Chapter 11

The effect of defoliation on persistence of white clover

11.1 Introduction

Any consideration of the persistence of perennial ryegrass also needs to consider the persistence of white clover, which is usually its sown companion species. Although perennial ryegrass/white clover are usually re-established because the ryegrass population is so low as not to warrant retention, the persistence of white clover is still generally limited to 3 or 4 years. Perennation of irrigated white clover usually depends overwhelmingly on stolon survival (Hollowell 1966; Fulkerson and Slack 1996a), with seedling recruitment assuming greater importance under dryland conditions (Jones 1982; Garden 1988; Archer and Robinson 1989).

Summer growth is thought to be a useful criterion for survival of white clover over the stress period of summer by reducing the opportunity for tropical grasses to invade sown temperate pasture (Fulkerson et al. 1993a). Fulkerson et al. (1993c) showed that the summer-active white clover variety Osceola when grown in monoculture, was better able to resist tropical grass incursion than the more commonly sown variety Haifa, with kikuyu yield making up only 9% of DM in the Osceola pasture over the first summer, compared to 55% in the Haifa pasture. The survival of white clover may also be improved with adequate irrigation over summer.

The present study sought to determine the effect of irrigation and defoliation intensity during summer on yield of Osceola and its ability to slow tropical grass incursion in a mixed sward with perennial ryegrass.

11.2 Materials and methods

The experiment was blocked within the studies reported in Chapters 4 and 10. This Chapter deals predominantly with the white clover component of mixed pasture.

11.2.1 Experimental design

The site was prepared and sown as outlined in Chapter 4, section 2.2, the only differences being that the plots were 5 x 3 m, were replicated 4 times within 2 blocks, and were sown with 10 kg perennial ryegrass and 6 kg white clover/ha. Plots received 40 kg urea/ha in June.

Treatment details were as follows:

Defoliation intensity: Plots were defoliated from 1 December 1994 to 31 March 1995 at 50 mm *or* 120 mm height, *or* not defoliated to 4 March. Before and after this period, all plots were defoliated at 50 mm height.

The defoliation interval was based on the time taken for the lower leaves of clover to begin to senesce, and was approximately equivalent to the time taken for 3 leaves/tiller to expand in the accompanying perennial ryegrass.

Irrigation was carried out as detailed in Chapter 4, section 2.2.

11.2.2 Measurements

DM yield, botanical composition and seed germination were all measured as described in Chapter 4, section 2.3.

11.2.2.1 Plant density and growing points. The number of individual ryegrass and tropical grass plants per 0.09 m^2 quadrate, placed at random in 2 locations per plot, was recorded in November 1994 and April 1995. The number of growing points which intersected a 1 m ruler randomly placed twice in each plot was determined in November 1994, and in February and April 1995.

11.2.2.2 Stolon development. On 26 October, 18 individual clover stolons were marked with coloured wire hoops from plants along an identifiable transect within one plot from each treatment. These stolons were monitored fortnightly until June 1995. At each monitoring event, the production of nodes along each stolon and the amount and timing of stolon breakup, branching and death was recorded.

11.2.2.3 Seedling recruitment. In May 1995, the number of new clover seedlings in 4 permanent 0.09 m² quadrates in each plot, were counted and marked with coloured wire loops. In June, the survival of these seedlings and any new seedlings which had germinated, was recorded.

11.2.2.4 Plant-parasitic nematodes. In September and December 1994, and February and April 1995, 9 soil cores (80 mm diameter, 150 mm deep) were taken, to each include the taproot of a clover plant. Cores were combined into 3 large samples (of 3 cores), and underwent analysis for identification of plant-parasitic nematodes associated with the soil and roots of the plants.

11.2.3 Statistical analyses

Comparisons between treatment means were tested by l.s.d. following analysis using the general linear model package in Minitab (Ryan *et al.* 1985).

11.3 Results

11.3.1 Plant density of grasses and growing point density of clover

Over the summer period, the number of tropical grass plants increased from 18 to 47 plants/m². Defoliation significantly increased (P<0.01) tropical grass plant density compared with no defoliation (51 vs. 39 plants/m²). Defoliation height had no significant effect (P>0.05) on the number of clover growing points (7 ± 1 (mean ± se)/m) in autumn of year 2. Ryegrass plant density at this time was significantly greater (P<0.001) when plants were defoliated to 120 mm height than under other treatments (25 vs. 5 plants/m², respectively).

11.3.2 DM yields

Ryegrass DM yield over summer (7 November 1994 to 31 March 1995) was decreased (88 vs. 621 kg DM/ha; P<0.05) by ceasing defoliation, compared to plots defoliated to 50 or 120 mm. A lax defoliation regime (to 120 mm height, or no defoliation) also reduced tropical grass DM yield, compared to close defoliation (1,913 vs. 2,632 kg DM/ha; P<0.05) (Figure 11.1).
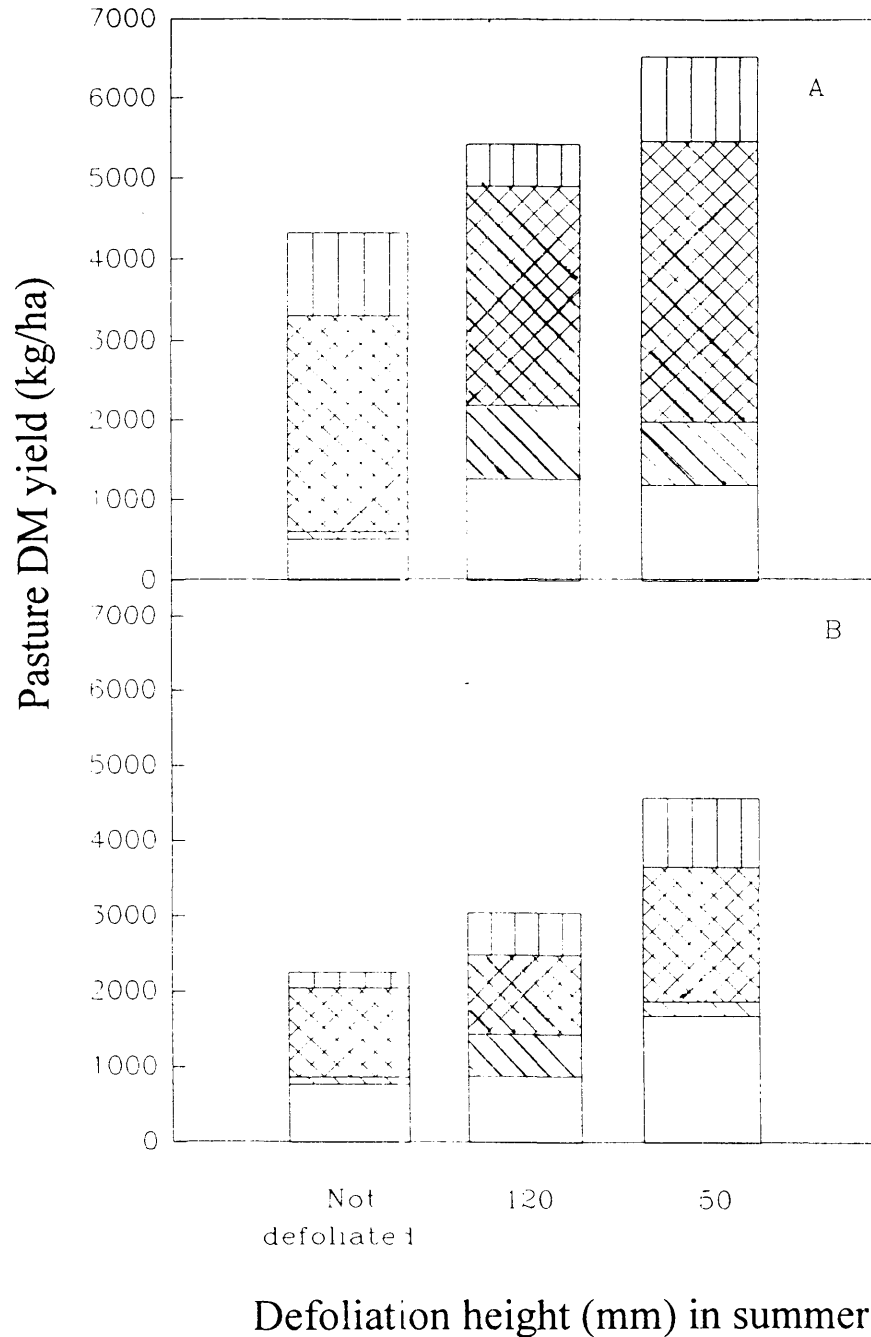

Figure 11.1. Yield (kg DM/ha) from November 1994 to April 1995 of white clover (□), ryegrass (▨), tropical grass (▨) and weed (▥), in plots (A) irrigated or (B) not irrigated over the summer.

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Over the summer, irrigation significantly increased (P<0.001) tropical grass yield (2,963 vs. 1,342 kg DM/ha), but had no significant effect (P>0.05) on the yield of any other pasture component.

The DM yield from April to June in year 2 (Figure 11.2) reflected the density of clover growing points and ryegrass plants in April.
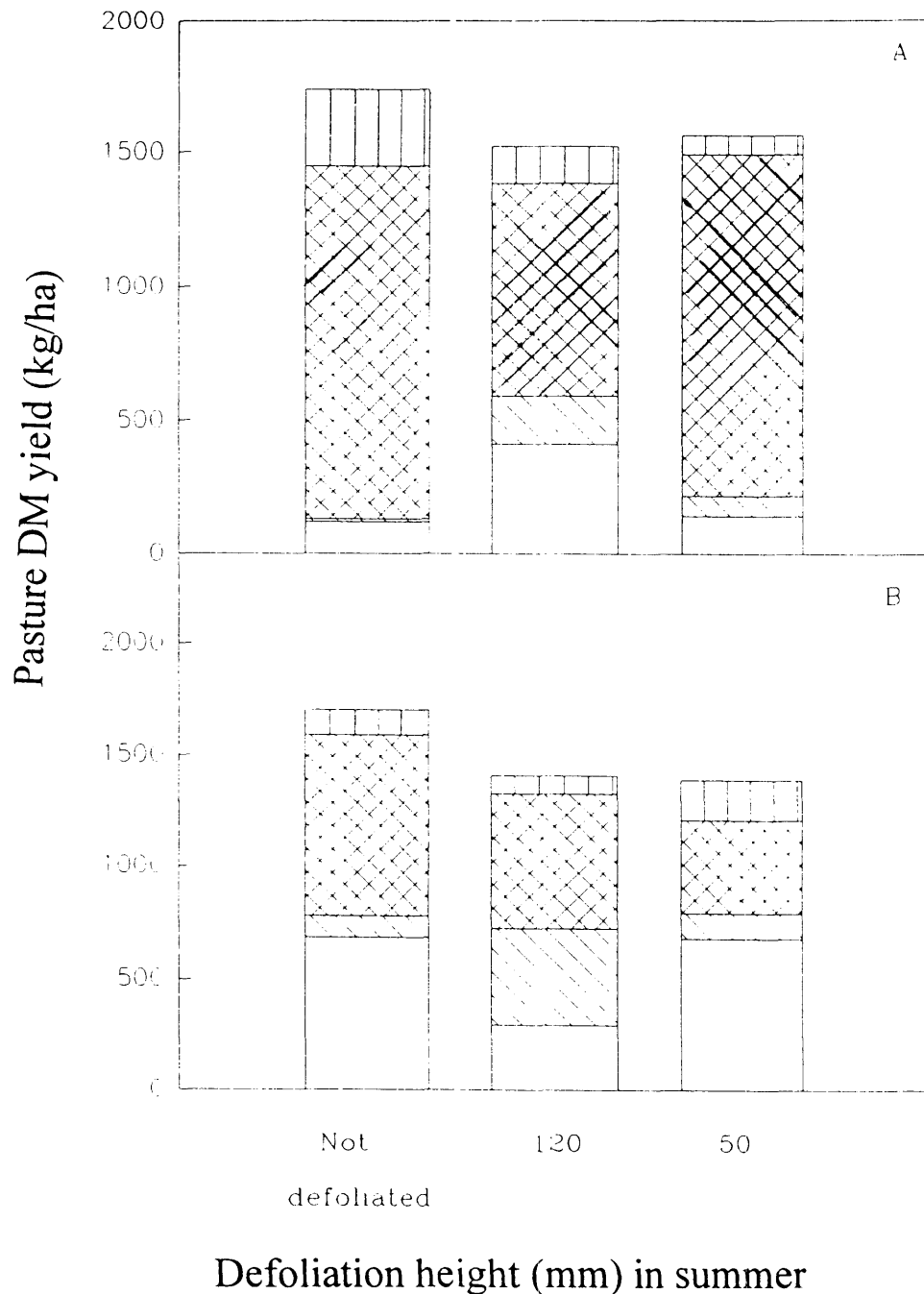

Defoliation height (mm) in summer

Figure 11.2. Yield (kg DM/ha) from April to June 1995 of white clover (□), ryegrass (▨), tropical grass (▩) and weed (▥), in plots (A) irrigated or (B) not irrigated over the summer.

Defoliation height had no significant effect (P>0.05) on yield of clover, ryegrass or weed, but defoliation to 120 mm height significantly reduced (P<0.05) total yield of tropical grass (June 1994 to June 1995) (2,586 vs. 3,310 kg DM/ha).

Irrigation had no significant effect (P>0.05) on the number of ryegrass or tropical grass plants present in summer, but significantly reduced (P<0.001) the number of clover growing points (3 vs. 11 growing points/m).

11.3.3 Clover stolons

From October 1994 to June 1995, Osceola produced a cumulative mean (± s.e.) over all treatments of 58 ± 2 nodes/stolon, with no significant difference between treatments (P>0.05).

The fragmentation process began in late December, with a major peak in early January, and this was independent of treatments (Figure 11.3).
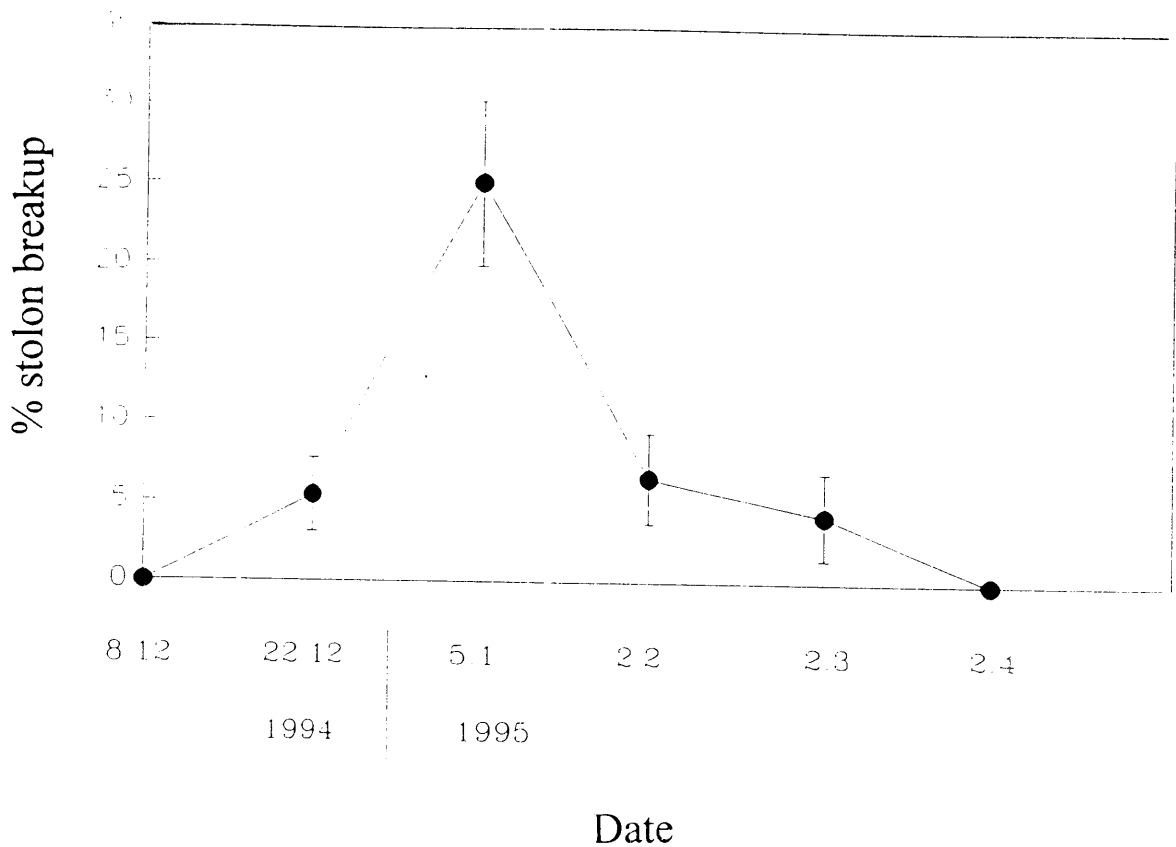
Figure 11.3. The percentage of white clover stolons, marked on 26 October 1994, which underwent breakup over summer. Standard errors of the means are indicated by vertical bars.

This peak in stolon breakup was followed by a peak in stolon death; 71% of the clover stolon fragments died within a month of breakup. By April, none of the original taprooted parent plants remained in the pasture.

11.3.4 Seed viability and seedling recruitment

At temperatures above 20°C, 92 ± 1 (mean ± s.e.) % of harvested clover seed did not germinate, and even at the lowest temperature regime (15°C), 82 ± 3 (mean ± s.e.) % of seed still did not germinate. Chilling did not improve the percentage germination of seeds.

In May of year 2, there were 1.51 ± 0.44 (mean ± s.e.) clover seedlings/m^2, with no significant difference (P>0.05) between treatments. By June, 46% of seedlings had died, whilst 0.45 new seedlings/m^2 had established since May.

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11.3.5 Plant-parasitic nematodes

The number of nematodes in roots of clover plants was not affected by defoliation or irrigation treatments. Root-knot nematodes were in moderate levels in September, increasing to high levels in December and February, then declining to moderate levels again in April. Spiral nematode (*Helicotylenchus* spp.) had built up to high levels in April only, while clover cyst nematode was present in moderate levels at this time. The high levels of nematodes measured would be expected to detrimentally affect white clover growth and performance (L.J. McLeish, personal communication).

11.4 Discussion

Defoliating to a height of 50 mm over summer slightly increased white clover yield compared to defoliating to 120 mm. This is consistent with results from a number of studies in temperate regions (Reid 1959, 1962; Appadurai and Holmes 1964; Frame and Boyd 1987; Brink 1995), which state that clover DM yield and content in pasture may be increased by close defoliation, provided that the interval between defoliations allows the plant adequate time to recover. It is possible that the yield of Osceola in the current study may have been greater, had the 50 mm defoliation height not also promoted growth of, and hence competition from, tropical grasses and weeds.

The detrimental effect of tropical grass on white clover survival in a ryegrass/clover pasture has been shown by Lambert *et al.* (1979) in a 7 year grazing study in New Zealand. Inclusion of kikuyu in the pasture mix reduced clover DM yield by up to 37%, while more erect tropical grasses, such as paspalum, had a negligible effect on clover, but reduced ryegrass DM yield.

When white clover was sown into an established kikuyu pasture in the subtropics, Fulkerson and Slack (1996a) found that defoliating in spring to 120 mm height allowed the tropical grass to dominate the clover earlier in the season compared to defoliating to 50 mm height, reducing clover yield by 21% (18,306 vs. 23,231 kg DM/ha, respectively). In the present study, lax defoliation or no defoliation during summer slowed tropical grass invasion,

compared to close defoliation. However, in the present study, tropical grass plants had only germinated around September to October, and may have been vulnerable to shading by the more mature ryegrass and clover pasture species.

Irrigation decreased the number of clover growing points, probably because it promoted a larger tropical grass canopy; the resultant shading leading to depressed clover stolon branching (Robin et al. 1994a, b), lowering the density of stolons and hence growing points (Wilman and Acunap 1993). We have previously concluded (Chapter 4) that infrequent irrigation during summer only benefited deeper-rooted plant species such as tropical grasses and weeds. Furthermore, waterlogging due to large rainfall events in mid-summer, and accentuated by irrigation, would be of further detriment to the shallow-rooted clover stolon fragments at this time of the year.

The low seedling recruitment of white clover, and poor survival of white clover seedlings, reinforce the view that perenniality of white clover under irrigation relies almost exclusively on stolon survival (Hollowell 1966; Fulkerson and Slack 1996a). This low level of recruitment was mainly due to poor seed germination which was possibly overestimated, as some of the seedlings observed in year 2 could have come from hard seed sown the previous year. Low germination of seed set by white clover plants has previously been reported in the subtropics (Fulkerson and Slack 1996a) and in temperate areas (Archer and Rochester 1982), with greater than 80% of seed in both these studies classified as hard.

In contrast to temperate regions, where white clover stolons undergo breakup in spring (Brock et al. 1988), in the current study, breakup occurred in summer. Previous work with white clover in the subtropics under dryland conditions (Irwin and Jones 1977; Jones 1982; Garden 1988) indicated that stolon death reaches a peak during summer, associated with the death of primary taproots (Jones 1980; Garden 1988). However, in these studies, the advent and extent of breakup was not measured, and stolon death was attributed mainly to moisture stress and pathogen damage.

Following breakup, the fragmented white clover stolons are assumed to be vulnerable to environmental and managerial stress (Brock et al. 1988). This was supported by the high

death rate of fragmented stolons over summer in the current study, and highlights the need to determine appropriate management procedures which will maximise clover stolon survival through summer in the subtropics. Although poor survival rates of recruited seedlings and stolon fragments may be attributable to harsh climatic conditions during breakup (high temperature, waterlogging), the effects of pathogens such as nematodes and fungi on clover stolons in the subtropics may also be substantial (Irwin and Jones 1977; Fulkerson and Slack 1996a, b, see Plate 12), and requires further investigation.

Plate 12. The effect of root-knot nematodes on growth of white clover seedlings after 4 weeks. Seedlings were grown in nematode-free soil (left), or in soil containing root-knot nematode (centre and right) (Source: W.J. Fulkerson, unpublished data).

In the current study, nematode analysis was only qualitative, but still indicated that nematode numbers increase substantially during summer. As with breakup, measurement of nematodes needs to be repeated to obtain more precise data on seasonal burden of nematodes.

Chapter 12

The fate of white clover stolons over summer under grazing

12.1 Introduction

Results of *cut-plot* studies reported in Chapter 11 support previous observations (Hollowell 1966; Fulkerson and Slack 1996a) that perenniality of irrigated white clover relies on vegetative propagation via stolon fragmentation, and the survival of those stolon fragments over summer. There is limited seedling recruitment due to minimal germination of seed, and poor survival of the few seedlings recruited. Under subtropical conditions, stolon breakup was most pronounced during mid-summer, in contrast to spring in temperate areas (Brock *et al.* 1988). The combination of harsh climatic conditions and a buildup of plant-parasitic nematodes at this time resulted in a high death rate (greater than 70%) of stolon fragments (Chapter 11). This is consistent with earlier research in subtropical Australia under dryland conditions (Irwin and Jones 1977), which showed that nematodes and fungi cause extensive death of white clover stolons over summer.

The present study sought to monitor white clover survival over summer *under grazing*, with emphasis on stolon dynamics, and to identify the factors affecting survival of fragmented stolons. This study comprised 2 parts: the first determined the effect of grazing frequency in early spring in a first year pasture on white clover survival over summer, and the second monitored second and third year white clover plants over summer.

12.2 Materials and methods

This experiment was blocked within the study reported in Chapter 5; this Chapter deals predominantly with the white clover component of the mixed pasture.

12.2.1 Measurements

12.2.1.1 Study 1. Effect of grazing frequencies in spring. DM yield and botanical composition were measured as described i ı Chapter 5, section 2.2.2a.

a. Growing point density. The number of clover growing points in a 120 mm diameter soil plug were recorded from 15 soil plugs, taken at random positions within each plot in October, November and December 1995 and March 1996.

b. Stolon development. On 2 October, 30 individual clover stolons were marked with coloured wire hoops from plants along an identifiable transect within each plot, and monitored monthly until 2 January 1996. At each monitoring event, the production of nodes along each stolon and the amount and timing of stolon breakup, branching and death were recorded.

c. Root development. On 5 October, ten 80 mm diameter x 300 mm soil cores were taken at random positions within the plots to include the taproot of a clover plant, and root DM determined as described in Chapter 3, section 2. From the time of widespread breakup of clover (February), root samples were taken at random points each month until May 1996, to monitor the developing root system. As breakup was sporadic, there were both fragmented and whole plants present in the pasture in February and March, and at these times, root samples were taken from 10 whole and 10 fragmented plants, chosen at random.

d. Fungal analysis. In February 1996, fragmented sections of clover stolon were assayed for fungal pathogens. The method of isolation of fungal colonies from stolons is outlined in Chapter 3, section 5.

12.2.1.2 Study 2. Monitoring clover plants of different ages. The experimental design is described in Chapter 5, section 2.3. Measurements taken were as described in section 2.1.1 of the current study, with the exception that DM yield, root development and fungal analysis were not repeated.

Growing point density was measured from 20 soil plugs taken at random positions within the pasture.

a. Plant-parasitic nematodes. In September 1995, 15 soil cores (80 mm diameter, 150 mm deep) were each taken to include the taproot of a clover plant chosen at random in each of the first, second and third year pastures, and from first year pastures only in March 1996. Cores were combined into 3 large samples (of 5 cores), and underwent analysis for identification of plant-parasitic nematodes associated with the soil and roots of the plants.

12.2.2 Statistical analyses

Comparisons between treatment means, for all parameters except data from individually-marked stolons and clover root DM, 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 stolons were made using the student t-test.

12.3 Results

As study 2 was such a small part of the overall study, and results from marked stolons were not significantly different (P>0.05) between the ages of pasture, the results were combined.

12.3.1 Growing point density and pasture composition

In contrast to ryegrass, more frequent grazing significantly increased (P<0.01) the density of growing points and the proportion of clover DM in first year pasture in spring (743 vs. 380 growing points/m^2 and 11% vs. 1%, respectively) compared with less frequent grazing. However by autumn, there was no significant difference (P>0.05) between plots (301 vs. 318 growing points/m^2 and 4% vs. 2% by DM, in frequently and infrequently defoliated plots, respectively).

In spring, there was no significant difference (P>0.05) between second and third year pasture in clover growing point density or proportion of pasture DM, however both growing point density and DM composition were significantly higher (P<0.01) than in first year pasture

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(1,114 vs. 967 growing points/m^2, 27 vs. 24% by DM, respectively). By autumn, there was no significant difference (P>0.05) between second and third year pasture in the density of growing points or proportion of clover DM, with 93 ± 30 (mean ± s.e.) growing points/m^2, and 1% by DM across both pastures.

12.3.2 Stolon dynamics

The initiation of new nodes and the death of individually-marked clover stolons did not vary significantly (P>0.05) over spring and summer either with grazing interval in first year pasture, or between years, and the combined means are presented in Figure 12.1.
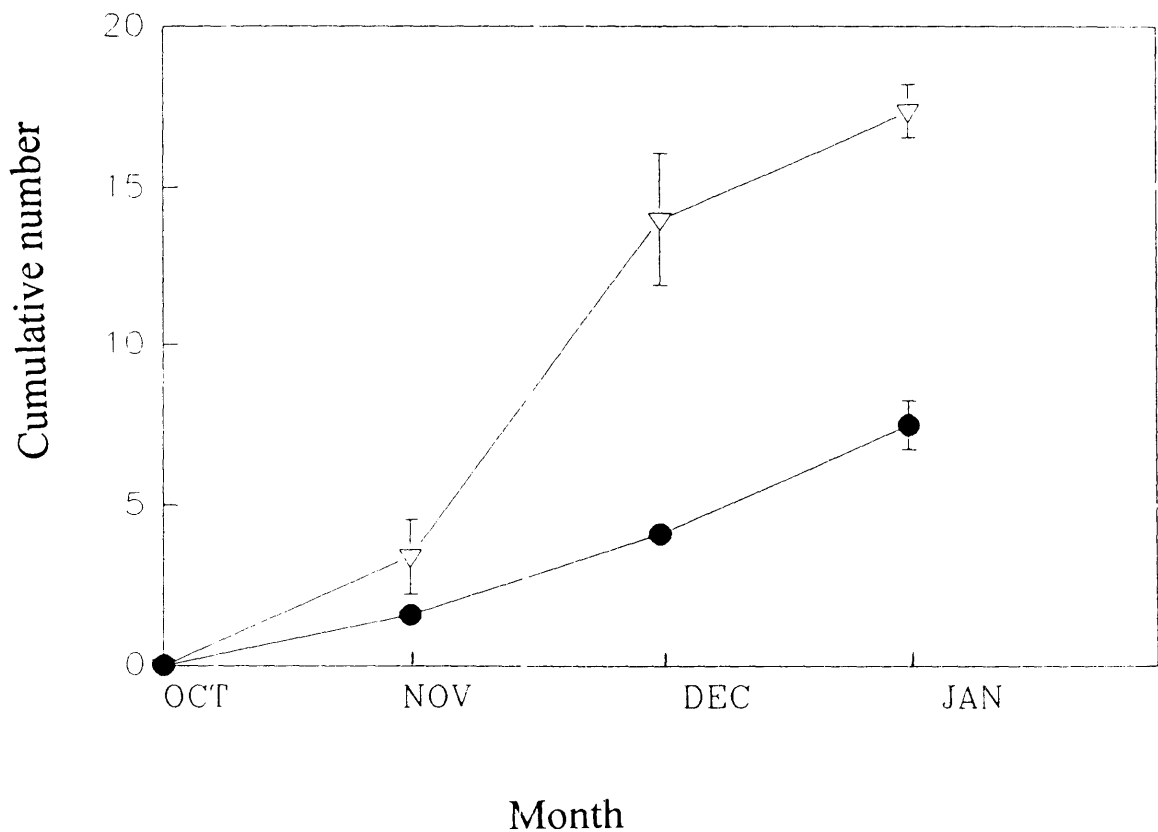

Figure 12.1. Cumulative number of nodes/live stolon (●) and cumulative number of dead stolons (▽), over spring and summer on individually-marked clover stolons in studies 1 and 2. Standard errors of the means are indicated by vertical bars.

By early January 1996, twice as many stolons had branched under more frequent, than under less frequent, grazing.

12.3.3 Stolon breakup

Clover plants began to fragment sporadically in early January (7% of the marked stolons had broken up, with no difference between treatment or years), and by late March, no whole plants were observed in any pasture. The development of the clover root system in whole and fragmented plants and in relation to top DM (combined leaf, petiole and stolon) are shown in Figure 12.2.

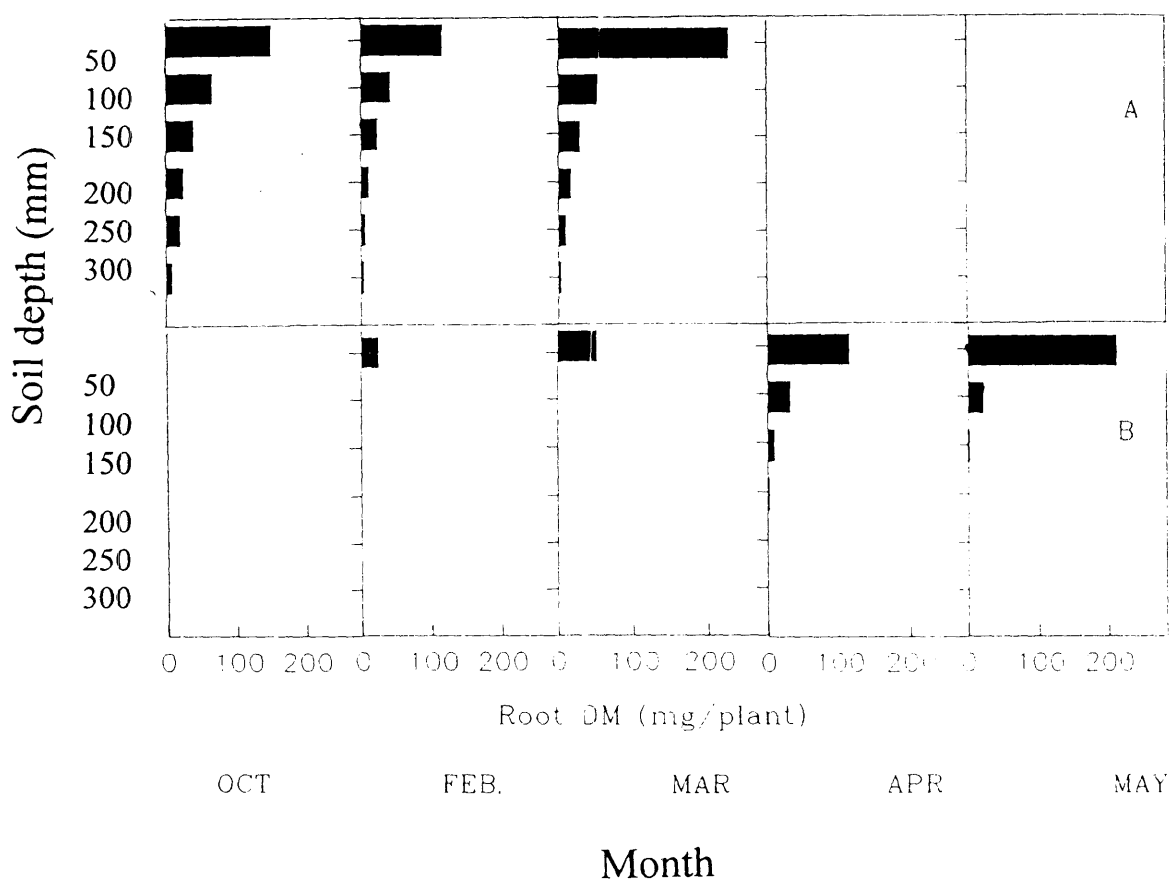| | Top DM (mg/plant) | | | | |
| | | Month | | | |
	October	February	March	April	May
Figure A	850	780	1160	-	-
Figure B	-	30	50	370	390

Figure 12.2. White clover root DM (mg/plant) with soil depth (50 mm increments) and top DM (mg/plant) in first year pasture. Data are from (A) whole plants, or (B) fragmented sections of stolon.

By early autumn, the root system of these small stolon fragments had only penetrated to one-sixth of the soil depth of the parent taprooted plants, and comprised only 17% of the root DM of the parent plant prior to breakup.

12.3.4 Pathogens

Following isolation from primary cultures, *Fusarium* fungi were present in 29% of stolon samples, with *F. solani* and *F. oxysporum* identified. *Rhizoctonia solani* was identified in 54% of stolon samples. A large amount of nematode-trapping fungi were also observed to be associated with clover stolon fragments.

The number of nematodes associated with clover roots increased over the summer period in first year pastures, and was greater in older pasture (Table 12.1).

Table 12.1. The mean (± s.e.) number of plant-parasitic nematodes/10 g white clover root in first, second and third year pastures in spring, and first year pasture in autumn.

	Nematodes/10 g root			
Nematode genera	**Spring**			**Autumn**
	First year	**Second year**	**Third year**	**First year**
Meloidogyne (root-knot nematode)	205 ± 170	316 ± 183	1,403 ± 709	1,230 ± 384
Ditylenchus (stem nematode)	21 ± 21	50 ± 50	61 ± 48	0
Pratylenchus (root lesion nematode)	0	0	0	27 ± 20

The number of root-knot nematodes in roots was considered to be high across all pastures, and would have been expected to detrimentally affect clover growth and performance (G.N. Berg, personal communication). Clover cyst nematode, although present in soil, was absent from roots of sampled plants.

12.4 Discussion

In first year pasture, more frequent grazing doubled clover density in spring - this was achieved, not through greater stolon growth, or less stolon death, but through increased

branching of stolons, presumably due to the greater penetration of light (Dennis and Woledge 1985; Robin *et al.* 1994a, b) through more frequent defoliation. However by autumn, clover density had declined by 59% in pastures frequently grazed the previous spring, but only by 16% when pastures were infrequently grazed, giving a similar density for both treatments. This higher mortality of frequently-grazed white clover plants may be due to lowered energy reserves and associated restricted root system (see Chapter 2, section 3.3), making these plants more vulnerable to climatic stress, as was the case with ryegrass. In addition, the ingression of tropical grasses, greater under frequent grazing (Chapter 5), would have made clover survival more difficult, as white clover in mixed pasture appears less successful than ryegrass in competing with tropical grasses over the summer period (Chapter 11). Furthermore, as white clover is more adversely affected than perennial ryegrass by the effects of selective grazing (Frame 1966a, b; Evans and Williams 1987), and soil compaction (Edmond 1964; Frame 1966b), more frequent grazing may also have reduced white clover density in this manner.

The current study confirms under grazing our previous observations under cutting (Chapter 11) - white clover stolons begin to breakup in early to mid-summer, and it appears that the stolons fragment in a similar way each summer. The climate during this breakup period is characterised by above-optimal temperatures, and a high likelihood of waterlogging. In addition to these stresses, the number of root-knot nematodes in roots increases over summer (by 6-fold in this study). If the number of nematodes increased by a similar degree over the second and third year summers - especially in third year pasture where the nematode burden in spring was already 7 times that of first year pasture - this may explain the decline of white clover in 2 or 3 year old pastures commonly observed in the subtropics (Garden 1988; Fulkerson and Slack 1996a, b). Infestation of root-knot nematodes has been reported to severely reduce white clover growth and function in subtropical Australia (Colman 1964; Irwin and Jones 1977) and New Zealand (Sarathchandra *et al.* 1995; Van Den Bosch and Mercer 1996a, b).

The effect of fungi on white clover is less clear. Sarathchandra *et al.* (1995) found *Fusarium oxysporum* to be the most common fungus isolated from white clover roots in a glasshouse trial in New Zealand, however it did not appear to be pathogenic under their conditions.

Similarly, Irwin and Jones (1977) considered neither *F. oxysporum* nor *Rhizoctonia solani* to be pathogenic to white clover seedlings. Thus, although *F. oxysporum* and *R. solani* were isolated from more than half of the stolon samples tested in the current study, their lack of pathogenicity in these other studies means any detrimental effect on stolons is not conclusive. On the other hand, Irwin and Jones (1977) reported that *Pythium* fungi were partly responsible for death of white clover roots and stolons over summer in subtropical Australia. *Pythium* was not isolated from stolons in the current study.

The present study indicates that frequent grazing may decrease white clover persistence, possibly in the same manner as it affects ryegrass (Chapter 5), through depletion of reserves and formation of a shallower root system. Stolon breakup occurs in summer each year, and the plants thus formed are small, with a much shallower root system than original plants, regrowing slowly over the next few months. It is during this time that white clover is vulnerable to environmental and managerial stresses, as a combination of harsh climatic conditions (high temperature, possibly waterlogging), and a buildup of nematodes and perhaps other pathogens in roots, would make survival difficult. Future studies should be undertaken to determine appropriate pasture management practices for white clover in summer which will maximise the survival of the fragmented plants.

Chapter 13

General discussion

The results of these studies have shown that a defoliation interval coinciding with regrowth of 3 leaves/tiller prior to summer, results in greater persistence and productivity of perennial ryegrass under irrigation in subtropical dairy regions of Australia, than more frequent defoliation. This confirms previous studies by Fulkerson *et al.* (1993b), Fulkerson and Bryant (1994) and Fulkerson and Slack (1994a), in which defoliation at or near this 3-leaf stage throughout the year increased the summer survival of perennial ryegrass plants, compared to more frequent defoliation. However, our studies have gone further, identifying the critical time for appropriate defoliation as being winter and early spring. Furthermore, this was also confirmed under grazing, whereas the previous studies were conducted under a cutting regime. Defoliation interval during late spring, which coincided with reproductive development of perennial ryegrass, had little or no effect on persistence of perennial ryegrass. This contrasts with results of Matthew *et al.* (1991), although their studies were under glasshouse conditions.

Defoliation at the 3-leaf stage from sowing to early spring led to greater survival of ryegrass plants, and less invasion of tropical grasses, than more frequent defoliation. This greater persistence of infrequently-defoliated ryegrass plants was attributed to greater survival of tillers over the summer, with these tillers being larger, having a more extensive root system, and accumulating higher levels of WSC reserves, compared to plants subject to more frequent defoliation. In the United Kingdom, Hughes and Jackson (1974) also found that 'hard' grazing decreased persistence of perennial ryegrass, and this was similarly associated with depleted WSC reserves, a reduced root system, and smaller tillers, compared to less severely grazed plants. Likewise, Thom *et al.* (1986) in temperate New Zealand, and McKenzie (1996, 1997) in subtropical South Africa, found that frequent grazing at 14 day intervals decreased tiller size, and reduced plant survival of perennial ryegrass over summer, compared to infrequent grazing at 28 day intervals. Thus infrequent defoliation, by maximising WSC levels and promoting overall plant size, creates a plant which can withstand the severe stress

of a subtropical summer.

The importance of WSC in plant survival through summer has previously been implicated in field studies in Europe with perennial ryegrass (Arcioni *et al.* 1980, 1985a, b, c) and cocksfoot (Volaire 1994b, 1995; Volaire and Thomas 1995; Volaire and Gandoin 1996). Under cutting, plant survival over summer was closely related to WSC content; furthermore, plants with higher WSC levels developed a larger root system (Arcioni *et al.* 1980, 1985a, b; Volaire and Thomas 1995), and maintained a greater density of live tillers during summer (Arcioni *et al.* 1985c; Volaire 1994b, 1995), than plants with lower WSC levels.

In the current studies, regrowth of perennial ryegrass following defoliation was strongly and positively correlated to the stubble WSC content at defoliation, with regrowth in the first 3 days completely reliant on WSC. Thereafter, the emerging leaves began to photosynthesise, and after one week of regrowth, one-third of leaf DM was attributed to WSC, and two-thirds to current photosynthate. However, the capacity of the plant to subsequently photosynthesise was also related to WSC, as high levels of WSC prior to defoliation led to regrowth of a larger leaf area than low WSC levels. Thus, it was found that after a full 3-leaf regrowth cycle, plants with initially high WSC levels at defoliation still maintained a higher level of regrowth than plants with low WSC levels, confirming a previous glasshouse study by Fulkerson and Slack (1995).

Thus, although the period of reliance of perennial ryegrass on WSC reserves post-defoliation is limited to the time taken for the emerging leaves to begin photosynthesising, the period of reliance on WSC could be expected to be longer if the plant was re-defoliated (Fulkerson 1994) or shaded (Alberda 1957) during this early stage of regrowth.

Replacement of dying tillers is vital for perennation of perennial ryegrass, since the lifespan of individual tillers is less than one year (Jewiss 1966; Langer 1979). Also, under subtropical conditions, seedling recruitment from commercial cultivars of perennial ryegrass is minimal (Fulkerson *et al.* 1993a; Lowe and Bowdler 1995). Perenniality is thus enhanced by both greater initiation and emergence of daughter tillers, and greater survival of tillers once emerged. Defoliation, through increasing light penetration into the pasture sward, generally

increases tiller emergence (Mitchell 1953a, b), however to maximise tiller survival, sufficient time is required between subsequent defoliations, to allow WSC levels to be replenished, and root growth to continue.

With regard to root growth, a number of studies have shown that the cessation of root growth following defoliation is exacerbated by more severe defoliation (Jacques and Edmond 1952; Crider 1955; Evans 1973), and those plants frequently defoliated develop a shallower root system than plants infrequently defoliated. Our studies showed that under grazing, this shallower root system results in greater plant loss through sod pulling, supporting results of Thom *et al.* (1986) in New Zealand. While plant losses due to sod pulling in our studies were less than 10% of the total number of plants lost over summer, even under frequent defoliation, the disturbance of soil through sod pulling provides ideal conditions for the germination and establishment of tropical grasses. Also, the shallow root system presumably makes those plants which survive pulling, more vulnerable to environmental stresses in summer.

Nevertheless, even under defoliation at 3 leaves/tiller, the root system of perennial ryegrass is shallow, with greater than 80% of root DM in the top 50 mm of soil in our studies, and this confirms previous observations by W.J. Fulkerson (unpublished data) on a range of soil types in the subtropics. It is unclear why rooting is restricted in this manner, but it may be partly due to:

1. The high temperatures experienced by the plant in spring to autumn, with the roots being more sensitive than top growth, since the optimal temperature for root growth is about 12°C, (Davidson 1969), while for top growth is about 18°C (Mitchell 1956).

2. Pathogens on roots of perennial ryegrass in this environment appear to be a greater burden than in more temperate regions, and this requires further investigation.

3. Even under a grazing interval which allows optimal levels of WSC to accumulate, the climatic situation in spring to autumn is such that solar radiation and hence WSC synthesis and accumulation, is reduced by extensive cloud cover (W.J. Fulkerson, unpublished data), and loss of WSC is high due to high temperatures, as respiration rises exponentially with temperature above the optimum for growth (Murata and Iyama 1963). Under these

conditions of C starvation, the poor root development could be explained if there was a priority for allocation of WSC, and the roots had a lower priority than top growth (Nielsen and Humphries 1966); this was found to be the case.

Quantitative evidence that a priority for allocation of WSC reserves exists during regrowth of perennial ryegrass, with roots and tillers deprived of WSC until leaf regrowth resumed, was found in the study reported in Chapter 7. Although a number of previous studies with perennial ryegrass (Evans 1972; Jacques and Edmond 1972; Ong 1978; Ong and Marshall 1979; Colvill and Marshall 1984; Danckwerts and Gordon 1987; see also Chapter 2, section 2.3) had indicated that a priority for WSC allocation existed, no effort had been made to qualify this by observing the sequential regrowth of leaves and roots, the initiation of tillers and replenishment of stubble WSC stores following defoliation.

Our studies have found that on a regrowth time scale, tiller initiation was most sensitive (lowest priority), root regrowth moderately sensitive, and leaf regrowth relatively insensitive, to a decrease in WSC prior to defoliation. The time of daughter tiller initiation also coincided with the start of replenishment of stubble WSC levels. In contrast to this *sequence* of events, the *absolute effect* of a reduction in WSC on growth was different, with root elongation and survival most affected by low WSC levels (lowest priority).

Thus, pre-summer defoliation of perennial ryegrass plants at 3 leaves/tiller to 50 mm stubble height, allows more rapid replenishment of WSC reserves, and enables a greater proportion of WSC to be allocated to maintain a more active root system, promote tillering, and result in greater leaf regrowth, compared to more frequent and close defoliation. However, even under optimal defoliation, a major limitation to persistence of perennial ryegrass may still be C starvation during summer.

A number of researchers have postulated that N reserves also play an important role in regrowth of grasses (Dilz 1966; Ourry *et al.* 1988, 1989a, b), particularly when WSC reserves are limiting, for example under severe defoliation (Davidson and Milthorpe 1966b). In support of this hypothesis, studies conducted in subtropical Australia (Fulkerson *et al.* 1993b; Lowe *et al.* 1995) indicated that N fertiliser applied during winter and spring improved

survival of perennial ryegrass plants over summer under *cutting*, presumably by increasing the N content of plants (Dilz 1966; Fairey 1985), and hence N reserve levels. However the current studies found no basis for this hypothesis under *grazed* conditions; it may be that other factors, particularly the effect of the grazing animal, may negate any positive effect of higher N reserves.

Given that even under optimal pasture management, low WSC levels may still limit survival of perennial ryegrass during summer, an alternative may be to induce plants to become dormant over summer, and this was approached in 2 ways. Firstly, varieties of perennial ryegrass which exhibited physiological summer dormancy, characteristic of Mediterranean ecotypes, were evaluated. Secondly, biennial ryegrass was used, and an attempt was made to perennate these plants and/or rely on seedling recruitment.

Summer dormancy has been shown to improve persistence of perennial ryegrass in temperate Australia (Silsbury 1961). However, results of our studies indicated that summer dormancy is unlikely to be a beneficial trait in the subtropics, since addition of water to dormant tillers caused over 85% of these tillers to regenerate, even at high temperature (38/20°C day/night). An erratic summer rainfall pattern in the subtropics would cause dormancy to break, and these regenerating plants would have little chance of surviving.

Likewise, although the biennial ryegrass genotype studied was capable of regenerating a pasture in the second year with similar production to the establishment year, this was achieved by deferring defoliation in late spring, with a loss of about 32% of edible DM. Also, as there was little high temperature dormancy of seed, it is expected that in a wet summer, this would result in few seeds remaining to germinate when cooler autumn conditions prevail. Furthermore, the management required to ensure survival of biennial ryegrass into late spring, to allow plants to set seed, is more critical than for perennial ryegrass, as the biennial genotype was observed to be more sensitive to frequent defoliation, and this confirms earlier glasshouse studies by Fulkerson *et al.* (1994).

Since white clover is the usual companion species for perennial ryegrass (Fulkerson *et al.* 1993a), any consideration of the persistence of perennial ryegrass must also consider the

persistence of white clover. The low seedling recruitment of white clover and poor survival of seedlings observed under subtropical conditions in our studies, reinforce the view that perenniality of white clover (particularly under irrigation) relies almost exclusively on stolon survival (Hollowell 1966; Fulkerson and Slack 1996a).

Fulkerson and Slack (1996a) defined an optimal defoliation interval for white clover in the subtropics which was based on the lower (younger) leaves of the plant beginning to senesce, since even moderate shading is detrimental to survival of white clover (Brougham 1958b; Ostrowski 1972; Brougham *et al.* 1978). Our studies indicated that for a large-leafed variety of white clover such as Haifa or Osceola, this interval was approximately equivalent to regrowth of 3 leaves/tiller of the companion perennial ryegrass. Thus defoliation at the 3-leaf stage of perennial ryegrass also appears to be optimal for white clover persistence.

More frequent grazing in spring increased the density of white clover growing points at this time, by the same process as it increased tiller density in perennial ryegrass - through allowing more light into the sward to stimulate the stolon branching process (Dennis and Woledge 1985; Robin *et al.* 1994a, b). However, less of these growing points survived the following summer; this may have been due to a decrease in white clover reserve levels and restricted rooting, as was the case with perennial ryegrass. Also, frequent defoliation would increase the effects of selective grazing (Frame 1966a, b; Evans and Williams 1987) and soil compaction (Edmond 1964; Frame 1966b), which are more detrimental to white clover than to perennial ryegrass. Furthermore, frequent defoliation would decrease white clover survival by allowing greater incursion of tropical grasses, since even a summer-active white clover cultivar like Osceola was not vigorous enough to out compete tropical grasses (primarily kikuyu). This is consistent with a previous study in New Zealand by Lambert *et al.* (1979), where DM yield of white clover was reduced by up to 37% in the presence of kikuyu.

Perennation of white clover from stolons relies on stolon breakup, which results in the loss of the primary taproot, and fragmentation of the plant at stolon internodes, to produce many small stolon fragments, reliant on development of nodal roots for survival. This breakup occurs in spring in temperate areas (Brock *et al.* 1988; Pinxterhuis *et al.* 1993; Brock and Hay 1996). The current studies identified, for the first time, that in the subtropics, stolon breakup

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is in the summer. This was noted under both cutting and grazing, in sequential summers for the 2 white clover varieties investigated.

The breakup period in the subtropics coincided with above-optimal temperatures, a high likelihood of waterlogging through summer rainfall, a buildup of nematodes in plant roots to levels likely to be detrimental to white clover growth and performance (L.J. McLeish and G.N. Berg, personal communications), and the presence of various species of fungi in stolon fragments. These factors may have combined to cause the high death rate (greater than 70%) of stolon fragments observed within the first month of breakup. Previous research in subtropical Australia under dryland conditions (Irwin and Jones 1977; Jones 1982; Garden 1988), showed stolon death of white clover peaked in the first summer, however this was attributed to moisture stress and pathogen damage, and not the physiological process of stolon breakup.

A more frequent defoliation interval than 3 leaves/tiller during late spring and early summer was shown to be beneficial for ryegrass persistence, but this is likely only if leaves are badly infested with rust, and/or are being shaded by tropical grasses. In these circumstances, a longer defoliation interval will be more detrimental to survival of perennial ryegrass, than the deleterious effects of frequent defoliation. However, this short defoliation interval will still reduce growth and persistence of the plant, as evidenced by Lancashire (1974) in New Zealand. He 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, but by up to 45% under a 40-day rotation. However where rust was negligible, the longer grazing interval gave a 150% higher DM yield than grazing at 10-day intervals. Also in New Zealand, Thom et al. (1986) prevented tropical grasses from shading neighbouring perennial ryegrass plants, by clipping potentially shading leaves of the tropical grasses 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.

Over summer, irrigation will only have a positive effect on perennial ryegrass and white clover if the interval between successive irrigations is short enough (less than one week) to benefit these shallow-rooted species, otherwise it will only benefit the deeper-rooted tropical grasses and weeds.

In an applied context, the results of these studies have shown that perennial ryegrass needs to be defoliated at about the 3-leaf stage of regrowth to optimise growth and persistence. This also applies to the companion white clover, especially if these are large-leafed varieties. The winter and early spring has been defined as the critical time for imposing a 3-leaf defoliation interval. In contrast, in late spring, survival over summer may be improved by reducing grazing interval to 1½ to 2 leaves/tiller, if leaf rust is severe, however, this will still reduce growth and persistence of the plant as evidenced in the absence of rust. The results also explain why grazing interval is so important for persistence.

Grazing at the 3-leaf stage results in greater survival of ryegrass plants, and less invasion of tropical grasses, than more frequent defoliation. This greater persistence of infrequently-defoliated ryegrass plants was attributed to greater survival of tillers over the summer, with these tillers being larger, having a more extensive root system, and accumulating higher levels of WSC reserves, compared to plants subject to more frequent defoliation.