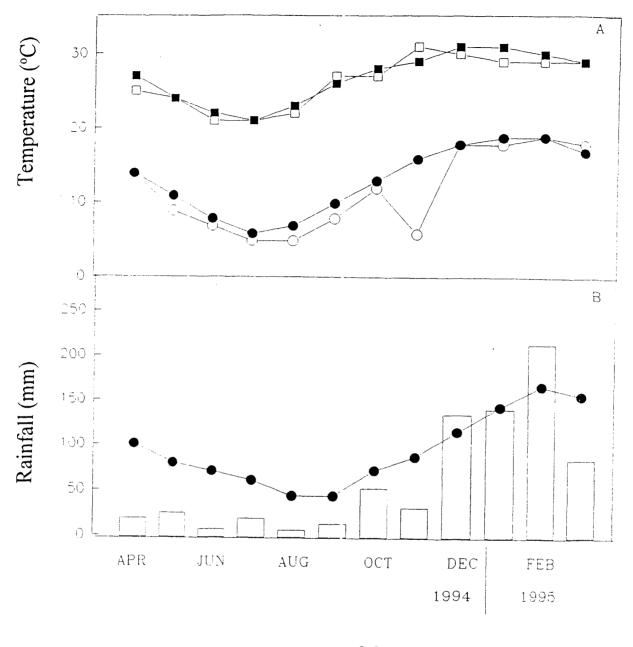
# Chapter 3

## **General methods**

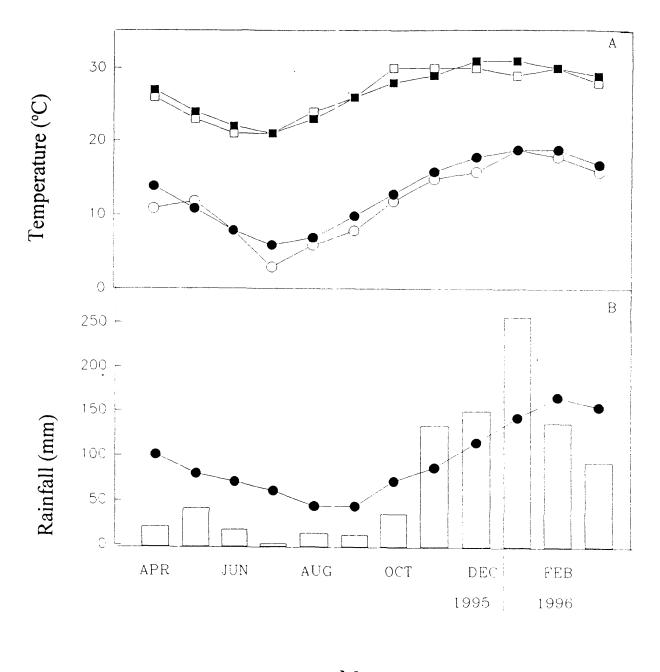
## 3.1 Details of Casino experimental site

The experimental site used for studies reported in Chapters 4, 5, 10, 11 and 12 was located on river flats near Casino, on the north coast of New South Wales (latitude 28°S). The climate is subtropical, with mean minimum and maximum temperatures in the warmest month (February) ranging from 19 to 30°C, and in the coldest month (July) from 7 to 21°C. Mean monthly maximum and minimum temperatures and rainfall over the experimental period, both actual and long-term for Chapters 4, 10 and 11, are shown in Figure 3.1, while those for Chapters 5 and 12 are shown in Figure 3.2.



Month

Figure 3.1. Mean monthly (A) maximum ( $\Box$ ) and minimum ( $\bigcirc$ ) temperatures (°C), and (B) rainfall (mm), for the Casino site. Data are from April 1994 to March 1995 (open), or long-term (132 year) average (shaded).



Month

**Figure 3.2.** Mean monthly (A) maximum ( $\Box$ ) and minimum ( $\bigcirc$ ) temperatures (°C), and (B) rainfall (mm), for the Casino site. Data are from April 1995 to March 1996 (open), or long-term (132 year) average (shaded).

The soil at the Casino site was a heavy clay with an undefined A horizon (classification Ug 5.4, Northcote (1971)). Analysis of the top 75 mm of soil gave pH  $_{(CaCl_2)} = 5.8$ ; phosphorus (Colwell) = 165 ppm; potassium = 1.33 meq/100 g soil; and aluminium = 0.16 meq/100 g soil.

### 3.2 Root DM determination

The root development of ryegrass and clover plants was determined in the field from an 80 mm (diameter) x 200 mm soil core taken to include the root system of a ryegrass or clover plant, and cut into 50 mm vertical sections. After soaking in Calgon® ( $Na_3PO_4$ ) and water for 12 hours, roots were separated from the soil using a Hydroelute Manifold root-soil separation system (Smucker *et al.* 1982; see Plate 5). The separated roots were placed in water, and any foreign organic matter, including roots of other plants, was removed. The entire washing process was completed within 6 hours. Roots were then dried at 80°C in a forced-draught oven for 24 hours to determine DM.

In the glasshouse, ryegrass plants in pots were cut to ground level, and the remaining root system washed free from potting mix as described above.

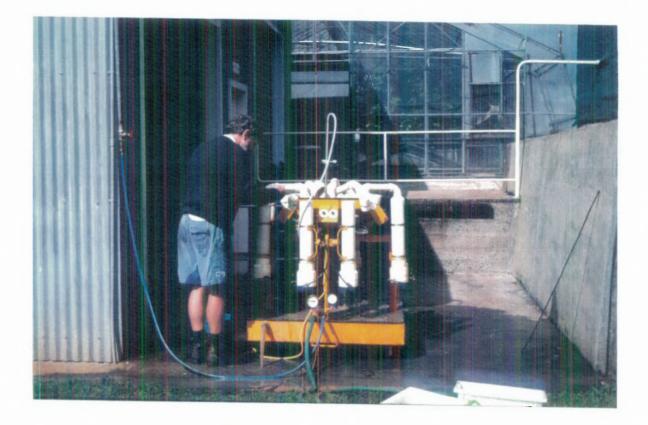


Plate 5. Operation of a Hydroelute Manifold root-soil separation system.

## 3.3 Seed germination tests

Ryegrass and clover seed germination was recorded after one week exposure to a day/night temperature regime of either 35/18, 30/18, 25/12, 20/10 or 15/8°C for 12 hours to determine the extent of dormancy. Day temperatures were set at 5°C intervals, and corresponding night temperatures were determined from historical weather data. Seeds were classified as germinated, dormant or dead, as defined by ISTA protocols (Anon 1985).

The chilling requirement was determined by refrigerating seeds at 5°C for 5 days before exposure to day/night (16 hours day/8 hours night) temperatures of 30/20°C.

## **3.4 WSC determination**

Plant DM was ground through a 1 mm sieve and WSC (% in DM) was determined by cold

extraction of plant material in a reciprocal shaker for one hour using 0.2% benzoic acidwater solution, and the hydrolysation of the cold water carbohydrates to invert sugar by 1 mol/L HCl. This was heated at 90°C, and the sugar dialysed into an alkaline stream of potassium ferricyanide, again heated at 90°C, and then measured using an autoanalyser (420 nm) (Technicon Industrial Method number 302-73A, derived from the method outlined by Smith (1969)).

## 3.5 Fungal isolation

Ryegrass roots or clover stolons were washed under running water, then cut into 10 mm sections. Half of the sections were surface sterilised by submerging in 10% sodium hypochlorite for 3 minutes. All sections were then dipped in sterile water, blotted dry on filter paper, and plated out onto either water agar or one-quarter strength potato-dextrose agar (Johnston and Booth 1983). Agar was checked every 24 hours, and over the next 4 days, fungal colonies observed were re-cultured onto the same agar, allowed to grow out, and then identified under a microscope.

## Chapter 4

## The effect of defoliation on persistence of perennial ryegrass

## 4.1 Introduction

Persistence of perennial ryegrass in the subtropics is closely associated with survival of plants through summer (Fulkerson *et al.* 1993b), hence management practices which enhance survival of ryegrass over summer will increase the persistence of these pastures. Research by Fulkerson *et al.* (1993b) and Fulkerson and Slack (1994a) has indicated that defoliation interval pre-summer has a major impact on ryegrass plant survival over summer. Frequent (2 week intervals), compared to infrequent (3-leaf stage, the actual interval varying from 26 to 51 days), defoliation reduced persistence of perennial ryegrass, possibly by retarding root growth (Fulkerson *et al.* 1993b), and by depleting plant WSC reserves (Fulkerson and Slack 1995). In addition, irrigation of a mixed ryegrass/white clover pasture during a relatively dry summer significantly increased survival and DM production of the perennial ryegrass component of the pasture (Fulkerson and Slack 1994a).

Results from detailed glasshouse studies by Matthew *et al.* (1991) suggested that timing of defoliation of ryegrass in relation to its reproductive phase in spring significantly affects tillering and ultimately persistence in a temperate environment. The premise for this was that defoliation at stem elongation diverts WSC, accumulated in the stems, to initiation of daughter tillers. In the subtropics, where reproductive development in most commercial perennial ryegrass cultivars is minimal (Fulkerson *et al.* 1993a; Lowe and Bowdler 1995), defoliation during the stem elongation phase of growth may have little effect on tillering. Furthermore, initiating daughter tillers in late spring prior to entering the harsh conditions of summer, may be detrimental to both the parent and daughter tillers as the latter are known to be reliant on the former, until 'hey develop their own roots and leaves (Colvill and Marshall 1981).

This study sought to define the timing of defoliation options before summer, and of irrigation management during summer, on survival of perennial ryegrass over summer. Timing of defoliation options was related to various ryegrass plant development stages such as stem elongation, seed set and leaf/tiller stage during the regrowth cycle, to identify the period during which defoliation has the greatest effect on persistence. The extent of seedling recruitment the next autumn was also measured.

## 4.2 Materials and methods

#### 4.2.1 Site

The study was located on river flats near Casino on the north coast of New South Wales, and undertaken between March 1994 and June 1995. For details of soil and climate see Chapter 3, section 1. The pasture examined was perennial ryegrass cv. Yatsyn and white clover cv. Haifa. This Chapter deals predominantly with the ryegrass component of the pasture.

#### 4.2.2 Experimental design

Plots were laid out in a split plot design, with summer irrigation as the main treatment, and defoliation management arranged factorially as subtreatments and replicated 3 times within 2 blocks, with plot size  $2 \times 3 \text{ m}$  (Plate 6).



Plate 6. Field plots of perennial ryegrass/white clover.

The experimental site was sprayed with glyphosate (7 L/ha) to control tropical grasses (on this site, primarily kikuyu, paspalum, summer grass (*Digitaria sanguinalis* (L.) Scop.) and couch (*Cynodon dactylon* (L.) Pers.)), cultivated to a fine seedbed, and sown on 22 March 1994 with 20 kg perennial ryegrass and 5 kg white clover/ha. At sowing, 250 kg molybdenised superphosphate and 100 kg muriate of potash/ha were applied, with 80 kg urea/ha 2 weeks later. Plots received 80 kg urea/ha bi-monthly from May to November 1994. Muriate of potash and superphosphate were applied in September at rates calculated to replace nutrients normally returned through grazing (Fulkerson and Slack 1996a). Superphosphate (without molybdenum) and muriate of potash were again applied in April 1995.

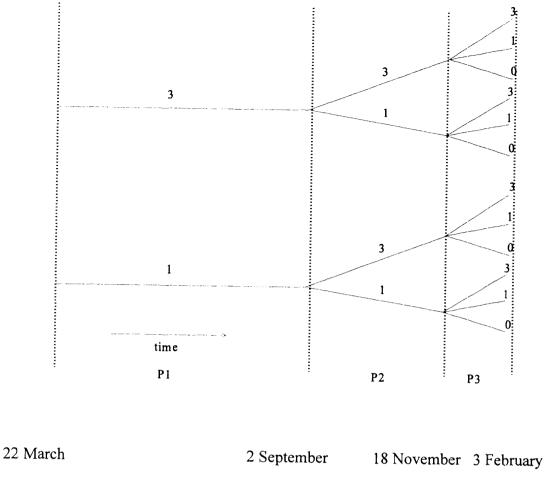
Treatment details were as follows:

*Defoliation interval*: Period 1 (P1) from 22 March 1994 to 2 September (estimated to be two 3 leaf/tiller regrowth cycles - subsequently referred to as regrowth cycles - prior to commencement of stem elongation), plots were defoliated at the 3 or 1 leaf /tiller stage of

regrowth.

Period 2 (P2) from the end of P1 until commencement of stem elongation (18 November) each treatment in P1 subject to defoliation at 3 *or* 1-leaf stages.

Period 3 (P3) from stem elongation until 3 February (2 regrowth cycles after stem elongation), all previous treatments again subject to defoliation at the 3 *or* 1-leaf stages, *or* not defoliated. All plots were defoliated at the 3-leaf stage for the remainder of the experimental period to 15 June 1995. The experimental design in terms of defoliation is shown in Figure 4.1.



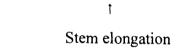


Figure 4.1. Schedule of defoliation treatments for ryegrass pasture, showing defoliation criterion  $(3 = 3 \text{ leaves/tiller}, 1 = 1 \text{ leaf/tiller}, 0 = no defoliation})$  and periods (P1 = 22 March to 2 September 1994, P2 = 3 September to 18 November 1994, P3 = 19 November 1994 to 3 February 1995) of imposition.

*Irrigation:* Irrigation was applied at 20 to 30 mm every 10 to 14 days, except from 30 November to 6 April, when this ceased in half of the plots. This was below the optimal irrigation interval defined by Fulkerson *et al.* (1993a) (see also Chapter 2, section 4.2.3).

#### 4.2.3 Measurements

**4.2.3.1 DM yield and botanical composition.** At harvest, forage was cut to a stubble height of 50 mm with a rotary mower, and weighed. A subsample was taken and dried at  $80^{\circ}$ C in a forced-draught oven for 24 hours to determine total DM yield. Before each 3-leaf harvest, 2 forage samples (100 x 300 mm) were cut at random positions within every plot to harvest height using hand shears, and separated into ryegrass, white clover, tropical grasses and weeds.

**4.2.3.2 Plant density.** The number of individual ryegrass and tropical grass plants per  $0.09 \text{ m}^2$  quadrate, placed at random in 2 locations per plot, was recorded monthly between 8 July and 18 November 1994, then on 6 April 1995.

**4.2.3.3 Seedling recruitment and tiller development.** Between 13 October and 3 March 1995, tiller density was measured monthly from 2 forage samples (100 x 150 mm), cut at random positions within each plot to ground level using hand shears. Twenty five tillers were randomly selected from each sample, and the number of nodes along each tiller recorded to establish the onset and extent of stem elongation. Two randomly selected plants/plot were cut to 50 mm stubble height, then to ground level monthly between 13 October and 31 March 1995, and dried at 80°C in a forced-draught oven for 24 hours, to obtain DM/tiller.

Approximately 10 individual ryegrass tillers were marked with coloured wire loops along an identifiable transect within one plot from each treatment. These tillers were monitored fortnightly from 10 November 1994 to 5 January 1995, then monthly until 14 June 1995. At each monitoring event, tillers were classed as vegetative, reproductive or dead, and the initiation of daughter tillers was also recorded.

**4.2.3.4 Root development.** On 19 November 1994 and again on 3 February and 31 March 1995, a ryegrass plant was chosen at random from one plot in each treatment, and root DM determined as described in Chapter 3, section 2.

**4.2.3.5 Seed germination.** Seed set and retained on unharvested plants was collected in January 1995, and germination was recorded as described in Chapter 3, section 3.

#### 4.2.4 Statistical analyses

Defoliation and irrigation treatment effects on plant density, plot yield and quality parameters were analysed by fitting mixed linear models (Searle 1971) which consisted of terms representing effects of direct interest (fixed effects) and terms not of direct interest (random effects). The models were fitted using restricted maximum likelihood estimation (Patterson and Thompson 1971) in the Genstat 5 statistical package (Genstat Committee 1993).

Spatial variation in the field was accounted for by including random block and column within block terms in each model, leading to an incomplete block analysis of the data. Fixed terms in the models were changed depending on the period of measurement. For P1, only the 2-level first defoliation treatment was included. Models for data from P2 included terms for the P1 and P2 defoliation treatments main effects and interaction. Models for data from P3 included terms for the P1, P2 and P3 defoliation treatments along with irrigation effects. All interactions between treatments up to the 3-way interactions were included in the models for the P3 data.

Significance tests for the fixed effects were carried out using Wald statistics (Genstat Committee 1993). Generalised least squares treatment means and standard errors (s.e.) were predicted from each model.

### 4.3 Results

Defoliation treatments during P2 had a significant effect (P<0.05) only on DM/tiller at stem elongation and on DM yield during P3, and so was taken out of the analysis for other treatment effects. Irrigation had an effect only on pasture DM yield.

#### 4.3.1 Plant density

Plant density at stem elongation was not significantly affected (P>0.05) by defoliation treatment during P1, with a mean of  $169 \pm 6$  (mean  $\pm$  s.e.) ryegrass, and  $14 \pm 1$  (mean  $\pm$  s.e.) tropical grass plants/m<sup>2</sup>. However, defoliating at 3 leaves/tiller resulted in significantly higher (P<0.01) ryegrass plant densities and significantly lower (P<0.01) tropical grass plant densities the following autumn, than more frequent defoliation (Table 4.1).

**Table 4.1.** Density (plants/m<sup>2</sup>) of ryegrass and tropical grass plants in autumn of year 2, as affected by defoliation treatment (where 3 = 3 leaves/tiller, 1 = 1 leaf/tiller, and 0 = no further defoliation) during P1/P3 in the previous year. Within columns, means with different superscripts are significantly different at P<0.05.

Defoliation treatment	Plant density (plants/m <sup>2</sup> )		
	Ryegrass	Tropical grass	
3/3	69 <sup>b</sup>	46 <sup>cd</sup>	
3/1	110ª	40 <sup>d</sup>	
3/0	44 <sup>c</sup>	53 <sup>bcd</sup>	
1/3	58°	67 <sup>ab</sup>	
1/1	63°	52 <sup>bcd</sup>	
1/0	42°	61 <sup>abc</sup>	

In contrast, ryegrass plant density was decreased by 26% when defoliated at 3 leaves/tiller during P3, and by 49% if defoliation ceased altogether, compared with defoliating at 1 leaf/tiller. Defoliation treatments during P3 had no significant effect (P>0.05) on tropical grass plant density the following autumn.

#### 4.3.2 Tiller dynamics

As with plant density, tiller density at stem elongation  $(5,023 \pm 129 \text{ (mean } \pm \text{ s.e.}) \text{ tillers/m}^2)$  was not significantly affected (P>0.05) by defoliation treatment up to that

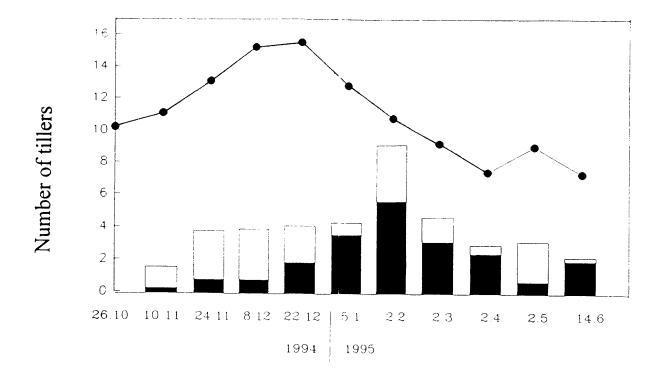
time, but DM per tiller was significantly reduced (P<0.01) by frequent defoliation during P2 (31 vs. 38 mg DM/tiller for plants defoliated at 1 or 3 leaves/tiller, respectively).

Tiller density in autumn was highest in plants infrequently defoliated during P1, then frequently defoliated during P3, while DM per tiller was lowest (P<0.01) when defoliation ceased during P3 (Table 4.2)

**Table 4.2.** Ryegrass tiller density (tillers/m<sup>2</sup>) and DM/tiller (mg) in autumn of year 2, and number of daughter and dead tillers to June of year 2 (per original tiller marked on 26 October 1994), following various defoliation treatments (where 3 = 3 leaves/tiller, 1 = 1 leaf/tiller, and 0 = no further defoliation) during P1/P3 in the previous year. Means with different superscripts within columns are significantly different at P<0.05.

Defoliation treatment	Tiller density/m <sup>2</sup>	DM/tiller (mg)	Tiller number/original tiller		
			Daughter tillers	Dead tillers	
3/3	2,437 <sup>ac</sup>	32	2.1	2.6	
3/1	2,591ª	32"	2.8	2.2	
3/0	$1,442^{bc}$	24 <sup>1</sup>	1.5	2.2	
1/3	1,359 <sup>b</sup>	31 <sup>ab</sup>	1.1	1.5	
1/1	1,579 <sup>ab</sup>	29 <sup>ab</sup>	1.3	1.5	
1/0	1,196 <sup>b</sup>	24 <sup>b</sup>	1.4	1.9	

The tiller density was a reflection of daughter tiller initiation and death observed in marked plants. The '3/1' defoliation treatment had the highest tiller density and was also the only treatment in which tiller initiation exceeded death. While defoliation treatment affected the magnitude of tiller initiation and death, the pattern of change over time was similar for all treatments, and hence mean values are shown in Figure 4.2.



## Date

**Figure 4.2.** Total number of live tillers (original marked tillers and daughters) ( $\bigcirc$ ), number of daughter tillers initiated ( $\square$ ) and number of dead tillers ( $\blacksquare$ ), from 26 October 1994 to 14 June 1995.

Continuing to irrigate over summer decreased tiller initiation by 38%, but had no effect on tiller death, with the result that tiller density in autumn was 21% lower (1,527 vs. 1,941 tillers/m<sup>2</sup>; P=0.08) in irrigated than in unirrigated plots, respectively.

#### 4.3.3 Seed set and seedling recruitment

There was a mean of 0.67 seedlings  $m^2$  recruited over all plots in year 2, and no treatment effects were detected. At high temperature (greater than 30°C)  $11 \pm 2$  (mean  $\pm$  s.e.) % of ryegrass seeds were dormant. Chilling did not improve percentage germination of seeds.

#### 4.3.4 Root DM

The root DM in the top 50 mm of soil accounted for  $82 \pm 1$  (mean  $\pm$  s.e.) % of total root DM. Although defoliation had no significant effect (P>0.05) on total root DM at stem

elongation (2.51  $\pm$  0.17 (mean  $\pm$  s.e.) g/plant), plants defoliated at 3 leaves/tiller in P1 had twice the root DM (P=0.09) at 150 to 200 mm soil depth, as plants cut at 1 leaf/tiller. By February, plants defoliated continually at 3 leaves/tiller in P1/P3 had significantly more (P<0.05) root DM in the top 50 mm of soil than plants subjected to 1/3 or 1/1 defoliations only. Main effect means for P1 showed that root DM of plants defoliated at 3 leaves/tiller (Table 4.3).

**Table 4.3**. Ryegrass root DM (g/plant) in the top 50 mm of soil in February 1995, following various defoliation treatments (where 3 = 3 leaves/tiller, 1 = 1 leaf/tiller, and 0 = no further defoliation) during P1/P3 in the previous year. Means with different superscripts are significantly different at P<0.05.

Defoliation treatment	Root DM (g/plant)	
3/3	3.54ª	
3/1	2.99 <sup>ab</sup>	
3/0	2.56 <sup>ab</sup>	
1/3	1.70 <sup>b</sup>	
1/1	1.50 <sup>b</sup>	
1/0	$1.78^{ab}$	

Total root DM decreased by 76% over the summer to  $0.61 \pm 0.01$  (mean  $\pm$  s.e.) g/plant in autumn; the difference between treatments was not significant (P>0.05).

#### 4.3.5 DM yield

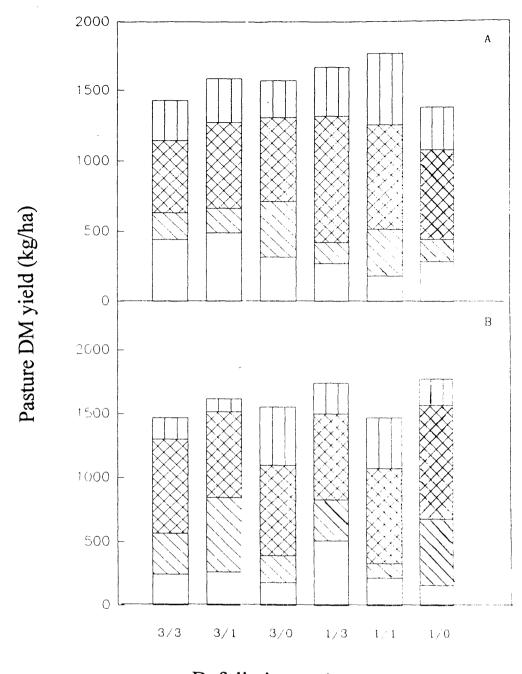
The effect of defoliation over the full experimental period (22 March 1994 to 15 June 1995) shows that infrequent defoliation during P1 significantly increased ryegrass DM and decreased tropical grass DM compared to frequent defoliation. However, during P3, infrequent defoliation actually decreased ryegrass DM (Table 4.4).

**Table 4.4.** The effect of main defoliat on treatments (where 3 = 3 leaves/tiller, 1 = 1 leaf/tiller, and 0 = no further defoliation) during P1, P2 and P3 on yield of pasture components (kg DM/ha) from 6 June 1994 to 15 June 1995. Means with different superscripts within species type and periods are significantly different at P<0.05, P<0.01 (\*), or P<0.001 (\*\*).

Defoliation treatment (leaves/tiller)		Yield (kg DM/ha)				
		Ryegrass	Clover	Tropical grass	Weed	
P1	1	5,520 <sup>a</sup> **	2,044 <sup>a</sup> *	2,015 <sup>a</sup>	1,159	
	3	7,120 <sup>b</sup>	1,792 <sup>b</sup>	1,806 <sup>b</sup>	1,153	
P2 1 3	1	6,074ª	1,593***	2,179 <sup>a</sup> **	1,201ª	
	3	6,566 <sup>b</sup>	2,243 <sup>b</sup>	1,642 <sup>b</sup>	1,111 <sup>b</sup>	
Р3	1	6,778 <sup>a</sup> *	1,909	1,640 <sup>a</sup> **	1,206 <sup>a</sup> *	
	3	6,249 <sup>b</sup>	1,984	1,909 <sup>b</sup>	1,051 <sup>b</sup>	
	0	5,934 <sup>b</sup>	1,862	2,183°	1,210ª	

Over the summer (stem elongation to April 1995), irrigation had no significant effect (P>0.05) on ryegrass or clover DM yield, but increased tropical grass yield by 35% (1,364 vs. 1,008 kg DM/ha; P<0.05) and weed yield by 54% (861 vs. 559 kg DM/ha; P<0.01), compared to not irrigating.

The DM yield of ryegrass over the auturn of year 2 (Figure 4.3) reflected plant and tiller densities in April, with the exception of defoliation treatment 1/3 (P1/P3).



Defoliation regime in P1/P3

**Figure 4.3.** Yield (kg DM/ha) of ryegrass ( $\Box$ ), white clover ( $\boxtimes$ ), tropical grasses ( $\boxtimes$ ) and weeds ( $\blacksquare$ ) from April to June in year 2, in plots (A) irrigated or (B) not irrigated over the summer, and defoliated during P1/P3; 3 = 3 leaves/tiller, 1 = leaf/tiller, 0 = no further defoliation.

The interaction between defoliation and irrigation was significant (P<0.001) for DM yield of all pasture components (Figure 4.3). Generally, an increase in tropical grass DM

yield under any particular treatment was at the expense of ryegrass DM yield, and viceversa.

### 4.4 Discussion

A lack of vernalisation in the subtropical environment meant that little seed was set and hence seedling recruitment was minimal. As a consequence, production of perennial ryegrass pastures in year 2 was reliant almost exclusively on individual plants surviving the summer.

Defoliation frequency had a variable effect on survival of ryegrass plants through summer. Compared to defoliating frequently at the 1-leaf stage, infrequent defoliation at the 3-leaf stage of regrowth from March to September (P1), led to 33% more ryegrass plants surviving summer and 23% less tropical grass plants invading the plots. This is consistent with previous studies, in which infrequent defoliation (Fulkerson et al. 1993b; Fulkerson and Bryant 1994) over the entire growing season, improved persistence of perennial ryegrass. Similarly in South Africa, McKenzie (1996) found an increase in vigour of perennial ryegrass tillers as defoliation interval increased from 3.5 (continuous grazing) to 28 days, also in a subtropical environment. The greater colonisation of swards by tropical grasses in plots frequently defoliated in P1 is again consistent with results of Fulkerson et al. (1993b), who found a significant negative relationship between ryegrass and tropical grass plant density. Field observations suggest that the tropical grasses colonise bare soil left by the death of ryegrass plants in response to more light penetrating into a more frequently-cut sward, which would stimulate germination of This mechanism also appears in temperate climates, with 'hard' grazing seed. (defoliated to 30 mm stubble height each 14 days) in spring increasing the paspalum component in a ryegrass/paspalum pasture in New Zealand (Percival and McClintock 1982).

The effect of frequent defoliation in winter on plant survival over summer appears to be, in part, due to suppression of root growth, with total root DM tending to be lower in November (2.4 vs. 2.6 g DM/plant) and substantially lower in February (2.1 vs. 3.5 g DM/plant), compared to less frequent defoliation. This is consistent with results of Evans (1972) who found the effect of defoliation on weakening the root system of perennial ryegrass to be cumulative. Furthermore, the loss of plants *under grazing*, rather than cutting as in the present study, would be expected to be greater as the weakened root system might increase sod pulling.

Frequent defoliation during P1 increased tiller density in spring, compared to infrequent defoliation (5,233 vs. 4,813 tillers/m<sup>2</sup>, respectively) presumably due to a more favourable light environment at the base of the canopy (Colvill and Marshall 1984). However these frequently-defoliated tillers were smaller, and less survived to the following autumn (1,373 vs. 2,123 tillers/m<sup>2</sup>, respectively), possibly due to a lower stubble WSC level (Alberda 1957, 1966b; Colvill and Marshall 1984). This has also been observed by McKenzie (1996) in a subtropical environment.

Surprisingly, defoliation treatment from 3 September to 18 November (P2) had no effect on plant or tiller survival. Matthew et al. (1991) have previously shown in glasshouse studies, that defoliating ryegrass plants at the late stem elongation to early seed-head stage increased daughter tiller initiation. The premise for this is that WSC reserves, accumulated in elongating stems of the reproductive tiller, are made available to initiate daughter tillers if these reproductive tillers are defoliated below the elevated growing point and hence die. Colvill and Marshall (1984) claimed that perennation of perennial ryegrass grown in field plots in the United Kingdom depends upon survival of tillers formed during or after flowering. However in the subtropics, this period (early December) is characterised by an increase in temperature to levels above optimum for ryegrass growth (see Chapter 2, section 1.2). Since the smallest tillers are sacrificed in favour of parent tiller survival when perennial ryegrass is stressed (Ong 1978), there may be no benefit in a flush of young tillers following flowering in the subtropics. In addition, there were few reproductive tillers under the conditions of the current study (3% with more than 4 nodes or seed-heads), and overall this would have had an insignificant effect on tiller initiation.

In contrast to the situation in P1, infrequent defoliation in P3, from 19 November to 3 February, decreased perennial ryegrass survival over summer but had no effect on tropical grass plant density. This effect is believed to have been indirect through shading of ryegrass plants by tropical grasses and weeds and/or through maintaining a dense canopy conducive to infestation with leaf rust.

Although other factors could be involved, the assumption that shading of ryegrass by tropical grasses decreased its survival is supported by regression analysis which showed a significant negative relationship (P<0.001) between ryegrass plant density (plants/m<sup>2</sup>) in autumn, and both tropical grass plant density (plants/m<sup>2</sup>) in autumn (TGD) and DM yield (kg DM/ha) of tropical grass (TGDM) over summer as shown below:

Ryegrass plant density = 137 - 0.55 TGD - 0.03 TGDM (Adj r<sup>2</sup> = 0.37)

Studies in New Zealand by Thom *et al.* (1986) also confirm the importance of shading on plant survival in a tropical/temperate grass association. They prevented paspalum from shading neighbouring ryegrass plants by clipping potentially shading leaves of paspalum in early summer, which led to a 230 to 300% increase in ryegrass tillers/plant and a 3-fold reduction in plant deaths over summer. The beneficial effect of clipping on ryegrass could also be partly due to decreased root competition from paspalum, brought about by more frequent defoliation.

The pasture in the present study was visibly affected by leaf rust during P3; this was presumably implicated in the favourable response to frequent defoliation at that time of year. In a temperate environment, Lancashire (1974) showed that rust infestation in a perennial ryegrass/white clover pasture decreased ryegrass DM yield by up to 15% under a 10-day grazing rotation, and by up to 45% under a 40-day rotation. However, if rust was negligible, the longer grazing interval gave a 150% higher DM yield than at 10-day intervals. Likewise, in a rust-free ryegrass/paspalum pasture, Thom *et al.* (1986) found that short grazing intervals (14 days) over late spring/early summer led to a rise in ryegrass plant deaths over summer. These studies support the hypothesis that increased frequency of defoliation will be beneficial only if it reduces rust infestation or prevents

tropical grasses shading ryegrass.

In the present study, irrigation over summer had no effect on perennial ryegrass DM yield or plant density, but there was a significant increase in yield of tropical grasses and weeds, indicating that the sub-optimal irrigation imposed only benefited these deeperrooted and more summer-active species. Also, the large rainfall events in mid-summer left irrigated plots waterlogged for several weeks, to the detriment of the shallow-rooted ryegrass and clover. The high probability of large rainfall events during the subtropical summer is the main reason farmers are cautious of irrigating in summer. Lowe and Bowdler (1984) obtained similar small, but variable, yield responses (5 to 13%) by reducing irrigation interval during summer from between 14 to 20 days, to around 7 to 10 days, with the highest responses coming in dry years. However, the shortest intervals imposed were still far too long based on present recommendations which take evapotranspiration and effective rooting zone into consideration (Fulkerson et al. 1993a). In confirmation of this, Fulkerson and Slack (1994a) obtained higher yield responses to summer irrigation under drier conditions than in the current study (380 vs. 615 mm rainfall from November to March, respectively). Ryegrass/white clover yield was increased by 95% over summer and by 68% in autumn/winter.

The current study confirms the impact of pre-summer defoliation on survival of ryegrass over summer and identifies the specific time when defoliation is most important. Thus, defoliation of perennial ryegrass at the 3 leaf/tiller stage of regrowth in winter to early spring improves ryegrass plant survival through summer and lowers the influx of tropical grasses. More frequent defoliation at this time probably weakens ryegrass plants, letting more light into the canopy and allowing tropical grasses to establish. Frequent defoliation at this time is associated with reduced root DM of ryegrass in summer and this would be expected to decrease persistence. Conversely, defoliation frequency in spring immediately prior to stem elongation appears relatively unimportant for survival of ryegrass. After stem elongation, frequent defoliation increased ryegrass plant survival due probably to reduced effects of rust infestation, and less competition (shading) from the tropical grasses (due to more frequent removal of shading leaves).

The results indicate that irrigation of ryegrass over summer in situations prone to waterlogging, will only be of benefit in dry years and if scheduling is frequent enough to benefit ryegrass rather than tropical grass.