** On 18 November 1995 and 6 March 1996, 10 ryegrass plants were chosen at random from each plot, and root DM determined as described in Chapter 3, section 2.

**e. WSC determination.** The WSC content of leaves and stubble of ryegrass was determined from 6 randomly selected plants/plot in November and December 1995 and February 1996. Approximately 3 hours after sunrise (in view of the known diurnal variation in WSC content (Fulkerson et al. 1994)), plants were cut to a stubble height of 50 mm (leaf sample), then cut to ground level (stubble sample), and immediately packed in ice for transportation to a freezer. After 24 hours in a freezer, samples were freeze-dried, and WSC levels determined using the procedure described in Chapter 3, section 4.

**f. Fungal analysis.** The roots of several ryegrass plants pulled out of the soil by grazing stock were assayed for fungal pathogens, in particular *Rhizoctonia*, which had previously been found to be associated with roots of pulled ryegrass plants (W.J. Fulkerson, unpublished data). The method of isolation of fungal colonies from roots is outlined in Chapter 3, section 5.

### 5.2.3 Study 2. Monitoring ryegrass plants of different ages

**5.2.3.1 Experimental design.** In both a second and third year pasture (pastures sown around March-April), 40 wooden stakes were driven into the ground along one identifiable transect, leaving no more than 15 mm surface exposed, which then formed points for fixed quadrates. Pasture was grazed at approximately the 3-leaf stage of regrowth for the duration of the experimental period.

**5.2.3.2 Measurements.** All measurements were as described in section 2.2.2 of the current study, with the exception that DM yield, post-grazing pasture residues and tiller DM were not measured, nor was fungal analysis performed on plant roots.
Pasture density was recorded from 40 fixed 0.09 m² quadrates/pasture; sod pulling from 20 fixed 1 m² quadrates/pasture; tiller density from 20 plugs chosen at random in each pasture, and root DM from 15 soil cores chosen at random in each pasture.

5.2.4 Statistical analyses

Comparisons between treatment means, for all parameters except data from individually-marked tillers, were tested by l.s.d. following analysis using the general linear model package in Minitab (Ryan et al. 1985). Comparisons between treatment means from individually-marked tillers were made using the student t-test.

5.3 Results

In late January 1996, a combination of high temperatures (greater than 40°C) and high rainfall (see Figure 3.2) proved disastrous for survival of much of the ryegrass component of pasture. The cooperating farmer sprayed the pasture with 2 L glyphosate/ha in late March (providing an effective kill of most ryegrass, tropical grasses and weeds, while leaving the white clover mainly intact) and then resowed ryegrass.

5.3.1 Study 1

In early November, warm, wet weather provided ideal conditions for germination of tropical grass seeds, and by late spring, the pasture had an average of 38 tropical grass seedlings/m², with no difference between treatments. Despite the severe climatic conditions, in early March 1996, the infrequently grazed plot had 55% higher ryegrass plant density, with each plant having double the number of tillers, and less tropical grass than the frequently grazed plot, although differences were not significant (P>0.05) (Table 5.2). This was consistent with the component yields in mid-summer, with the area grazed frequently in spring containing 46% ryegrass and 46% tropical grass by DM, whereas the area grazed infrequently had 63% ryegrass and 31% tropical grass. The infrequently-grazed plot yielded slightly more ryegrass DM over the period in which grazing treatments differed than the frequently-grazed plot.
Table 5.2. Plant and tiller density in early March 1996 for plants defoliated at the 1 or 3 leaf/tiller stage of regrowth in spring of the previous year.

<table>
<thead>
<tr>
<th>Defoliation treatment</th>
<th>Plant density (plants/m²)</th>
<th>Tiller density (tillers/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ryegrass</td>
<td>Tropical grass</td>
</tr>
<tr>
<td>1 leaf/tiller</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>3 leaf/tiller</td>
<td>17</td>
<td>55</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

In February, ryegrass plants grazed infrequently had significantly more (P=0.05) stubble WSC (% of DM), and significantly more (P<0.01) stubble WSC (mg/plant), than plants grazed frequently (Table 5.3). Although leaf WSC levels were, on average, 37% lower than stubble WSC levels, they followed the same trend (data not presented).

Table 5.3. Stubble WSC concentration (%) and content (mg/plant) in ryegrass plants grazed at the 1- or 3-leaf/tiller stage of the regrowth cycle in spring.

<table>
<thead>
<tr>
<th>Defoliation treatment</th>
<th>WSC in stubble</th>
<th>November 1995</th>
<th>December 1995</th>
<th>February 1996</th>
<th>l.s.d. (P=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>12</td>
<td>15</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>mg/plant</td>
<td>156</td>
<td>140</td>
<td>31</td>
<td>94</td>
</tr>
<tr>
<td>3 leaves/tiller</td>
<td>%</td>
<td>18</td>
<td>18</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>mg/plant</td>
<td>238</td>
<td>235</td>
<td>97</td>
<td>94</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>%</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg/plant</td>
<td>128</td>
<td>114</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

Over summer, WSC content (mg/plant) fell by 60% in plants grazed infrequently, and by 80% in plants grazed frequently. The high WSC content in November in plants grazed infrequently was due to higher stubble WSC%, as tiller number/plant (50) and DM (28 mg/tiller) were identical between the 2 treatments. In December and February, higher
WSC content was a combined effect of higher stubble WSC%, more tillers/plant (55 vs. 28 in December, 35 vs. 30 in February, *P* > 0.05) and heavier tillers (26 vs. 22 mg/tiller in December, 23 vs. 15 mg/tiller in February, *P* > 0.05), compared to plants grazed frequently.

More frequent grazing in spring resulted in plants entering summer with a smaller root system than plants grazed less frequently (Figure 5.1), and although the greater root DM of plants grazed infrequently was consistently greater than for plants defoliated frequently, differences were only significant (*P* < 0.001) in the top 50 mm of soil. This top 50 mm soil layer accounted for 79 ± 2 (mean ± s.e.) % of total root DM in spring for both treatments.

<table>
<thead>
<tr>
<th>Spring defoliation interval (leaves/tiller)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

![Ryegrass root DM (g/plant)](image)

**Spring 1995**  
**Autumn 1996**

**Figure 5.1.** Root DM (g/plant) with depth (0 to 200 mm with 50 mm increments) for samples taken in spring 1995 and autumn 1996, from plants subject to defoliation at 1 or 3 leaves/tiller.
In autumn, roots of frequently-grazed plants were restricted to the top 50 mm of soil, while roots of infrequently-grazed plants still penetrated to 150 mm depth. Root DM had declined to $27 \pm 2$ (mean ± s.e.) % of the spring DM value, irrespective of treatment.

The number of individual ryegrass plants pulled from the soil by grazing stock was significantly higher ($P<0.001$) following frequent grazing in spring. Sod-pulling reached a peak (16 vs. 1 plants/m² for plots grazed at 1 and 3 leaves/tiller, respectively) during the fourth and fifth episode of frequent grazing. By February 1996, the plot grazed frequently in spring had lost a total of 23 plants/m² through sod-pulling by stock, compared to 4 plants/m² in the plot grazed less frequently. *Rhizoctonia solani* was identified in 28% of root samples from pulled plants following isolation from primary cultures.

Frequent grazing led to a slightly denser, but not significantly different ($P>0.05$), ryegrass pasture in October (3,917 vs. 3,475 tillers/m² for pasture grazed frequently and infrequently, respectively). However, this trend was reversed by December (3,325 vs. 4,527 tillers/m², respectively, $P>0.05$), due to tiller death exceeding tiller initiation during this period (Figure 5.2), and confirms results of the plot-cut study described in Chapter 4. By January, tiller death exceeded tiller initiation in both plots, and total tiller population thus declined.
Figure 5.2. Commencing with 30 individually-marked tillers, the total number of (A) live tillers (original and daughter), is the difference between (B) number of daughter tillers initiated, and (C) number of dead tillers over spring and summer in pastures grazed at 1 (■) or 3 (□) leaves/tiller in spring.

5.3.2 Study 2

The ryegrass plant density was significantly higher (P<0.001) in first (study 1), compared to second and third (study 2) year pasture (259, 94 and 89 plants/m², respectively). The number of tropical grass plants increased with age of pasture (38, 49 and 56 plants/m² in first, second and third year pasture, respectively), and although not significantly different (P>0.05), plants in first year pasture had just germinated, whereas plants in second and third year pasture were mostly mature plants.
In early spring, the ryegrass DM composition of first year pasture was 96%, compared to 58% and 39% in the second and third year pasture, respectively. By contrast, tropical grass DM was negligible in spring in first year pasture, but comprised 8% and 19% of DM in the second and third year pasture, respectively. By mid-summer, ryegrass comprised 63%, 44% and 25%, and tropical grass 31%, 35% and 50% of DM in first, second and third year pasture, respectively.

There was greater root DM in the top 50 mm of soil in second year pasture than in third year pasture (Figure 5.3).

**Figure 5.3.** Root DM (g/plant) with depth (0 to 200 mm with 50 mm increments) for samples taken in spring 1995 and autumn 1996 from plants in a second and third year perennial ryegrass and white clover pasture.

In autumn, root penetration had declined to half of the soil depth, and a mean of 16% of DM, compared to spring.

Over the spring and summer, 3 ryegrass plants/m² were physically pulled from the soil by grazing stock in both second and third year pasture, mostly in early summer. The
proportion of total plant loss over summer attributable to sod pulling was 3 ± 0 (mean ± s.e.) % for both pastures.

Ryegrass tiller density varied significantly (P<0.001) both between and within years (Table 5.4), with tiller density increasing from spring to summer in third year pasture, and decreasing in second year pasture.

Table 5.4. Tiller density (tillers/m²) of the second and third year perennial ryegrass/white clover pasture.

<table>
<thead>
<tr>
<th>Pasture age (years)</th>
<th>Tiller density (tillers/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October</td>
</tr>
<tr>
<td>2</td>
<td>4,014</td>
</tr>
<tr>
<td>3</td>
<td>1,427</td>
</tr>
</tbody>
</table>

l.s.d. (P=0.05) = 1,268 between years, and sample dates

Tiller dynamics did not differ significantly (P>0.05) between second and third year pasture, and so values were combined (Figure 5.4).
Figure 5.4. Commencing with 30 individually-marked tillers, the total number of live tillers (original and daughter) (●), is the difference between number of daughter tillers initiated (■), and number of dead tillers (□) in second and third year perennial ryegrass/white clover pasture.

Figure 5.4 indicates that the tiller population increased in second and third year pasture over summer due to tiller initiation exceeding tiller death at this time.

5.4 Discussion

In confirmation of previous glasshouse studies (Fulkerson and Slack 1995), frequent grazing did not allow sufficient time for the plants to replenish plant reserves, as indicated by the 34% lower stubble WSC content (mg/plant) in spring and 68% less in summer, compared to infrequently grazed plants. This was associated with a smaller root system (38% less DM in spring and 46% less in autumn), resulting in 6 times more plants pulled from the soil by stock in spring, and presumably, also made the surviving plants more vulnerable to environmental stresses in summer. It is interesting to note that
under frequent grazing, nearly twice as many ryegrass plants were pulled from the soil in spring as survived to early March 1996. There was a dramatic peak in plants pulled from the soil after 5 short grazing sequences (2 x 1½ leaves/tiller and 3 x 1 leaf/tiller stage of regrowth) and this cumulative effect of defoliation on weakening the root system over time is consistent with previous studies by Crider (1955), Evans (1971) and Hodgkinson and Baas Becking (1977). In Chapter 4 we showed a marked effect of defoliation frequency in winter and early spring on root DM, and the effect also appeared to be cumulative over several months. In New Zealand, Thom et al. (1986) showed that frequent, compared to infrequent grazing (14 vs. 28 day intervals) from late winter to autumn, more than doubled the loss of perennial ryegrass plants from sod pulling (17 vs. 7% of total plants lost, respectively).

Although root DM only differed in the top 50 mm of soil in spring, it is possible that sod pulling is a function of root strength rather than rooting depth. In addition, the method of determining root DM did not differentiate between live and dying roots. Notwithstanding this, it was calculated that sod pulling accounted for 10% of the total plant loss between spring and autumn in pasture grazed at 1 leaf/tiller, compared to 4% of a smaller number of plants lost in pasture grazed less frequently. *Rhizoctonia* fungus was found on the roots of only a proportion of pulled plants, indicating that this may not be a causative factor in sod pulling.

There is evidence that regrowth of perennial ryegrass is reduced proportionally when stubble WSC concentration falls below about 15% (Davies 1965; Alberda 1966b). This may partly explain the poor performance of plants grazed at 1 leaf/tiller in spring, as WSC concentration would then be considered marginal in November and December, and critical in February, whereas plants grazed less frequently only had marginal WSC levels in February. The higher WSC levels were associated with the initiation of 71% more daughter tillers from October to January. The association between WSC and tiller number is consistent with results by Alberda (1957, 1966b), Colvill and Marshall (1984) and Volaire (1995), who found that higher levels of photosynthate were associated with greater production and survival of daughter tillers. In mid-summer, when WSC levels declined in both pastures, presumably due to a higher respiratory load at the high
temperatures (Murata and Iyama 1963; Wilson 1975), daughter tiller initiation was also low. In practice, there may be no overall benefit from increased tiller initiation during summer, as these developing tillers would be a burden on the parent tiller (Ong 1978; Ong and Marshall 1979) during a time of major stress, and hence not survive.

As expected, ryegrass plant density in first year pasture was greater than second or third year pasture, although plants were smaller. As seedling recruitment from perennial ryegrass plants in the subtropics is minimal (Fulkerson et al. 1993a; Lowe and Bowdler 1995; Chapter 4), plants present in second and third year pasture had survived from the original sowing, and while there was a substantial loss of plants over this time, the remaining plants were larger (more tillers/plant, and having a more extensive root system) than first year plants. Tropical grass plants in the first spring were small, individual seedlings, whereas in second and third year pastures, these plants had formed large, established structures, especially the stoloniferous species like kikuyu and couch. As pastures age, the competition for resources by these tropical grasses, with their larger root system (Fulkerson and Lowe 1994) and faster growth rate than ryegrass during the summer months (Colman and Lazenby 1970; Downes 1970; Wilson and Ford 1971), increases, making survival of ryegrass plants more difficult.

Results from previous studies indicate that the optimal cutting interval for ryegrass pastures is between 2.5 to 3.5 leaves/tiller, since more frequent defoliation decreases ryegrass persistence and yield (Chapter 4; Fulkerson et al. 1993b; Fulkerson and Bryant 1994; Fulkerson and Slack 1995). The present study extends this work under grazing. Although the present study was limited by a lack of replication of treatments, in Study 1, areas were matched using DM production and botanical composition as covariates, to reduce the randomness of results, and sufficient data was collected in both studies to make statistically significant predictions. Given the lack of statistical power, care must be taken in interpretation of results, however the results obtained are consistent with the hypothesis that decreased survival of ryegrass over the summer is a consequence of frequent grazing, and this is associated with a lower WSC content and a shallower root system.
Chapter 6

The effect of defoliation on WSC and subsequent root and top
growth of perennial ryegrass

6.1 Introduction

Previous studies (Fulkerson et al. 1993b; Fulkerson and Bryant 1994) have shown that frequent defoliation of perennial ryegrass pre-summer, specifically in winter and early spring (Chapter 4), reduces its survival over summer in a subtropical environment. Frequent defoliation has been shown to lower WSC reserves in ryegrass (Davies 1966; Fulkerson and Slack 1994b) and other grass species (Bommer 1966; Bartholomew and Booysen 1969). Pastures grazed frequently pre-summer, enter summer with lower WSC plant reserves (Chapter 5), and the root systems of plants surviving to the following autumn are not as well developed as the root systems of plants defoliated less frequently at the 3-leaf stage of regrowth (Chapters 4 and 5).

There is empirical evidence to suggest that plant reserves are important in plant survival through, and recovery from, periods of stress such as drought, frost or heat (Julander 1945; Weinmann 1952). In this regard, Arcioni et al (1980, 1985a, b, c) have shown an association between WSC and plant survival, within varieties of perennial ryegrass, over summer in a Mediterranean environment. Similar results have been obtained in Europe with cocksfoot, by Volaire (1994b, 1995), Volaire and Thomas (1995), and Volaire and Gandoin (1996). It could be suggested that WSC plant reserve levels in ryegrass are even more important in the subtropics during summer, when cloud cover reduces incoming C (through reduced photosynthesis) and the warm nights increase respiration, and hence outgoing C (see also Chapter 2, section 2.4.1).

The aim of the present study was to determine the relative importance of WSC plant reserves on top and root growth, and on tiller dynamics of perennial ryegrass. WSC
reserve levels were varied by imposing different defoliation frequencies (Davies 1966). The relative importance of current photosynthate and WSC reserves on regrowth was then determined by monitoring growth in light and darkness (etiolated growth). Etiolated growth has previously been used as a measure of potential plant vigour and hence plant reserve levels (Edwards 1965).

6.2 Materials and methods

6.2.1 Site
The experiments were conducted in a glasshouse under natural light and normal temperature between March and September 1994 at the Wollongbar Agricultural Institute, New South Wales (latitude 28°S). Perennial ryegrass cv. Yatsyn was sown in pots (100 mm diameter x 230 mm high) containing a potting mixture composed of 50% composted sawdust, 30% compost and 20% sand. Plants were fertilised monthly with Nutricote® controlled release fertiliser (Arthur Yates Pty. Ltd., Australia) to ensure adequate nutrient availability, and watered 3 times daily to replace evapotranspiration losses unless otherwise indicated.

6.2.2 Experimental design
The pots, each containing one plant, were arranged to give 2 miniswards (studies 1 and 2) of plants in the glasshouse at a density of 96 plants/m², simulating the plant density of a perennial ryegrass pasture in the spring of its establishment year on the subtropic north coast of New South Wales (Fulkerson et al. 1993b). Edge effects were minimised by placing reflective aluminium foil around each minisward, with the foil adjusted to remain 50 mm below the top of the ryegrass canopy, and by discarding the results of the row of plants around the perimeter of the sward (‘buffer’ row).

When the plants had grown to about 30 tillers/plant (approximately 12 weeks), the last fertiliser application was made and all plants were defoliated to a stubble height of 50 mm at that time (termed H₀) and at all other harvests thereafter, and treatments commenced. Defoliation removed all non-sheath leaf tissue. Leaf material was dried at
80°C in a forced-draught oven for 24 hours, to determine DM yield/plant, and this was used for subsequent analysis of covariance.

6.2.2.1 Study 1. Excluding the buffer row of plants, the remaining 312 plants were divided into 4 groups distributed randomly throughout the minisward following Ho; one group was defoliated 3 times at the 1-leaf stage (3 x 1), each 1-leaf stage being 16 ± 0.3 (mean ± s.e.) days at this time. The second group was defoliated twice, once at the 1-leaf and once at the 2-leaf stage (1,2). The third group was also defoliated twice, once at the 2-leaf and once at the 1-leaf stage (2,1). The last group was defoliated once at the 3-leaf stage (1 x 3). Harvest of the last group, termed H1, coincided with the final harvest of each of the first 3 groups.

At H1, all plants were defoliated (all leaf removed) and leaf material from individual plants was dried in a forced-draught oven at 80°C for 24 hours to obtain DM, and then analysed for WSC using the method outlined in Chapter 3, section 4.

Plants from within each treatment group were allocated at random into 4 equal groups; the first group was destructively harvested at H1, to obtain DM and WSC of stubble and roots. The second and third groups were destructively harvested at 3 or 6 days after H1, respectively, to obtain DM and WSC of leaf and stubble; the fourth group was removed to a box (800 x 1750 x 500 mm high) designed to exclude all light, in order to obtain an indication of plant reserves from etiolated growth. Fans operated to circulate and equilibrate the air inside and outside the box. All plants in the box were watered to maximum saturation, allowed to drain until no water leaked, then a representative sample of 10 pots were weighed. Each 2 days thereafter, these 10 pots were rewatered to their previous weight, and the same volume of water added to all pots in the dark box. Half of the plants were defoliated at weekly intervals until no further leaf extension was observed (4 weeks), at which time all plants were destructively harvested to obtain DM and WSC of leaf, stubble and roots. Leaf DM and WSC were measured at each weekly harvest of plants grown in the dark. The number of tillers/plant were counted at each destructive harvest in all treatments. The mean daily maximum and minimum temperatures during the regrowth period were 19.2 and 10.2°C, respectively.
Root DM was obtained using the method outlined in Chapter 3, section 2, and samples were then analysed for WSC. The root WSC levels estimated by this procedure may have underestimated actual WSC, due to respiratory losses which would occur during the lengthy washing process.

6.2.2.2 Study 2. At H₀, the 324 plants in the second minisward were divided into 3 groups within 4 replicate blocks after allowing 58 plants as buffers around the perimeter, and were defoliated either 3 times at the 1-leaf stage (3 x 1), once at the 1-leaf and once at 2-leaf (1,2), or once at the 3-leaf stage at H₁ when all plants were defoliated (1 x 3). At H₁, 3 plants selected at random from within each treatment by replicate block, were defoliated to a 50 mm stubble height, then cut to ground level, and the resultant ‘leaf’ and ‘stubble’ were dried for determination of DM then analysed for WSC. Then, at 6 day intervals to 36 days after H₁, 4 plants from each treatment by replicate block were defoliated to give leaf and stubble DM yield and WSC content.

6.2.3 Statistical analyses
Comparisons between treatment means, for all variables, were tested by l.s.d. following analysis using the general linear model package in Minitab (Ryan et al. 1985). DM yields at H₀ were used as a covariate factor for analysis of variance of yields between H₀ and H₁, and at H₁. The relationships between WSC at H₁ and subsequent root, leaf and stubble DM and between leaf and stubble WSC content were determined using linear regression analysis.

6.3 Results

6.3.1 Study 1
Imposition of increased frequency of defoliation prior to H₁ caused a significant decline (P<0.05) in stubble WSC (% of DM), and more so in stubble WSC (mg/plant) at H₁. A similar trend was observed in leaf and root WSC (mg/plant) (Table 6.1). There was a significant linear relationship (P<0.001) between WSC (mg/plant) in leaf and stubble, as
described by the following regression equation:

\[
\text{Leaf WSC} = -7.2 + 1.92 \times \text{Stubble WSC} \quad (\text{Adj } r^2 = 0.94)
\]

**Table 6.1.** WSC (% of DM) in stubble and WSC (mg/plant) in the stubble, leaf, and root, and DM of stubble and root (g/plant) at H₁ in relation to defoliation treatment (3 x 1 = 3 times at 1 leaf/tiller; 1,2 = once at 1 and once at 2 leaves/tiller; 2,1 = once at 2 and once at 1 leaf/tiller; 1 x 3 = once at 3 leaves/tiller) prior to H₁.

<table>
<thead>
<tr>
<th>Defoliation treatment prior to H₁</th>
<th>Stubble WSC (% of DM)</th>
<th>Stubble WSC (mg/plant)</th>
<th>Leaf WSC (mg/plant)</th>
<th>Root WSC (mg/plant)</th>
<th>Stubble DM (g/plant)</th>
<th>Root DM (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 x 1</td>
<td>5.2</td>
<td>53</td>
<td>43</td>
<td>16.2</td>
<td>0.93</td>
<td>1.52</td>
</tr>
<tr>
<td>1,2</td>
<td>14.9</td>
<td>138</td>
<td>179</td>
<td>18.0</td>
<td>1.19</td>
<td>1.81</td>
</tr>
<tr>
<td>2,1</td>
<td>2.8</td>
<td>26</td>
<td>35</td>
<td>7.5</td>
<td>0.79</td>
<td>1.18</td>
</tr>
<tr>
<td>1 x 3</td>
<td>19.6</td>
<td>305</td>
<td>579</td>
<td>47.5</td>
<td>1.45</td>
<td>1.60</td>
</tr>
</tbody>
</table>

*ls.d. (P=0.05)* 3.6 191 184 15.7 0.32 0.90

Stubble, but not root, DM varied significantly (P<0.05) with treatment (Table 6.1). However, root DM (g/plant) was significantly related (P<0.01) to both root and stubble WSC (mg/plant) at H₁, as described by the following regression equations:

\[
\begin{align*}
\text{Root DM} &= 0.89 + 0.03 \times \text{Root WSC} \quad (\text{Adj } r^2 = 0.50) \\
\text{Root DM} &= 1 + 0.004 \times \text{Stubble WSC} \quad (\text{Adj } r^2 = 0.42)
\end{align*}
\]

**6.3.1.1 Regrowth in light.** At 3 days after H₁, the leaf DM of the 1 x 3 leaf treatment was significantly greater (P<0.05) than the other defoliation treatments. There were no significant differences (P>0.05) between the remaining treatments (Table 6.2).
Table 6.2. Leaf DM (g/plant), leaf WSC (mg/plant) and stubble WSC (mg/plant) for plants at 3 days and 6 days after H, in relation to defoliation treatment (3 x 1 = 3 times at 1 leaf/tiller; 1,2 = once at 1 and once at 2 leaves/tiller; 2,1 = once at 2 and once at 1 leaf/tiller; 1 x 3 = once at 3 leaves/tiller) prior to H,.

<table>
<thead>
<tr>
<th>Defoliation treatment prior to H,</th>
<th>Days After H,</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf DM (g/plant)</td>
<td>Leaf WSC (mg/plant)</td>
<td>Stubble WSC (mg/plant)</td>
</tr>
<tr>
<td>3 x 1</td>
<td>0.07</td>
<td>2.0</td>
<td>6.8</td>
</tr>
<tr>
<td>1,2</td>
<td>0.07</td>
<td>1.1</td>
<td>8.3</td>
</tr>
<tr>
<td>2,1</td>
<td>0.06</td>
<td>1.1</td>
<td>5.6</td>
</tr>
<tr>
<td>1 x 3</td>
<td>0.11</td>
<td>2.6</td>
<td>48.3</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>0.04</td>
<td>2.2</td>
<td>46.5</td>
</tr>
</tbody>
</table>

The 86 ± 3 (mean ± s.e.) % decline in stubble WSC (mg/plant) between H, and the harvest 3 days after H, was not significantly different (P>0.05) between treatments. Similarly, the stubble DM (0.54 ± 0.07 (mean ± s.e.) g/plant) and tiller number/plant (43 ± 3 (mean ± s.e.) tillers) were not significantly different (P>0.05) between treatments.

At 6 days after H, plants defoliated at the 2 or 3 leaf stages prior to H, (1,2 and 1 x 3) had approximately twice the leaf DM of plants defoliated at the 1-leaf stage prior to H, (3 x 1 and 2,1) (Table 6.2). Averaged across all treatments, the mean content of WSC (mg/plant) in the leaves at 6 days after H, was 200% higher than that in plants defoliated 3 days previously (Table 6.2), with the 1 x 3 leaf treatment being significantly higher (P<0.05) than the 2,1 and 3 x 1 treatments at that time. Stubble WSC (mg/plant) had also begun to rise between 3 and 6 days after H, except in plants defoliated at the 3-leaf stage, although these plants still had the highest WSC levels. Linear regression analysis showed that there was a significant relationship (P=0.03) between stubble WSC (mg/plant) at H, and leaf DM regrowth (g/plant) at 3, but not 6, days after H, as follows:
Leaf DM = 0.051 + 0.000184 Stubble WSC  (Adj r²= 0.92)

6.3.1.2 Regrowth in darkness (etiolated growth). After 7 days in darkness, the regrowth of plants defoliated at the 2- or 3-leaf stage of regrowth prior to H₁ (treatments 1,2 and 1 x 3) had, as for plants kept in light for 6 days, more than twice the leaf DM regrowth of plants defoliated at the 1-leaf stage (Table 6.3).

Table 6.3. Leaf DM (g/plant) after 7 days in darkness, stubble WSC (mg/plant)†, leaf DM (g/plant) from photosynthesis‡, and root DM (g/plant) of plants cut each week, after 4 weeks in darkness, in relation to defoliation treatment (3 x 1 = 3 times at 1 leaf/tiller; 1,2 = once at 1 and once at 2 leaves/tiller; 2,1 = once at 2 and once at 1 leaf/tiller; 1 x 3 = once at 3 leaves/tiller) prior to H₁.

<table>
<thead>
<tr>
<th>Defoliation treatment prior to H₁</th>
<th>Leaf DM (g/plant)</th>
<th>Estimated stubble WSC (mg/plant)</th>
<th>Estimated increase in leaf DM (g/plant) due to photosynthesis one week after H₁</th>
<th>Root DM (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 x 1</td>
<td>0.06</td>
<td>4.55</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>1,2</td>
<td>0.15</td>
<td>5.17</td>
<td>0.27</td>
<td>0.48</td>
</tr>
<tr>
<td>2,1</td>
<td>0.06</td>
<td>4.50</td>
<td>0.10</td>
<td>0.36</td>
</tr>
<tr>
<td>1 x 3</td>
<td>0.12</td>
<td>4.58</td>
<td>0.24</td>
<td>0.86</td>
</tr>
</tbody>
</table>

l.s.d.(P=0.05)* 0.08 N/A N/A 0.58

† derived from leaf WSC (mg/plant).
‡ estimated from the difference between leaf DM at 6 days in light and 7 days in darkness.
* The number of samples for each treatment being unequal, individual l.s.d. values were calculated, and an average obtained.

Lacking sufficient plants for destructive harvest, stubble WSC (mg/plant) at 7 days in darkness was derived from leaf WSC (mg/plant) based on the relationship between these 2
variables in light (see section 3.1). Although estimated stubble WSC levels at 7 days in darkness fell to values which were similar between treatments, the far higher loss of WSC in plants initially high in WSC (133 and 300 mg/plant for plants defoliated at 2 and 3 leaves) as opposed to those low in WSC (48 and 21 mg/plant for plants defoliated at 3 x 1 or 2,1) up to, and including H1, are consistent with their greater regrowth response.

Root DM at the end of 4 weeks in darkness was significantly related (P<0.001) to WSC (mg/plant) in roots (Adj r² = 0.99) at that time and to WSC at H1. Despite this, only the root DM of the 1 x 3 treatment was significantly greater (P<0.05) than the 3 x 1 treatment; corresponding root WSC levels were 3.2 and 0.9 mg/plant, and significantly different (P<0.05).

6.3.1.3 Relative contribution of current photosynthate and plant reserves on regrowth. The relative contribution of current photosynthate to regrowth was derived from the difference between leaf DM regrowth after 7 days in darkness and after 6 days in light (Table 6.3). Thus current photosynthate and WSC plant reserves were estimated to contribute 67% and 33%, respectively, towards regrowth in the first week with the proportions being relatively constant between treatments. The contribution of current photosynthate, in terms of estimated DM, was also related to WSC levels at H1.

6.3.2 Study 2
Study 2 confirmed the short-term regrowth response to defoliation found in study 1, but also followed the longer term response (see Figure 6.1).
Figure 6.1. Leaf regrowth (g DM/plant) of plants defoliated 3 times at 1 leaf/tiller (●), once at 1 and once at 2 leaves/tiller (▼), or once at 3 leaves/tiller (▼). Standard errors are shown as vertical bars.

Thus, at 6 and 12 days after H₁, leaf DM yields (g/plant) differed significantly (P<0.01) between all treatments, and were significantly related (P=0.07) to stubble WSC levels (% of DM) at H₁, according to the following equation for 12 days:

$$\text{Leaf DM}_{12 \text{ days}} = 0.28 + 0.04 \text{ Stubble WSC} \quad (\text{Adj } r^2 = 0.97)$$

After 12 days at approximately the 1-leaf stage of regrowth, plants defoliated at 2 leaves/tiller at H₁ were able to retain a similar growth rate to plants defoliated at 3 leaves/tiller. As a consequence, after 36 days of regrowth, the leaf DM yield of the 1 x 3 and 1,2 leaf stage treatments were not significantly different (P>0.05), but both were significantly higher (P<0.001) than plants defoliated more frequently (3 x 1).
6.4 Discussion

Ryegrass plants defoliated to 50 mm stubble height relied on WSC reserves for the first 3 days of regrowth as evidenced by the huge drain on stubble WSC. In the next 3 days, the emerging leaf must have begun to photosynthesise, causing an increase in leaf WSC and a commencement in replenishment of stubble WSC. This depletion in stubble WSC immediately after regrowth in ryegrass is consistent with previous studies by Davies (1966), Prud’homme et al. (1992) and Fulkerson and Slack (1994b). ¹⁴C tracer studies in ryegrass by Danckwerts and Gordon (1987) confirm that stored non-structural carbohydrates are in fact exported from the stubble to the newly expanding regrowth leaf immediately after defoliation. However, comparison of regrowth in light and darkness, in the present study, has allowed us to estimate that one-third of the regrowth up to 7 days after defoliation was derived from WSC reserves and two-thirds from current photosynthate. Furthermore, the potential to photosynthesise was also related to WSC content of stubble at defoliation.

Comparison of the regrowth response to WSC in light and dark is based on predicting stubble WSC in the dark from leaf WSC in the dark, using a relationship determined in light. Although we have no data that this is valid, it seems a fair assumption as maintenance respiration (hence WSC loss) is a function of temperature (Murata and Iyama 1963; McWilliam 1978), and the temperature in the dark was continually equilibrated with outside temperature. It is also possible that WSC allocation may change as plants are deprived of light; for example, less WSC may be partitioned to root growth, and hence the technique used may underestimate the contribution of current photosynthesis to leaf growth in light. Despite such limitations, there is still merit in the estimations presented.

Reliance on reserves for immediate post-defoliation growth could be expected to be much longer if the plant was unable to effectively photosynthesise. This would be the case with shading (Alberda 1957; Ludlow 1978), either from competing plants in the canopy, or from cloud cover. Alternatively, if the first regrowth leaf was removed by regrazing, subsequent regrowth would be expected to be reduced (Fulkerson 1994) in response to
depleted reserves.

There is evidence of a positive relationship between WSC levels in stubble and regrowth, (Davies 1965; Alberda 1966b) but apparently only if stubble WSC levels are below about 15%. Presumably, once WSC levels are greater than 15%, they have no further positive effect on growth and other factors (e.g. nutrients, water) may then become limiting. This hypothesis is consistent with results of the present studies. For example, in study 1, plants defoliated at the 1-leaf stage at H, had a mean stubble WSC concentration of only 4%, and had consistently lower regrowth than plants defoliated at the 2- and 3-leaf stages at H, with WSC concentrations of 15 and 20%, respectively. This also applied in study 2 up to 12 days regrowth. However if given adequate time to regrow, plants defoliated at 2 leaves/tiller could compensate for an initial low stubble WSC content, with leaf DM yield at 36 days being comparable to plants defoliated at 3 leaves/tiller (3.8 ± 0.3 vs. 4.3 ± 0.2 (mean ± s.e.) g DM/plant, respectively), despite the initially lower WSC concentration (8 vs. 17% of DM, respectively). After 36 days, DM regrowth (2.46 ± 0.18 (mean ± s.e.) g DM/plant) of plants defoliated 3 times at 1 leaf/tiller, remained significantly below that of plants defoliated at the 2- or 3-leaf stages, reflecting the low WSC concentration (1% of DM) of these plants. Hume (1991) has reported a close relationship between WSC concentration in stubble and subsequent regrowth in annual and perennial ryegrass and prairie grass (Bromus unioloides Kunth.) under field conditions, but again WSC% values were all below 15%.

From a pasture management perspective, the 3-leaf stage of the ryegrass regrowth cycle is regarded as the optimum grazing interval, since defoliating at or near this stage allows replenishment of WSC reserves and has been shown to increase both yield and persistence of the pasture compared to defoliating at shorter intervals (Fulkerson et al. 1993b; Chapters 4 and 5), with the 1-leaf stage regarded as the time the plant is most vulnerable to re-defoliation (Fulkerson and Slack 1995).

Lack of persistence of temperate grasses appears to be causally linked to poor root development (Hughes and Jackson 1974; Arcioni et al. 1980, 1985a, b; Fulkerson et al. 1993b; Chapters 4 and 5) and so defoliation practices which impede root development
may jeopardise the long-term persistence of perennial ryegrass pastures in the subtropics. The impact of more frequent defoliation on persistence may be mediated via WSC levels to root growth. This is supported by Evans (1972) who found that depletion of soluble carbohydrates in roots of perennial ryegrass was the main cause of depression of root growth. Frequent defoliation has been shown to retard root growth in the field under both cutting (Chapter 4) and grazing (Chapter 5), with a similar trend in the present study.

Results from this study provide an explanation of why frequent grazing may decrease persistence of perennial ryegrass in the subtropics. Frequent defoliation leads to lower levels of WSC plant reserves and poorer regrowth of leaves and roots, which would put these plants at a disadvantage in competing with other plants in the pasture. The reliance on plant reserves may be particularly relevant during a typical subtropical summer, when frequent cloud cover may act to lower photosynthesis (Evans et al. 1964; Ludlow 1978) and therefore retard the buildup of WSC in the plant (W.J. Fulkerson, unpublished data), while concurrent high temperatures increase energy loss through respiration (Murata and Iyama 1963); it is possible that plants entering this period with low WSC levels would not survive. This may explain the high loss of ryegrass plants observed (Fulkerson et al. 1993b; Fulkerson and Bryant 1994; Chapters 4 and 5) during summer when plants are frequently defoliated in spring.