Chapter 7

A priority for allocation of WSC in regrowth of perennial ryegrass following defoliation

7.1 Introduction

Studies outlined in Chapter 6 with perennial ryegrass showed a significant positive linear relationship between stubble WSC at defoliation, and subsequent regrowth. This relationship held for WSC expressed as a concentration (% of DM) or total amount (mg/plant), generally being stronger for the latter, and this confirms earlier studies of Davies (1966) and Fulkerson and Slack (1995). In a glasshouse study with timothy, Smith and Jewiss (1966) concluded that the content (weight) of WSC in stubble was more important that the concentration (%) of WSC, as an indication of the total energy available to the plant in periods of stress, again reflecting the increased importance of total reserves per plant. Research by Davies (1965) and Alberda (1966b) indicated that while regrowth of perennial ryegrass declined with decreasing stubble WSC *concentrations* below about 15%, there was no further increase in regrowth when WSC concentrations were much above this 'critical' level.

In a survival context, it seems logical for re-establishment of the photosynthetic canopy after defoliation to assume a higher priority than root growth or daughter tiller initiation and development, and it then follows that preferential allocation of WSC may exist in regrowth. If this hypothesis holds, the needs of the strongest sink (leaf) will be met while secondary sinks (roots, tillers) will be deprived of reserves. Tracer studies using ^{14}C in perennial ryegrass (Danckwerts and Gordon 1987), biennial ryegrass (Marshall and Sagar 1965; Gifford and Marshall 1973), fescue (Johansson 1993) and barley (Ryle and Powell 1975) indicated that defoliation induces an increase in WSC allocated to initiate and maintain leaf regrowth, at the expense of WSC allocated to initiate and maintain root and tiller growth, and to replenish reserves in the stubble.

In further support of the hypothesis that tillers and roots assume a lower priority for WSC allocation is the observation by Ong and Marshall (1979) that in partially-shaded plants, unshaded tillers supplied shaded tillers with WSC, but when the whole plant was shaded, the smallest tillers actually died, as export of WSC from larger tillers ceased. Ong (1978) also found that the smallest tillers were first to die when plants were subject to either shading or nutrient stress. Furthermore, Jacques and Edmond (1952), when studying the effects of defoliation on perennial ryegrass and cocksfoot, found that as frequency and/or closeness of defoliation increased, so too did the tendency for plants to produce new leaves before root growth resumed.

If leaf regrowth takes priority over root growth, it may explain the mortality of ryegrass plants over summer when the WSC reserves may be critically low due to high temperature and cloud cover, particularly when defoliation has been frequent.

Although these studies have indicated that a preferential allocation of reserves may exist in grasses, no effort has been made to quantify this by observing the sequential regrowth of leaves and roots, and the initiation of new tillers following defoliation, in relation to reserve levels.

The aim of this study was to determine the priority of the ryegrass plant for leaf and root growth and daughter tiller initiation after defoliation, in relation to level of WSC reserves at defoliation. These variable levels of stubble WSC reserves were achieved by varying the defoliation interval (Davies 1966) and ambient temperature (Alberda 1957) before defoliation, and the harvest height at defoliation (Fulkerson and Slack 1995).

7.2 Materials and methods

7.2.1 Site

The experiment was conducted in 2 chambers in a controlled-temperature glasshouse under natural light between February and July 1997 at the Wollongbar Agricultural Institute, New South Wales. In each chamber, perennial ryegrass cv. Dobson was sown

into one wooden and 4 glass boxes containing potting mixture comprising 60% composted sawdust, 30% compost and 10% sand. The wooden box, measuring 1100 mm x 1100 mm x 600 mm deep, was separated by wooden partitions into 8 compartments (100 mm x 1100 mm x 600 mm deep) and the 4 glass boxes, 100 mm x 1500 mm x 600 mm depth, were mounted in parallel on a frame so that each box could be separated to observe root extension and be held at a 25° angle to the vertical. When the glass boxes were pushed together they formed a minisward of 4 x 15 ryegrass plants at a density of 100 plants/m^2, 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). The wooden box formed a similar minisward to that in the glass boxes; the plants in the wooden boxes provided the largest proportion of plants harvested during the regrowth cycle. At a 25° angle, the geotropic growth of the roots along the walls of the glass boxes made them easy to monitor (Hodgkinson and Baas Becking 1977). When observations were not being made, the outsides of the glass boxes were covered with sheets of reflective aluminium foil to exclude light. After germination, plants were fertilised each 2 weeks with Aquasol® liquid fertiliser (Hortico Australia Pty. Ltd.), to ensure adequate nutrient availability, and watered twice daily to replace evapotranspiration losses. One of the glasshouse chambers is shown in Plate 9.

Plate 9. One of the 2 chambers in the glasshouse, showing plants grown in glass (covered with reflective aluminium foil) and wooden boxes.

Plants were defoliated to a stubble height of 50 mm approximately 8 weeks after sowing, to promote tillering. When plants had grown to about 20 tillers/plant (approximately 12 weeks from sowing), all plants were again defoliated (termed H_0) to a stubble height of 50 mm. All non-sheath leaf tissue was removed, and the DM yield/plant of leaf material was determined at this and at all subsequent harvests by drying samples at 80°C in a forced-draught oven for 24 hours. Leaf DM and tiller number/plant at H_0 were used for subsequent analysis of covariance.

7.2.2 Experimental design

Treatments began after H_0 within each glasshouse chamber. Defoliation interval was the main treatment, after which defoliation height was imposed as a subtreatment, factorially arranged and randomly allocated into 3 blocks within the glass boxes and 2 blocks within the wooden box. After H_0, half of the plants in each of the glass and wooden boxes in each chamber were defoliated on each of 3 occasions at the 1-leaf stage, each Li being approximately 9 days under the 25/15°C day/night temperature regime imposed. The other

half of the plants were defoliated once at the 3-leaf stage of regrowth (termed H_1) which coincided with the final harvest of the first group. Defoliation at all times was to a stubble height of 50 mm.

One week prior to H_1, the night temperature in one chamber was reduced to 8°C, and that in the other chamber raised to 20°C; day temperatures remained unchanged. At H_1, leaf DM above the 50 mm stubble height was obtained from all plants; 6 plants per defoliation interval treatment were selected at random from the wooden box in each chamber and cut to ground level to obtain DM and WSC of the stubble. Half the plants in each of the defoliation interval groups in each chamber were further defoliated to a stubble height of 20 mm, and this stubble dried to determine DM and then analysed for WSC. This harvest and all subsequent harvests involving WSC analysis were performed approximately 3 hours after sunrise because of the known diurnal variation in WSC content (Fulkerson *et al.* 1994). Stubble samples were immediately packed on ice for transportation to a freezer, and following 24 hours in a freezer, were freeze-dried.

Thus, there were 8 treatments (2 night temperatures by 2 defoliation intervals by 2 defoliation heights), and a total of 35 plants/treatment - 15 grown in glass boxes and 20 in wooden boxes.

Four, 6, 8, 12 and 18 days following H_1, 3 plants per wooden box and one plant per glass box from each defoliation interval by height treatment were likewise destructively harvested. All remaining plants (12/treatment) were harvested to ground level 27 days (approximately the 3-leaf stage of regrowth) after H_1. At each of these harvests, those plants which had been defoliated to 20 mm height at H_1 were first defoliated to 50 mm along with all other plants, then further defoliated to 20 mm, and DM and WSC obtained from both 0 to 20 mm and 20 to 50 mm stubble fractions. The number of tillers/plant was counted at each destructive harvest.

7.2.3 Leaf and root extension

At H_1, one tiller from each of 10 plants per defoliation interval by height treatment in the glass boxes, and 2 plants/treatment in the wooden boxes were marked with a coloured wire

loop. Leaf elongation rate was measured on these marked tillers every 2 days from the first day following H_1 using a ruler and with minimum disturbance of the plant. On the side of the glass boxes at H_1, a wax pencil was used to mark the position of 20 roots/treatment, and root length recorded from the pencil line each day over the next 10 days.

7.2.4 Tiller dynamics

At H_1, one tiller was marked in the same manner as for leaf elongation, and its status (live -with or without a daughter tiller, or dead) monitored every second day for 24 days. Also at H_1, young daughter tillers less than 10 mm long (measured from the leaf axil of the parent tiller), were marked with wire loops, and their survival monitored daily for 12 days.

7.2.5 WSC determination

The WSC levels of ryegrass stubble were determined using the procedure described in Chapter 3, section 4.

7.2.6 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 yield and tiller number/plant at H_0 was used as a covariate factor for analysis of covariance of yields between H_0 and H_1, and tiller number/plant at H_1. The relationships between WSC at H_1 and all other variables were determined by regression analysis using TableCurve™ (Anon 1992b).

The assumption that plant growth would be similar in both wooden and glass boxes was verified by statistical analyses which found no significant difference ($P>0.05$) within treatments between tiller number/plant at H_1, or leaf DM or stubble WSC during the regrowth period following H_1.

7.3 Results

7.3.1 Effect of pre-treatment on WSC

More frequent defoliation prior to H_1 resulted in a significantly lower ($P<0.01$) stubble WSC (mg/tiller), and WSC% ($P<0.001$) at H_1, than the less frequent defoliation regimes. The interaction between defoliation interval and height was significant for WSC% ($P<0.05$) and mg/tiller ($P=0.09$) at H_1 (Table 7.1).

Table 7.1. Stubble WSC concentration (%) and content (mg/tiller) at H_1 following defoliation at 3 (3) or 1 (1) leaf/tiller, and to $50_{(50)}$ or $20_{(20)}$ mm stubble height.

Defoliation treatment	WSC	
	%	mg/tiller
3_{50}	12.5	1.21
3_{20}	8.5	0.39
1_{50}	1.5	0.07
1_{20}	2.6	0.09
l.s.d. (P=0.05)	*2.3*	*0.53*

The combined effect of defoliation frequency and height treatments resulted in an 8-fold difference in WSC%, and a 17-fold difference in WSC (mg/tiller), with WSC decreasing with more frequent and close defoliation. The change in night temperature significantly altered ($P<0.01$) WSC% at H_1, but only in plants defoliated at 3 leaves/tiller. At H_1, plants subject to low night temperature (8°C) contained 14.5% WSC, while plants subject to high night temperature (20°C) contained 6.5% WSC. There was no significant effect ($P>0.05$) of night temperature on WSC (mg/tiller) at H_1, or on any regrowth parameter thereafter.

After 27 days of regrowth, plants defoliated to 20 mm stubble height at H_1 had significantly less ($P<0.001$) WSC (mg/tiller) than plants defoliated to 50 mm for both 1 and 3 leaf defoliations prior to H_1 (Figure 7.1).

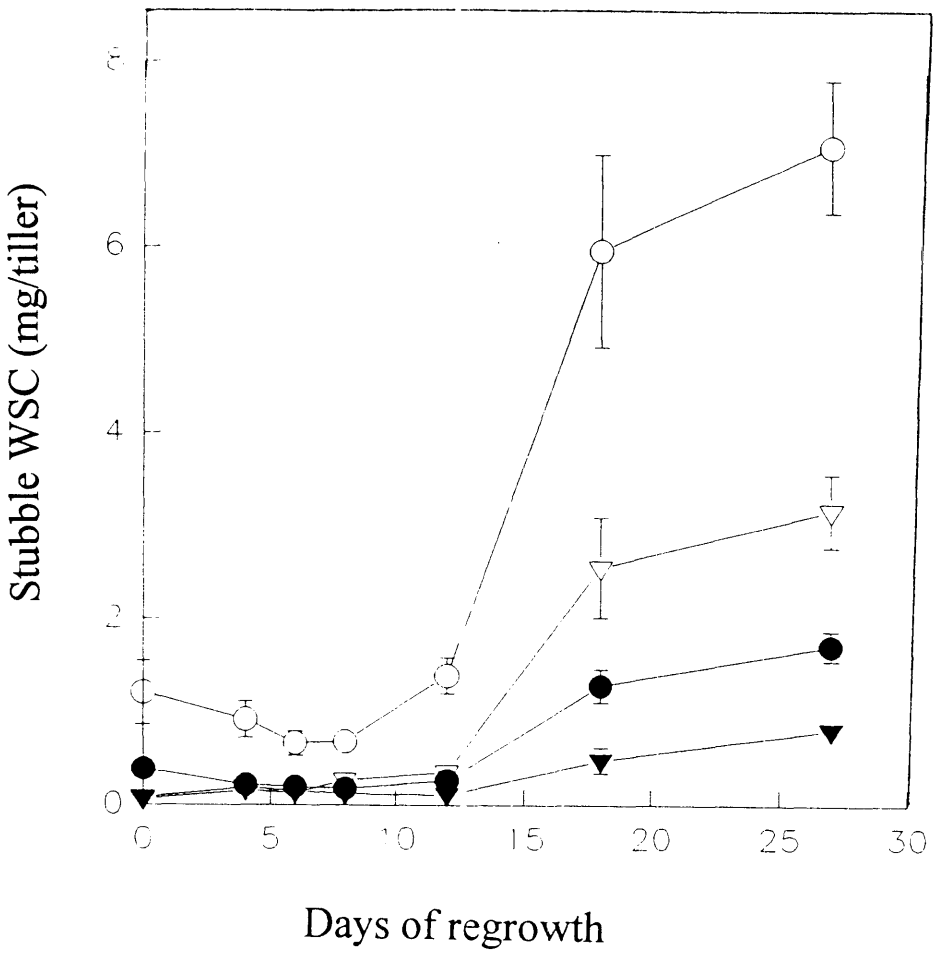

Figure 7.1. The pattern of stubble WSC (mg/tiller) depletion and replenishment over the 27 days of regrowth from H_1, following defoliation at 3 (○) or 1 (▽) leaf/tiller, and to 50 (open) or 20 (shaded) mm stubble height. Standard errors are shown as vertical bars.

Plants defoliated at 3 leaves/tiller to 50 mm (3_{50} treatment) also began to replenish their stubble WSC levels earlier, with an increase in WSC occurring between days 8 and 12 from H_1, compared to after 12 days for all other treatments.

7.3.2 Tiller dynamics

At H_1, plants had a mean (± s.e.) of 31 ± 0.9 tillers/plant. After 27 days of regrowth, plants defoliated at 3 leaves/tiller prior to H_1 had significantly more ($P<0.001$) tillers/plant than plants defoliated more frequently (73 vs. 37 tillers/plant, respectively). The larger increase in tiller number under 3-leaf defoliation was due partly to greater initiation of daughter tillers (1.2 vs. 0.4 daughter tillers initiated per parent tiller, respectively), and

partly to fewer deaths among the daughter tillers. In this regard, only 62 ± 2 (mean ± s.e.) % of daughter tillers marked at H_1 survived to 27 days of regrowth in plants defoliated at 1 leaf/tiller to 20 mm (1_{20} treatment), compared to a mean (± s.e.) of 98 ± 2 % for all other treatments.

The mean time to initiation of the first daughter tiller was 10 days earlier in plants defoliated at 3, compared to 1, leaf/tiller (5.5 ± 0.5 (mean ± s.e.) vs. 16 ± 1 days from H_1). The number of days to initiation of the first daughter tiller was significantly related ($P<0.01$) to stubble WSC (mg/tiller) at H_1 (SWSC), as follows:

$$\text{Number of days} = 6.9 + 71\ (\text{SWSC})^2 - 50\ e^{\text{SWSC}} \quad (\text{Adj } r^2 = 0.69)$$

Site filling (number of daughter tillers which emerge per Li) was significantly higher ($P<0.001$) in plants defoliated at 3 leaves/tiller, than in plants defoliated more frequently (54 vs. 16%, respectively).

The number of daughter tillers initiated per marked tiller over the regrowth period, and % site filling were both significantly related ($P<0.01$) to stubble WSC (mg/tiller) at H_1, as follows:

$$\text{Number of daughter tillers/tiller} = 0.46 + 0.77\ \text{SWSC} \quad (\text{Adj } r^2 = 0.69)$$

$$\%\ \text{site filling} = 19 + 35\ \text{SWSC} \quad (\text{Adj } r^2 = 0.64)$$

Daughter tiller survival was not related to stubble WSC at H_1.

DM/tiller at H_1 was significantly greater ($P<0.05$) in plants subject to 3_{50} defoliation than all other treatments, and after 27 days of regrowth, was still significantly higher ($P<0.001$) in these plants (Table 7.2).

Table 7.2. Tiller DM/tiller (mg) at H_1 and after 27 days of regrowth, mean leaf extension rate (mm/day) over the initial 11 days of regrowth, and leaf DM (g/plant) 27 days after H_1, following defoliation at 3 (3) or 1 (1) leaf/ tiller, to 50 ($_{50}$) or 20 ($_{20}$) mm stubble height.

Defoliation treatment	Tiller DM (mg/tiller) at:		Leaf extension rate	Leaf DM 27 days after H_1
	H_1	27 days after H_1	(mm/day)	(g/plant)
3_{50}	10	20	21	2.2
3_{20}	5	6	16	2.0
1_{50}	4	13	18	1.0
1_{20}	3	4	10	0.7
l.s.d.(P=0.05)	*3*	*2*	*2*	*0.4*

A significant interaction (P<0.001) between defoliation heights and regrowth was observed in respect of tiller DM. The tiller DM of plants defoliated to 50 mm stubble height at H_1 increased by a mean of 138% over the next 27 days of regrowth, but only by 23% in plants defoliated to 20 mm.

7.3.3 Leaf dynamics

Following defoliation at H_1, expansion of the remnant leaf (the youngest leaf emerging from the stubble sheath at the time of defoliation) began immediately in all plants. However, the time taken for the first new leaf to emerge was significantly increased (P<0.001) in plants defoliated to 20 mm, compared to 50 mm, stubble height at H_1 (9 vs. 7 days, respectively). The fact that leaf emergence was measured at a lower height on plants defoliated to 20 mm underestimates this time taken to appearance. If measured at 50 mm height, the leaf tip would have an extra 30 mm of sheath to travel through before emerging, and at a mean (± s.e.) leaf elongation rate of the remnant leaf of 11.5 ± 0.7 mm/day, plants defoliated to 20 mm at H_1 would take an extra 2.6 days before the appearance of their first leaf was noted, thus the time taken would be 12 vs. 7 days, respectively. There was no significant effect (P>0.05) of any treatment on subsequent leaf appearance intervals. The mean leaf extension rate over the first 11 days of regrowth was

significantly reduced ($P<0.05$) by more frequent and close defoliation (Table 7.2).

There was a significant linear relationship ($P<0.05$) between leaf extension rate (mm/day) over the first 11 days of regrowth and stubble WSC (mg/tiller) at H_1, as follows:

$$\text{Leaf extension rate} = 13.6 + 5.9\ \text{SWSC} \quad (\text{Adj } r^2 = 0.42)$$

Leaf regrowth after 11 days is shown in Plate 10.

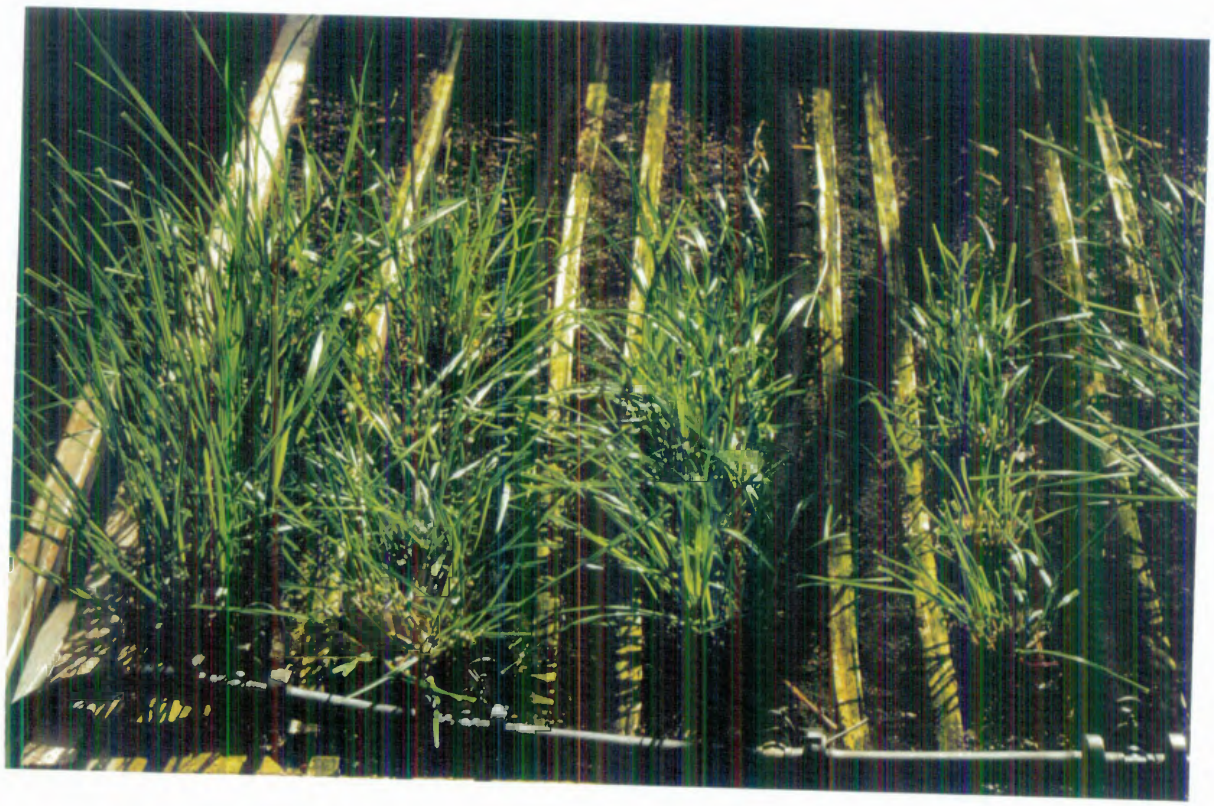

Plate 10. Leaf regrowth after 11 days, for, from left to right, plants subject to 3_{50}, 3_{20}, 1_{50} and 1_{20} defoliation treatments.

After 27 days of regrowth, plants defoliated at 3 leaves/tiller prior to H_1 had produced significantly more ($P<0.001$) leaf DM than plants defoliated at 1 leaf/tiller (2.1 vs. 0.9 g, respectively) (Table 7.2). Neither night temperature nor defoliation height had any significant effect ($P>0.05$) on leaf DM regrowth.

There was a significant linear relationship ($P<0.05$) between leaf DM (g/plant) after 27 days of regrowth and stubble WSC (mg/tiller) at H_1, as follows:

$$\text{Leaf DM} = 1.04 + 0.99\ \text{SWSC} \quad (\text{Adj } r^2 = 0.52)$$

7.3.4 Root dynamics

Root elongation, which ceased in all plants at defoliation, began significantly earlier ($P<0.001$) in plants subject to 3_{50} defoliation, than in plants subjected to all other treatments; these plants also had a significantly higher ($P<0.001$) mean elongation rate (mm/day) after initiation of root growth (Table 7.3).

Table 7.3. Mean number of days to root initiation, elongation rate of roots (mm/day) and survival of roots (%) over the initial 10 days of regrowth, following defoliation at 3 (3) or 1 (1) leaf/tiller, and to 50 ($_{50}$) or 20 ($_{20}$) mm stubble height.

Defoliation treatment	Number of days to commencement of root growth	Elongation rate (mm/day)	% roots surviving after 10 days
3_{50}	4	1.32	100
3_{20}	8	0.19	93
1_{50}	7	0.22	73
1_{20}	8	0.02	55
l.s.d. (P=0.05)	*2*	*0.28*	N/A

Nearly half of the marked roots of plants under 1_{20} defoliation died in the first 10 days of regrowth, while all roots of plants under 3_{50} defoliation survived over this period. The mean (± s.e.) number of days to death of roots for treatments 3_{20}, 1_{50} and 1_{20} was 8 ± 0.3, with no significant difference ($P>0.05$) between treatments.

Both the number of days taken for roots to begin elongation, and their mean elongation rate (mm/day) from the initiation of growth were significantly related ($P<0.01$) to stubble

WSC (mg/tiller) at H_1, as follows:

Number of days = 8.49 - 3.08 SWSC (Adj $r^2 = 0.77$)

Root elongation rate = 0.04 + 0.91 SWSC (Adj $r^2 = 0.69$)

Survival of roots over the first 10 days of regrowth was likewise significantly related ($P<0.01$) to stubble WSC (mg/tiller) at H_1, as follows:

% roots surviving = 113.1 - 13.3/$(SWSC)^{0.5}$ (Adj $r^2 = 0.74$)

The effect of defoliation interval on root growth is shown in Plate 11.

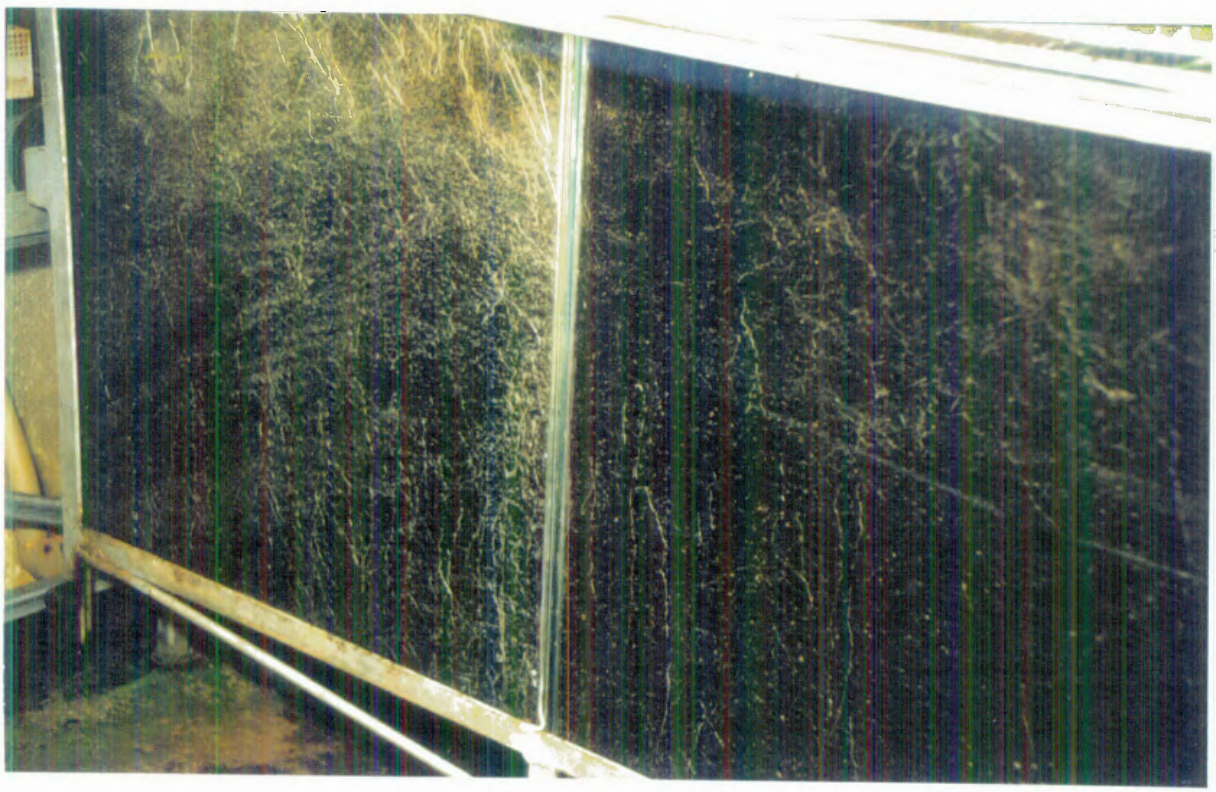

Plate 11. Root growth after 11 days, of plants defoliated to 50mm stubble height at 3 (left) or 1 (right) leaf/tiller.

7.4 Discussion

The present study provides quantitative evidence for the existence of a priority in allocation of WSC reserves in perennial ryegrass following defoliation. In a time sequence, leaf regrowth assumes the highest priority, as evidenced by immediate extension of leaves in all plants. This is followed by commencement of root regrowth, then daughter tiller initiation. In plants with initially high levels of WSC, the first daughter tiller was initiated just days after root growth resumed, but in plants with low WSC, tiller initiation took place at least a week after root growth. Thus, on a regrowth time scale, tiller initiation was most sensitive, root regrowth moderately sensitive, and leaf regrowth relatively insensitive, to a decrease in WSC. The time of daughter tiller initiation also coincided with replenishment of stubble WSC levels.

Stubble WSC levels, and hence subsequent regrowth, were reduced by a combination of frequent (1 vs. 3 leaves/tiller) and close (20 vs. 50 mm height) defoliation. The observation that an increase in night temperature only decreased WSC in combination with defoliation at 3, but not 1, leaf/tiller probably reflects greater use of WSC for respiration by these larger plants. Regression analyses indicated that for all regrowth variables tested, there was a stronger correlation to stubble WSC mg/tiller than to stubble WSC % of DM at H_1, supporting previous studies with perennial ryegrass (Davies 1966; Fulkerson and Slack 1995; Chapter 6), timothy (Smith and Jewiss 1966; Smith 1974) and tall fescue (Booysen and Nelson 1975), and indicating that the regenerative capacity of a ryegrass plant following defoliation is best expressed by the total amount of WSC in the tiller.

The sequence of events for WSC allocation during regrowth may be illustrated by comparing the regrowth of plants with the highest and lowest total stubble WSC at H_1 (1.52 vs. 0.05 mg/tiller, respectively). The 'high WSC' level was achieved through 3_{50} defoliation, under 8°C night temperature during the week prior to H_1, while the 'low WSC' level was achieved through 1_{20} defoliation, under 20°C night temperature.

In contrast to the sequence of regrowth events following defoliation, in which timing of

tiller initiation was most sensitive to WSC, the absolute effect of growth was different, with elongation and survival of roots most affected by reduced WSC. A 30-fold difference in stubble WSC at H_1 between high and low WSC plants produced only a 4-fold increase in leaf DM after 27 days, while tiller number/plant increased 6-fold. Root elongation rate was 59 times higher in the high, than the low, WSC plants.

The greater leaf DM after 27 days of high, than of low, WSC plants was partly due to a doubling of the leaf elongation rate, partly to an 82% increase in the number of tillers/plant, and partly to an increase in tiller DM (by 150% in high, and 33% in low, WSC plants).

The larger increase in tiller numbers in high WSC plants was likewise due both to a 3-fold increase in daughter tiller initiation, and to 1.7 times greater survival of young daughter tillers. Because of the greater initiation of daughter tillers, site filling in high WSC plants was almost 4 times that of low WSC plants, and high WSC plants began to replenish stubble WSC at least 4 days earlier than low WSC plants.

The observation that number of tillers/plant and site filling were both decreased by frequent and close defoliation supports previous work by Hume (1991). In that study, perennial ryegrass was defoliated at 1, 2 or 4 week intervals over 8 weeks (4 weeks approximately coinciding with the 3-leaf stage of regrowth), to 30 or 60 mm stubble height. Defoliating plants at the equivalent of 1 leaf/tiller (weekly) to 30 mm stubble height, almost halved site filling, and reduced number of tillers/plant by 86% (25 vs. 175 tillers), compared to defoliating at 3 leaves/tiller to 60 mm. Over the 8 week experimental period, stubble WSC (%) rose slightly from 18 to 22% in plants defoliated at 3 leaves/tiller to 60 mm, but fell to 6% in plants defoliated at 1 leaf/tiller to 30 mm, and consistent with results of the present study, this was significantly related to regrowth.

In the current study, a low WSC status was associated with increased mortality of daughter tillers, and this is in line with previous observations in perennial ryegrass (Alberda 1957, 1966b), timothy (Colby *et al.* 1974) and cocksfoot (Volaire 1995). This clearly indicates that daughter tillers assume a lower priority for WSC allocation than the parent tiller in

times of stress (e.g. severe defoliation, low light levels) which reduce WSC.

The perenniality of perennial ryegrass depends on its capacity to replace dying tillers (Colvill and Marshall 1984; Marshall 1987), and to a lesser extent, on seedling recruitment. Tiller regeneration is particularly important in the subtropics, where seedling recruitment from perennial ryegrass cultivars is minimal (Fulkerson *et al.* 1993a; Lowe and Bowdler 1995). Thus, from results of the present study, frequent and close defoliation of perennial ryegrass, and the associated depletion of WSC reserves, prejudices the survival of the plant by retarding the tiller initiation process and increasing death of younger tillers.

It has previously been postulated that the mechanism by which root growth is retarded following defoliation is the reduced supply of WSC from the tiller (Brouwer 1966; Langer 1979). Evans (1972) reported that defoliating perennial ryegrass plants to a stubble height of 25 mm resulted in a decrease in root WSC levels and an associated suppression of root elongation, compared to undefoliated plants. This suppression was largely countered by addition of sugar to the root medium, indicating that WSC depletion was the main cause of suppression of root elongation.

In the current study, the time taken for roots to resume growth following defoliation was increased by more severe defoliation (decreased interval and height). This was also found by Evans (1973), where complete or near complete cessation of root elongation occurred when young perennial ryegrass plants were defoliated every second day to a stubble height of 25 mm, while root elongation continued when plants were defoliated every second day to stubble heights of 50 and 100 mm, albeit at reduced rates compared to undefoliated plants. In this same experiment, reducing defoliation height from 50 to 25 mm increased the percentage of dead roots 9-fold, and this is also in line with results from the current study. Similarly, Davidson and Milthorpe (1966b) found that defoliating young cocksfoot plants to a stubble height of 25 mm led to an immediate and almost total reduction in root elongation and respiration. In that study, the root elongation rate fell to about 1%, and after 8 days was still only 5%, of the rate prior to defoliation.

Also, the smaller root system resulting from frequent defoliation would presumably place these plants at greater risk of sod pulling, as was found by Thom *et al.* (1986) in New Zealand (see also Chapter 5). In the study by Thom *et al.* (1986), 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). Poor rooting of perennial ryegrass plants is causally linked to poor persistence (Hughes and Jackson 1974; Arcioni *et al.* 1980, 1985a, b; Fulkerson *et al.*1993b), and in the subtropics, the action of defoliation in restricting root growth and function may partly explain the observation that less perennial ryegrass plants survived summer under frequent, compared to infrequent, winter and spring defoliation (Fulkerson *et al.* 1993b; Fulkerson and Bryant 1994; Chapters 4 and 5). Any factor which retards root growth has a carry-over effect on the rest of the plant's growth, due to restriction in uptake of water and nutrients (Davidson and Milthorpe 1966b; Clement *et al.* 1978; Langer 1979) and may further impact on plant survival.

A priority for reserve allocation in perennial ryegrass is logical in terms of its survival as a species on at least 2 counts. Firstly, it is important that tillers assume a low priority during regrowth, since initiation of tillers too soon after defoliation when WSC levels are low, will only jeopardise survival of both the parent and daughter tiller, as daughter tillers are reliant on the parent until they develop their own root system and leaves. To illustrate this, Colvill and Marshall (1981) found that the first daughter tiller to emerge became independent from its parent when it reached a dry weight of around 25 mg, which was equivalent to the time taken to expand 2 leaves/tiller and produce adventitious roots. After this stage, however, there was still some transfer of WSC from parent to daughter until the daughter was greater than 50 mg in DM. Secondly, root regrowth must take a second priority behind leaf extension and the establishment of renewed photosynthetic capability (leaves), otherwise the plant will rapidly deplete WSC through ongoing respiration, and die.

From a pasture management perspective, the current study confirms previous conclusions (Fulkerson *et al.* 1993b; Fulkerson and Slack 1995; Chapters 4, 5 and 6) that defoliation coinciding with the 3-leaf stage of regrowth and around a stubble height of 50 mm

optimises persistence and productivity of perennial ryegrass. However the current study goes further in providing an understanding of the mechanism by which this takes place. By allowing more rapid replenishment of WSC reserves, this optimal defoliation strategy enables a greater proportion of WSC to be allocated to maintain a more active root system, and promote tillering, compared to under more frequent and close defoliation.

Chapter 8

The impact of fertiliser N application in spring on summer survival of perennial ryegrass under grazing

8.1 Introduction

Previous studies (Julander 1945; Weinmann 1952; Arcioni *et al.* 1980, 1985a, b, c; Volaire 1994b, 1995; Volaire and Thomas 1995; Volaire and Gandoin 1996) have suggested WSC reserves in grasses play a role in plant survival through, and recovery from, stress periods such as high or low temperatures and drought. Previous studies reported in Chapter 5 show a positive relationship between WSC pre-summer, and survival of perennial ryegrass over summer. WSC appears to be the primary energy source during the early stages of regrowth following defoliation, but N reserves may assume importance when WSC reserves are low (White 1973). Thus, Davidson and Milthorpe (1966b) concluded that the decline in stubble WSC following defoliation of cocksfoot accounted for C used in respiration and regrowth. However, when defoliation increased in severity, even high concentrations of WSC did not meet C usage, and other substances, presumed to be N compounds, must have been remobilised for use in regrowth and respiration.

Recent research by Ourry *et al.* (1988, 1989b) provides evidence that N is remobilised from roots and stubble to the regrowing leaves following defoliation of perennial ryegrass. However, as with similar research on WSC, the reliance of regrowth on reserves has still to be conclusively proven. Previous research in subtropical Australia reported that monthly applications of N fertiliser from May to November (winter and spring) improved survival of ryegrass plants over summer in a perennial ryegrass/white clover pasture (Fulkerson *et al.* 1993b) or in a perennial ryegrass pasture grown in a monoculture, and fertilised with N (Lowe *et al.* 1995). These observations are consistent with the hypothesis that N reserves may be important at a time when WSC reserve levels are low (Davidson and Milthorpe 1966b), in this case due to high rates of respiration and/or low

rates of photosynthesis during a subtropical summer (see Chapter 2, section 2.4.1). Both these studies were conducted under a cutting regime, and it is possible that as N application may encourage surface rooting (Fulkerson *et al.* 1993b; Oswalt *et al.* 1959), that under grazing, plant loss may be greater due to sod pulling by stock (Tallowin *et al.* 1986), negating any positive effects of higher N reserve levels.

Application of N fertiliser increases the N content in ryegrass (Dilz 1966; Fairey 1985; McGrath 1992; Leon *et al.* 1995), and presumably also the pool of N reserves. Therefore, plants fertilised with N in spring would be expected to have greater N reserve levels in summer. Also plants low in N reserves would presumably rely more on uptake of soil N as nitrates and ammonia for synthesis of protein. The uptake and conversion of nitrates to protein is approximately one-third more costly, in terms of C used, than the direct conversion of amino acids to protein (I.R. Johnson, personal communication). Thus in summer, regrowth of plants low in N reserves may increase WSC demands from already low WSC levels, and jeopardise plant survival.

There is also a negative relationship between N application and level of WSC in the plant (see Chapter 2, section 2.4.6). If N is a factor limiting growth, moderate applications of fertiliser N increase plant growth, and the increase in energy demand for increased protein synthesis decreases WSC levels (Auda *et al.* 1966). In the longer term, however, the larger leaf area produced may cause an increase in WSC levels, through increased photosynthetic capacity (Chapter 6). On the other hand, high applications of fertiliser N may stimulate synthesis of amino acids, the C content of which could be supplied by breakdown of WSC reserves (White 1973). During a subtropical summer, when WSC levels are already low, irrespective of management applied (see Chapter 5), there would be no benefit to plant persistence in further decreasing WSC, therefore the amount of N fertiliser applied, and the timing of such applications, may be critical.

This study aimed to investigate, under grazing, the effect of timing, and mechanism of action, of N fertiliser application in spring, on the survival of perennial ryegrass during summer. WSC levels in ryegrass plants entering the summer were varied by imposing different defoliation frequencies (Davies 1966).

8.2 Materials and methods

8.2.1 Site

The experiment was located at the Wollongbar Agricultural Institute, New South Wales, and undertaken between September 1996 and May 1997.

In contrast to the Casino site (Chapters 4 and 5), the soil was a red krasnozem, of basaltic origin. The pasture examined was a mixed perennial ryegrass (cvv. Yatsyn and Dobson) and white clover (cvv. Haifa and Osceola), sown on 20 March 1996 with 10 kg Yatsyn, 10 kg Dobson, 2 kg Haifa and 2 kg Osceola/ha.

8.2.2 Experimental design

On 10 October, the pasture was grazed with Friesian cows, slashed to 50 mm stubble height, and thirty 3 m x 2 m plots laid down. Defoliation interval was the main treatment, with N fertiliser application factorially arranged as subtreatments, and replicated 3 times. All grazing took place with Friesian milking cows, at a stocking intensity of 90 cows/ha for a grazing period of 8 to 10 hours.

Defoliation interval treatments were:

Infrequent: Grazed twice at the 3-leaf stage.

Frequent: Grazed at the same time as the infrequently defoliated plots, but plots were cut to 50 mm stubble height with a rotary mower at each 1-leaf stage leading up to the 3-leaf grazings.

At a mean Li of 9.5 days, each 3-leaf stage was attained in approximately 28 days, and the total defoliation treatment period was 57 days. Duration of grazing at each 3-leaf stage was less than one day. After the treatment period, all plots were grazed at approximately the 3-leaf stage until the end of the experiment (from 3 December 1996 to 28 April 1997).

N fertiliser treatments were:

- no N applied,
- 100 kg urea/ha on 22 October,
- 100 kg urea/ha on 22 November,
- 100 kg urea/ha on 22 December,
- 100 kg urea/ha on 22 October, then the same on 22 December.

Prior to the experimental period, pasture had received 100 kg urea/ha, on 3 June.

For the duration of the study, irrigation was applied in the absence of rainfall at 20 to 30 mm each 4 to 6 days over summer, then about each 10 days the following autumn. This was within the optimal irrigation interval defined by Fulkerson *et al.* (1993a) (see also Chapter 2, section 4.2.3).

8.2.3 Measurements

8.2.3.1 Plant density and number of plants pulled by stock (sod pulling). Plant density was determined on 9 October 1996 and 28 April 1997 from the number of individual ryegrass and tropical grass plants in each of 3 fixed 0.09 m^2 quadrates/plot. Following each grazing between November 1996 and April 1997, the number of individual ryegrass plants pulled from the soil by stock were counted in each plot, then removed.

8.2.3.2 Tiller development. Tiller number and DM were obtained from 3 randomly selected plants/plot prior to each 3-leaf defoliation from November 1996 to April 1997. Five individual ryegrass tillers were marked with coloured wire loops along an identifiable transect within each plot on 11 October 1996, and monitored monthly until 11 April 1997. At each monitoring event, tillers were classed as being vegetative, reproductive or dead, and the initiation of daughter tillers was also noted.

8.2.3.3 Root development. On 31 December 1996 and 1 May 1997, 3 ryegrass plants were chosen at random from each plot, and an 80 mm (diameter) x 200 mm soil core taken

to include the root system of each plant, and cut into 50 mm vertical sections. After soaking in water for 12 hours, roots were separated from soil by washing through a 1 mm sieve. The separated roots were then placed in water, and any foreign organic matter, including roots of other plants were removed. Roots were then dried at 80°C in a forced-draught oven for 24 hours to determine DM.

8.2.3.4 WSC determination. The WSC content of leaves and stubble of ryegrass was determined from 3 randomly selected plants/plot on 8 November, 3 and 23 December 1996, and on 17 February and 4 April 1997. Stubble and leaf samples were obtained as outlined in Chapter 5, section 2.2.2e, and WSC levels determined using the procedure described in Chapter 3, section 4.

8.2.3.5 Fungal analysis. On 10 January, the roots of 6 ryegrass plants pulled out of the soil by grazing stock, along with the roots of 6 healthy ryegrass plants, underwent analysis for fungal pathogens. In addition, the effect of leaf rust fungus on plant WSC levels was investigated on 10 November by selecting 6 plants from each of the following: heavily affected with rust (estimated greater than 75% of leaf area infested), moderately affected (estimated between 30 and 50% of leaf area infested), and mildly affected (estimated less than 10% of leaf area infested). Stubble and leaf samples were obtained as outlined in Chapter 5, section 2.2.2e, and WSC levels determined using the procedure described in Chapter 3, section 4.

8.2.4 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.

8.3 Results

The addition of N fertiliser had no significant effect ($P>0.05$) on ryegrass plant density,

root growth, tiller dynamics or sod pulling.

In mid-spring (9 October), there were 282 ± 8 ryegrass plants and 8 ± 1 (mean ± s.e.) tropical grass plants/m^2 across all plots. By autumn, the infrequently-defoliated plots had 73% higher ryegrass plant density (88 vs. 51 plants/m^2, $P<0.001$) and 26% less tropical grass plant density (73 vs. 99 plants/m^2, $P<0.01$), than frequently-defoliated plots. There was a significant interaction ($P=0.05$) between defoliation frequency and N application on tropical grass plant density in autumn, with the highest density of tropical grass plants obtained under a combination of frequent defoliation and N fertiliser applied in October and November (Figure 8.1).

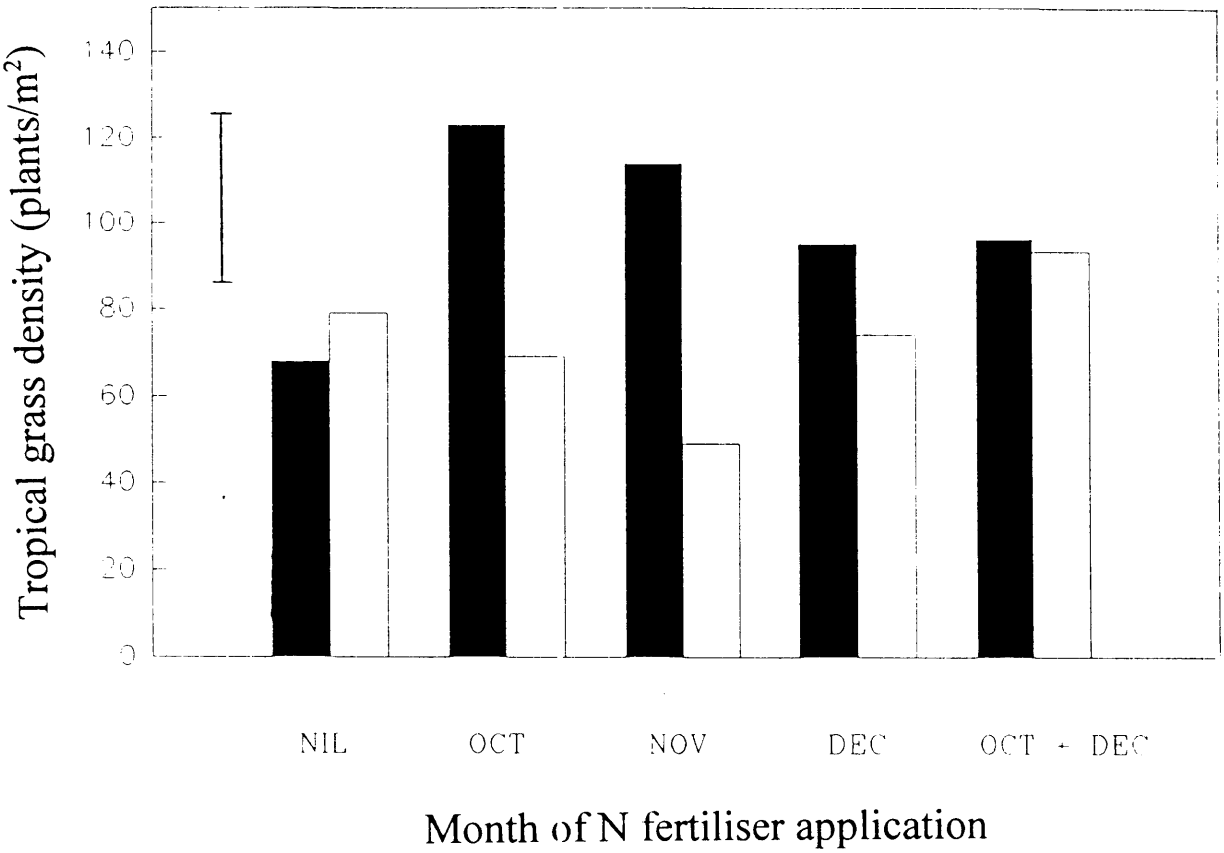

Figure 8.1. Tropical grass plant density (plants/m^2) in autumn of year 2, as influenced by N fertiliser application and defoliation at 1 (■) or 3 (□) leaves/tiller in spring. The l.s.d. for the interaction between N fertiliser application and defoliation is shown as a vertical bar.

Ryegrass plants defoliated infrequently had significantly more (P<0.01) stubble WSC%, and significantly more (P<0.001) stubble WSC (mg/plant) from spring through summer, than plants defoliated frequently (Table 8.1). By autumn, there was no significant difference (P>0.05) in WSC levels between treatments.

Table 8.1. Stubble WSC concentration (%) and content (mg/plant) in ryegrass plants defoliated at the 1- or 3-leaf/tiller stage of the regrowth cycle in spring.

Defoliation frequency (leaf stage/tiller)	**WSC in stubble**	**1996**		**1997**	
		November	**December**	**February**	**April**
1	%	19	16	15	17
	mg/plant	103	115	59	81
3	%	30	24	19	18
	mg/plant	201	191	101	100
l.s.d. (P=0.05)	*%*	*3*	*2*	*3*	2
	mg/plant	*48*	*39*	*24*	26

By early November, N applied in October had significantly decreased (P<0.01) stubble WSC% (19 vs. 28%) compared to other plots, which had not yet received N. By early December, only plots which had received N in November had significantly lower (P<0.001) WSC% (14 vs. 21%), than all other plots.

Frequent spring defoliation resulted in ryegrass plants entering summer with a smaller root system than plants defoliated infrequently (Figure 8.2), although DM difference was only significant (P<0.001) for roots in the top 50 mm of soil. This top 50 mm soil layer accounted for 94 ± 0.5 (mean ± s.e.) % of total root DM in summer and autumn for plants subjected to both defoliation treatments.

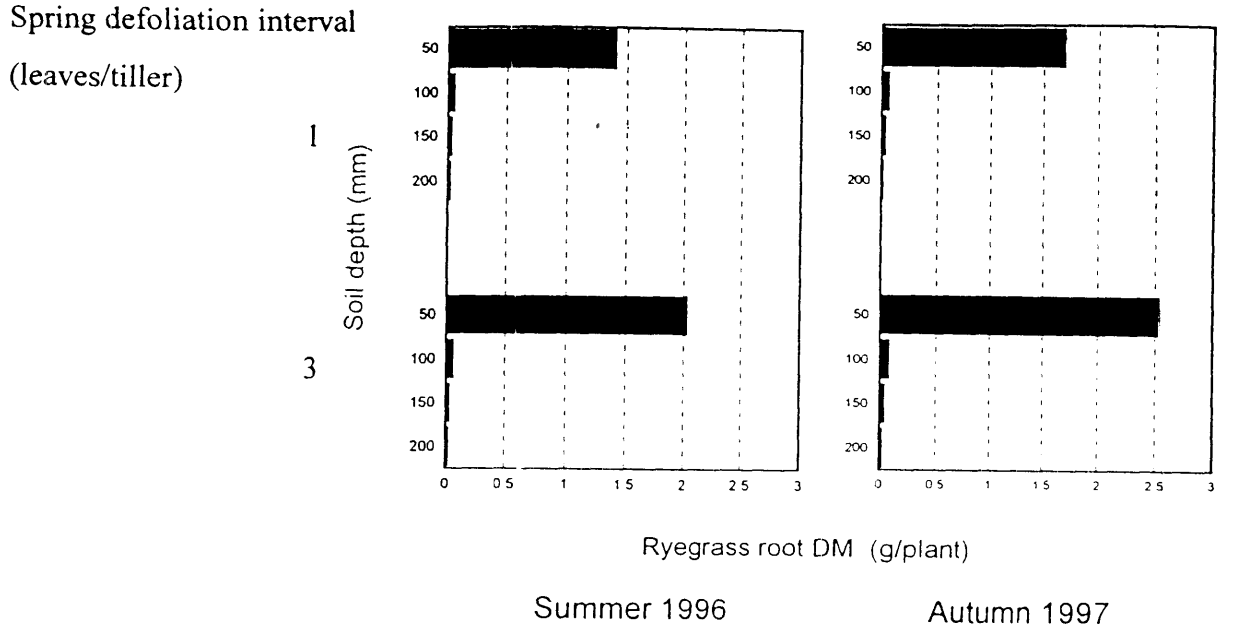

Figure 8.2. Root DM (g/plant) with depth (0 to 200 mm with 50 mm increments) for samples taken in spring 1996 and autumn 1997, from plants subject to defoliation at 1 or 3 leaves/tiller.

Plants defoliated infrequently in spring still had higher root DM than frequently-defoliated plants in autumn, and this difference was significant ($P<0.01$) only in the top 50 mm of soil, and below 100 mm ($P<0.06$) soil depth. By autumn, total root DM had increased from that measured in summer by 22 ± 3 (mean ± s.e.) % across both defoliation treatments.

The number of individual ryegrass plants pulled from the soil by grazing stock during December was significantly higher ($P<0.001$) under the frequent, than infrequent, defoliation (7 vs. 2 plants/m^2, respectively). By April, plots defoliated frequently in spring had lost a total of 17 plants/m^2 through sod pulling, compared to 12 plants/m^2 in plots defoliated less frequently ($P<0.05$). *Fusarium* fungi were identified on roots of both healthy and pulled plants, but *Rhizoctonia* fungi only on roots of pulled plants.

Puccinia rust significantly decreased ($P < 0.05$) WSC% in leaves, compared to leaves of rust-free plants (7 vs. 10%, respectively), but had no significant effect ($P > 0.05$) on stubble WSC%, with a mean of 30 ± 1 (mean ± s.e.) % WSC across all groups.

In November, the mean tiller density was 11,286 ± 564 (mean ± s.e.) tillers/m^2, with no significant difference ($P > 0.05$) due to defoliation frequency. In April, tiller density was significantly lower (2,750 vs. 4,691 tillers/m^2, $P < 0.001$), in plots defoliated frequently. This difference in tiller density was due to tiller death exceeding tiller initiation from January to March (Figure 8.3), whilst tiller initiation exceeded tiller death over the entire monitoring period in plots defoliated infrequently.

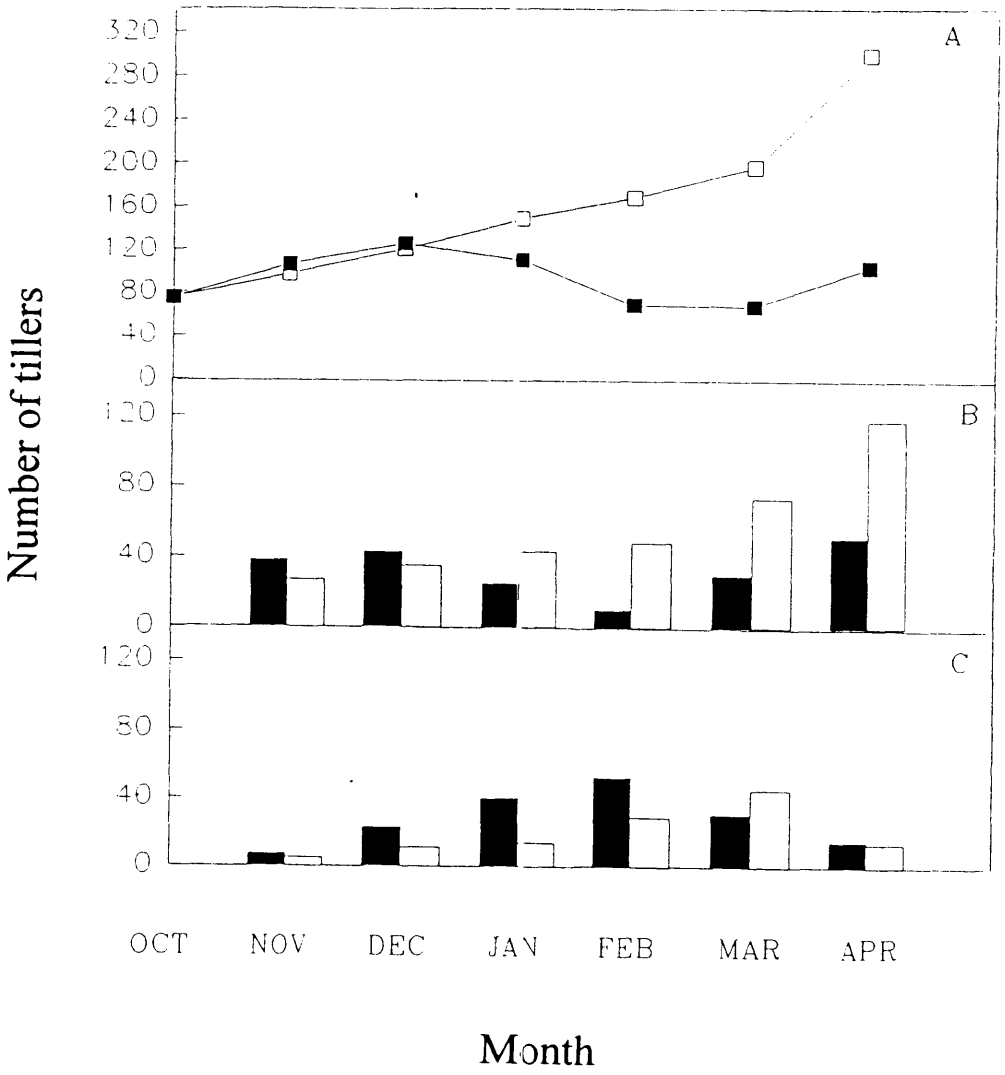

Figure 8.3. Commencing with 75 individually-marked tillers, the total number of (A) live tillers (original and daughter), is the difference between (B) number of daughter tillers initiated, and (C) number of dead tillers over spring and summer in pastures defoliated at 1 (■) or 3 (□) leaves/tiller in spring.

8.4 Discussion

In contrast to previous cut-plot studies (Fulkerson *et al.* 1993b; Lowe *et al.* 1995), under grazing, application of N fertiliser was found to have no significant effect on survival of perennial ryegrass plants over summer. On the other hand, defoliation had a marked effect on ryegrass persistence, with frequently defoliated plants having lower stubble WSC

(mg/plant) in spring (49% less than infrequently defoliated plants) and summer (42% less). The differential in WSC in this study was associated with a smaller root system (30% less root DM in spring and 33% less in autumn) and 29% more plants pulled from the soil by stock between November 1996 and April 1997. However, sod pulling accounted for only 6.5 ± 0.5 (mean ± s.e.) % of the total number of plants lost between spring and autumn, regardless of defoliation interval. These results are consistent with results from Chapter 5, where frequent grazing was associated with lower WSC, a smaller root system and more sod pulling, than infrequent grazing, although again, sod pulling accounted for no greater than 10% of plant loss between spring and autumn. In a field trial in the United Kingdom, Tallowin *et al.* (1986) found that sod pulling was more prominent during the summer months, and increased with amount of N applied (from 0 to 800 kg N/ha/year). However total herbage loss through sod pulling was still only minimal, with gaps created by sod pulling not exceeding 2% of any pasture area. In the current study, the effect of defoliation interval on various sward characteristics was not as marked as in Chapter 5, presumably due to milder weather conditions, and a more optimal soil moisture status, due to more frequent irrigation.

The lack of effect of N fertiliser on ryegrass plant survival or root DM in the current study, compared to the studies by Fulkerson *et al.* (1993b) and Lowe *et al.* (1995), is presumably in part due to the effect of the grazing animal, and perhaps due to differences in the number and timing of N fertiliser applications. In the studies by Fulkerson *et al.* (1993b) and Lowe *et al.* (1995), the pasture received monthly applications of N from May to November. In contrast, plants in the current study only received one application prior to the experimental period, which began in October. In addition, it is possible that in the studies by Fulkerson *et al.* (1993b) and Lowe *et al.* (1995), part of the observed increase in persistence of perennial ryegrass with application of N fertiliser was due to the deleterious effects of rust fungus being minimised under adequate N nutrition (Fulkerson *et al.* 1993a; Lowe and Bowdler 1995). In the current study, where rust infestation was minimal throughout spring and summer, N would have had little positive effect.

In the current study, N applied in October and November was observed to decrease WSC%, probably by increasing plant growth (Auda *et al.* 1996). After this time,

increasing temperatures would have become the major factor limiting ryegrass growth, with loss of WSC through respiration expected to increase substantially (Murata and Iyama 1963; McWilliam 1978). The limiting of growth by high temperature also explains the lack of effect of N on root growth. In addition, as the mechanism of retardation of root growth may be a reduced supply of WSC from the tiller (Brouwer 1966; Langer 1979), the lowering of WSC levels by N may have negated any positive effect N would otherwise have had on root growth. Thus our hypothesis, that N reserves may become more important during a period of low WSC levels, is rejected under the conditions of this study.

Although less ryegrass plants survived summer following frequent spring defoliation, the root DM of these plants still increased over this period. Also, WSC levels the following autumn were similar to those of plants defoliated infrequently, and this may indicate that only those plants with high WSC levels and larger root systems survived the summer.

The observation that *Rhizoctonia* fungus was associated with roots of pulled plants, but not with roots of seemingly healthy plants indicates that this fungus may have a role in sod pulling. While results from the current study and from Chapter 5 do not indicate that *Rhizoctonia* fungus is a causative factor in sod pulling, the observed pathogenicity of many strains of *Rhizoctonia* fungus (P.T. Wong, personal communication) indicates that this would place further stress on roots already damaged by frequent defoliation.

The present study provides further support for the hypothesis that survival of ryegrass plants over the summer is prejudiced by frequent defoliation, which is associated with a lower WSC content and a shallower root system (Chapter 5). Under grazing, sod pulling is a reflection of this weaker root system and contributes to plant mortality. There is no evidence from this study that ryegrass uses N reserves when WSC are low.