A STUDY OF SEASONAL ROOT AND TILLER DYNAMICS IN SWARDS OF PERENNIAL RYEGRASS (*Lolium perenne* L.).

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ABSTRACT

Objectives of this study were (i) to provide data on seasonal variation in root mass and root replacement in perennial ryegrass dominant swards, (ii) to simultaneously collect parallel data for above-ground parameters tiller population density, tiller natality, tiller mortality, herbage mass and herbage production, and (iii) to determine if such information on the behaviour of root and shoot systems and the inter-relation between the two could identify ways in which grazing management manipulation favouring root system development might subsequently result in pasture production increases.

Perennial ryegrass was chosen for study because it is the species most commonly used in new pasture sowings in New Zealand. Four field experiments and two glasshouse experiments are reported.

In the first field experiment, techniques for making measurements of root mass and root production in field swards were evaluated. Over 80 days from November 1985 to February 1986, total root mass measured by washing roots from "intact" soil cores did not change, but root mass in core-holes bored out and "refilled" with sand was 53% of that in intact cores. The refilled core technique was therefore adopted as a measure of "apparent" root production, and a later calibration study showed that measurements using the refilled core technique underestimate actual root growth. Using the refilled core technique, differences in root production were detected between six mowing treatments designed to allow varying degrees of reproductive development. Root growth was greater where mowing of swards was delayed sufficiently to allow reproductive growth until head emergence or anthesis than where seedheads were either removed before head emergence or left un-mown until seed-set. There was also evidence of increased tillering on treatments with the highest root growth.

In the second experiment (December 1986 to May 1988) plots were subjected to lax (LL) or severe (HH) grazing management or to cross-over LH or HL grazing managements. The cross-over date, December 7 1987, was timed to coincide with peak reproductive development. Swards in this study had approximately 100 m m^{-2} underground stolon, with a seasonal increase in late winter and higher stolon formation on LL plots than on HH plots. Apparent root growth rates exhibited marked seasonal variation, and were typically about 15% of above-ground net production. For 12 months from January 1987 to January 1988 apparent root growth averaged 8.4 and

7.3 kg DM ha^{-1} day^{-1} for LL and HH plots, respectively for 0 - 600 mm soil depth. Because of these relatively small differences in root growth, it was concluded that manipulation of root growth would not enable herbage production advantages to be achieved. However, after introduction of cross-over grazing managements, high herbage production was observed on LH plots and tissue turnover and herbage dissection measurements showed that this high herbage production was associated with high daughter tiller formation, probably from stubs of decapitated flowering tillers.

Experiment 3 (November 1988 to January 1989) comprised 3 plots under common grazing management, and was designed to provide detailed information on the location on the tiller axis of actively elongating roots, and to confirm seasonal patterns of root and tiller growth observed in Experiment 2. Root initiation normally occurred at the same node as leaf senescence, normally two roots formed at each node, and few active roots were found more than 10 nodes below the last leaf. Seasonal timing of peak root growth and tiller appearance was different from that in Experiment 2, however. This is believed to reflect genetic differences between the cultivars 'Ellett' used in Experiment 2 and 'Grasslands Ruanui' used in Experiment 3, but specifically designed controlled comparisons would be needed to confirm this.

Experiments 4, 5, and 6 were designed to provide more information on the reasons for high tillering on LH plots in Experiment 2, and investigated the number of daughter tillers formed by flowering tillers subjected to differing cutting treatments. In all three experiments the number and weight of daughter tillers formed was greatest where a degree of reproductive growth occurred, and was reduced where seedheads were cut closer to the ground or earlier, and where seedheads remained uncut to act as a competing sink. These observations indicate that assimilate from parent flowering tillers is important for daughter tiller formation and, in Experiment 6, a cutting treatment which increased translocation of carbon-14 tracer from labelled flowering tillers to daughter tillers also increased the number and weight of daughter tillers formed.

It is concluded that grazing management which exploits the potential for high tillering rates from stubs of flowering tillers could increase herbage production on many New Zealand farms by more than 0.5 t DM ha^{-1} over the summer/autumn period, and implications for farm practice are briefly discussed.

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CHAPTER 1: INTRODUCTION AND OBJECTIVES

1.1 Introduction

One major thrust of Agronomic research in New Zealand in the post second world war period has been to understand the responses of grass swards to manipulation by grazing management, with a view to optimising herbage production, within the constraints imposed by the animal component of the grazing system. Such research has not been unique to New Zealand, however. Sward behaviour was being researched in Britain and elsewhere much earlier, and research dating to the turn of the century is sometimes cited, still. What has happened in the last 40 years is that New Zealand research has focussed more sharply on questions relating to sward manipulation, as other avenues of more substantial advance, for example correction of soil trace element deficiencies in Central North Island volcanic soils, have become exhausted.

Much of this previous research has concentrated on aspects of tiller dynamics and there is as yet little information on the responses to grazing management of root systems of herbage grasses. In fact it has been a feature of agronomic research world-wide that behaviour of root systems of herbage grasses in the field is studied much less frequently than behaviour of shoot systems, and there is a disparity of knowledge about the above-ground and below-ground structure of grasslands. Davidson (1978) referred to root systems as the forgotten component of pastures and Bohm (1979) has said that root research under natural field conditions is "still a stepchild of science." Hunt & Easton (1989), in a major review of ryegrass research in New Zealand, dealt with the topic of roots in two short paragraphs, noting that there is an annual cycle of root replacement in late winter (Jacques, 1956) and that root growth is sensitive to defoliation (Evans 1971ab, 1973).

Furthermore, almost all previous studies of root behaviour of grass swards have lacked an integration of data on root dynamics with data on tiller dynamics. Root-shoot relationships are often modelled mathematically in the functional sense and Wilson (1988) has reviewed this subject, but theoretical studies on partitioning of mass between root and shoot at one point in time do not necessarily answer questions relevant to on-farm grazing management. The only previous study known to the author which has made

any attempt to link above-ground and below-ground data from field swards in the way outlined above was that of Garwood (1965). However, in Garwood's research, the emphasis was on describing irrigation effects on root development, and grazing management effects on root and tiller production were not studied.

Another consideration is the need to demonstrate that detailed information on sward behaviour can be used to generate pasture production advantages. For example, the New Zealand farmer would likely be more interested in the effects of alternative grazing management strategies on root development and on subsequent herbage production than in questions of root:shoot partitioning *per se*.

The current study therefore sought to draw together three rather disparate threads; namely the need for information on root growth responses following sward manipulation by grazing management; the need to consider the plant as a functional whole rather than to make measurements on the root system in isolation; and a pragmatic interest in identifying strategies for increased pasture production.

1.2 Objectives

Accordingly, the initial experimental objectives were:

i) To provide data on seasonal variation in root mass and root replacement for field swards of perennial ryegrass (*Lolium perenne* L.) subjected to contrasting lax and hard grazing management regimes at Palmerston North, New Zealand.

ii) To parallel root measurements with simultaneous measurements of above-ground parameters, especially seasonal variation in tiller population densities and in tiller appearance and death rates; and examine the link, if any, between root and shoot parameters.

iii) To explore the possibility of obtaining systematic pasture growth rate advantages if the dynamics of the root system and any seasonal interactions between root and shoot systems of field swards were understood and taken into account when formulating grazing management strategies.

Ryegrass was chosen because this species is used in the majority of new pasture sowings throughout New Zealand (Sangakkara *et al.*, 1982; Belgrave *et al.*, 1990). Potential increases in pasture productivity arising from varying grazing management strategy are generally held to be small. For example L'Huillier & Bryant (1987) reported that net herbage accumulation and per cow production was some 10% higher on swards grazed at 30 day intervals than on swards set stocked in spring, but this advantage was offset by corresponding decreases in production later in the season. Such findings need not be a deterrent to future research, however. New Zealand farmers currently trade in an environment of falling export prices and rising costs, so that any strategy leading to a reduced cost of production is likely to be of substantial interest to pastoral farmers. Furthermore, even a small percentage increase in pasture productivity on a farm scale has a large potential value on a national scale when it is considered that there are some 14 million ha of pastoral land in New Zealand, (Department of Statistics, 1989), of which some 9 million ha is sown pasture.

1.3 Overview of experimental programme

Following a review of the literature, six experiments are reported. The first (Experiment 1, November 1985 to February 1986) investigated techniques for root measurement and examined the effect on root growth of manipulation of sward reproductive growth. These results are presented in Chapter 3.

Experiment 2 (December 1986 to May 1988) monitored root growth and sward tiller dynamics for contrasting lax and hard grazing managements. For Experiment 2, results of root measurements are reported in Chapter 4, and results of above-ground measurements in Chapter 5. A follow-up experiment (Experiment 3, November 1988 to January 1990) was conducted to clarify certain aspects of the results from Experiment 2, and is reported in Chapter 6. Results from Experiments 2 and 3 were further analysed by multivariate analysis in order to provide a mathematical description of root-shoot relations (Chapter 7).

From results of Experiments 1, 2 & 3, a hypothesis was developed regarding a possible strategy for using grazing management to enhance sward productivity. A small field experiment (Experiment 4, November 1988 to January 1989) and two glasshouse experiments (Experiments 5 & 6, September to December, 1989) were conducted to test this hypothesis, and results from these three experiments are reported in Chapter 8. A final summary and conclusions are presented in Chapter 9.

CHAPTER 2: LITERATURE REVIEW.

2.1 Introduction and overview

In this review, a brief outline is given of important milestones in sward dynamics research affecting farm practice in New Zealand, with particular reference to research aimed at identifying ways to increase herbage production.

A convenient historical starting point for this review is the 1950's research carried on at DSIR, Grasslands Division, Palmerston North, which centred on grazing management for optimisation of herbage mass (Section 2.2.1) or sward photosynthetic capacity (Section 2.2.2). In the 1960's and 1970's a new focus emerged, namely research into factors governing the behaviour of tiller populations, and this topic is reviewed in more detail (Section 2.2.3). More recently, there has been interest in the effects of variation in grazing management during sward reproductive growth in spring (Section 2.2.4).

Against this background of extensive research into above-ground behaviour of grass swards, research on pasture root systems has been limited, and there are gaps in the knowledge (Section 2.3). Existing information on root-shoot relationships in grazed pastures is almost all theoretical or based on glasshouse studies, and it is concluded that further field research in this area would be desirable (Section 2.4). Finally, techniques appropriate to such a study are examined (Section 2.5), and the objectives for a first experiment identified (Section 2.6).

2.2 Increasing herbage production through manipulation of above-ground organs of grass swards

2.2.1 Manipulation of herbage mass

In New Zealand there has been a longstanding awareness of the link between grazing management and pasture production, and rotational grazing was trialled on dairy pastures at Massey University as early as 1938 (Riddet, 1951) with the expectation that the practice would improve pasture productivity and utilisation. However the first serious attempt to systematise and quantify the relationships between herbage mass and herbage accumulation was the work of Brougham in the 1950's.

Brougham (1955), for rotationally grazed pastures, found that accumulation of herbage mass over time, when corrected for effects of weather, followed a sigmoid trajectory. Subsequent studies examined the effect of season (Brougham, 1956a) and of different initial values of herbage mass (Brougham, 1956b), and both these studies confirmed the sigmoid pattern of herbage accumulation over time during the regrowth phase of rotationally grazed swards. Brougham therefore concluded that grazing managements which avoid either extremely short or extremely long grazing intervals should optimise herbage production by avoiding what he called Phase 1 and Phase 3 (Brougham, 1957), the exponential and asymptotic phases of a pasture regrowth curve.

However, subsequent experiments (Brougham, 1957, 1959, 1960a) showed that optimisation of herbage production in practice was complex, and was not always achieved by adhering to the theoretical guidelines which took account only of herbage mass. In particular, hard grazing in autumn was found to enhance, rather than suppress herbage accumulation (Brougham, 1960a). Even so, Brougham's research led to recognition that potential production was lost when swards remained ungrazed in winter (Brougham, 1970). Surprisingly, it was more than 20 years after Brougham's initial research that these changes in herbage accumulation at differing herbage masses were explained definitively, using tissue turnover techniques (Davies, 1981), in terms of an asymptotic relationship between leaf elongation and herbage mass and a linear or curvilinear relationship between leaf senescence and herbage mass (Bircham & Hodgson, 1983; Parsons *et al.*, 1983a,b). Although Brougham's (*loc. cit.*) studies related to rotationally grazed swards and those of Bircham & Hodgson (1983) and of Parsons *et al.* (1983a,b) were for continuously grazed swards, it has subsequently been demonstrated that average tissue flows for a rotationally grazed sward are approximately equal to those for swards continuously grazed at the same herbage mass as the average herbage mass for the rotationally grazed sward (Parsons *et al.*, 1988a).

During the interval between Brougham's research and the emergence of the understanding that the relationship between senescence and herbage mass is different from that between leaf elongation and herbage mass, a system of winter rotational grazing had evolved in New Zealand. Initially rotational grazing was advocated to farmers by extension officers on the basis that it improved herbage production, and white clover content of pastures (Smith *et*

al., 1976). Data of Parmenter & Boswell (1983) show that less frequent grazing of winter pastures, as occurs in "autumn-saved pasture" systems, results in increased senescence losses as described above, and also losses of spring potential productivity, possibly attributable to reduction in grass tiller density and clover stolon density following prolonged periods at high herbage mass (Section 2.2.3 below). In this sense the claims of Smith *et al.* (1976) that rotational grazing improves clover content of pastures would be valid, however, it is now generally accepted that the real benefit of rotational grazing in winter in the New Zealand context is control of herbage intake by animals (Sheath *et al.*, 1987). Thus, adoption of Brougham's ideas has been accompanied by a shift in thinking, towards a view that rotational grazing effectively allows transfer of surplus autumn feed forward in time without an accumulation of herbage mass on any one paddock.

Recently Parsons *et al.* (1988a) have pointed out that the relationship between growth and senescence in a rotationally grazed sward increasing in herbage mass is different from that in continuously grazed swards at constant herbage mass (Parsons *et al.*, 1983a) because when herbage mass is increasing, senescence rates are initially very low and there is a delay between growth and senescence. Parsons *et al.* (1988a) therefore claimed that the idea of a sigmoid pattern of regrowth is "fundamentally incorrect". While the argument of Parsons *et al.* (1988a) is technically correct, it should be noted that the real reason for the adoption of Brougham's ideas by New Zealand farmers is to achieve feed rationing, not to increase herbage production.

This question of whether or not there is a systematic difference in productivity between rotationally grazed and continuously grazed swards has been researched extensively in a number of different countries and in various contexts. In New Zealand, McMeekan (1960) and McMeekan & Walshe (1963) recorded little difference in pasture production between rotationally grazed and continuously grazed systems, but found that higher stocking rates, and hence higher rates of animal production, could be sustained on rotationally grazed dairy pastures. On the other hand Clark *et al.* (1982) found that rotational grazing in summer reduced live weight in ewes. In many cases such seemingly contradictory results can be reconciled by considering differences in herbage mass which appear over time as a result of different herbage intake by animals on different grazing management treatments. Hence, restriction of herbage intake by rotational grazing during a period when pasture growth is in excess of animal demand,

as in the case of Clark *et al.* (1982), can result in higher average herbage mass on a whole farm basis with a consequent increase in the proportion of dead material and loss of sward quality. Conversely, in winter, use of rotational grazing to restrict herbage intake at a time of low pasture growth, can prevent average herbage mass falling to levels so low that net pasture production is also reduced.

More fundamental comparisons of rotational and continuous grazing have also been conducted. Lantinga (1985) made detailed physiological measurements, and concluded that there was no intrinsic productivity difference between continuous and rotational grazing, but that continuous grazing is the best way to maintain high sward quality in the long term. Parsons *et al.* (1988 a,b) used a mechanistic model to examine the same question. These authors showed that rotationally grazed swards have a theoretical advantage during the regrowth phase because of the time lag between leaf elongation and senescence, arising from very low senescence rates among new leaves of recently grazed swards. However, they also showed that average values for leaf elongation and senescence in rotationally grazed swards are very similar to those of continuously grazed swards maintained at the same herbage mass, so that any theoretical advantage is small.

2.2.2 Sward light interception

Another approach to sward productivity has been to consider the capacity for light interception. This is logical, since all herbage accumulation derives ultimately from photosynthesis, and non-interception of some incident light implies that herbage accumulation may be below the optimum for the particular environmental conditions.

In this context, critical leaf area index has been defined (Brougham, 1958) as the leaf area at which 95% of incoming light is intercepted. Brougham (1958, 1960b) examined the leaf area index, chlorophyll content and light interception of swards of different herbage masses, but did not extend this work to establishing criteria for optimisation of herbage accumulation. The possibility that 95% light interception by the sward might be a useful criterion in defining optimum grazing strategy has been investigated by a number of workers, including Sheard & Winch (1966), Tainton (1974), and Korte *et al.* (1982). It was found that defoliation before 95% light interception reduced herbage accumulation (Sheard & Winch, 1966), and delaying defoliation for

two weeks after 95% light interception increased herbage accumulation in vegetative but not reproductive swards (Korte *et al.*, 1982). However, while these studies increase understanding of sward properties at differing levels of herbage mass, they do not offer the farmer a means to systematically increase pasture growth, beyond that already obtained through use of herbage mass criteria.

Factors affecting the photosynthetic capacity of individual leaves have also been studied and it is known that the developmental history of leaves may affect their photosynthetic capacity. Leaves developing in a high light environment attain higher photosynthetic capacity per unit area than leaves developing in a low light environment (Woledge, 1977; Parsons *et al.*, 1988b; Robson *et al.*, 1988) and photosynthetic capacity of leaves declines with age (Brown *et al.*, 1966; Woledge, 1972; Parsons *et al.*,1983a; Robson *et al.*, 1988). However, computer modelling of these responses has not led to identification of strategies for increased herbage production (Parsons *et al.*, 1988a,b).

2.2.3 Manipulation of tiller population density

One line of investigation of tiller dynamics has been to consider plant morphology and the number of sites available for tiller production (Mitchell, 1953; Booysen *et al.*, 1963; Davies, 1974), and this is discussed further below (Section 2.4.1), in conjunction with root production.

Another line of investigation has been to identify environmental and plant physiological factors which control tiller appearance. Such studies include those of Langer (1963), Jewiss (1972), Ong (1978a,b), Ong & Marshall (1979), and have shown that levels of light near the base of the tiller, nutrient supply, and high carbohydrate status are all major factors in promoting tillering. This understanding is important, but in general serves to predict effects of changes in the environment, as distinct from effects of manipulation by grazing management in a particular environment.

The effects on tiller population density of variation in grazing management have been reviewed by Arosteguy (1982), who notes that, compared to intermediate levels of defoliation, more severe or laxer levels of defoliation have been observed to result in lower tiller population density. Also, continuously grazed swards had higher tiller populations than similar rotationally grazed swards (Hodgson & Wade, 1978).

These responses represent the net effect of a number of different factors operating to either increase or decrease tiller natality or mortality. The more important of these factors appear to be:

(i) Stimulation of tiller appearance when light reaches the base of more closely grazed swards (Langer, 1963; Langer 1979). Recent evidence suggests this effect is phytochrome-mediated (Casal *et al.*, 1985).

(ii) Reduced tiller appearance (Mitchell & Coles, 1955; Davies *et al.*, 1983) and an increase in tiller mortality (especially young tillers, Ong, 1978b) if excessive shading occurs at high herbage masses.

(iii) Inhibition of tillering after more severe or repeated defoliation, partly resulting from low carbohydrate status (Auda, *et al.*, 1966; Davies, 1974).

(iv) Variation in numbers of tillers physically removed by animals during grazing or dying shortly after grazing. Removal is higher under rotational than continuous grazing (Hunt, 1989) higher under cattle grazing than sheep grazing (Arosteguy, 1982) and higher under more severe grazing (Bircham & Hodgson, 1983).

It is also clear that tiller populations are subject to size/density compensation (Lonsdale & Watkinson, 1982, 1983; Davies, 1988), and it is generally believed that size/density compensation largely negates any potential advantages from manipulating tiller density, although few studies have addressed this specific point. It is true that where a closely grazed sward with a high tiller population density is allowed an extended period of regrowth, gross leaf accumulation is higher than on a similar sward with a different grazing history and a lower tiller population density (Bircham, 1981; Parsons *et al.*, 1984; Grant *et al.*, 1988). However, such increases in herbage accumulation are temporary and tend to be offset by production decreases during the period of intensive grazing required to induce the high tiller density in the first place (Parsons *et al.*, 1984; Grant *et al.*, 1988). It remains to be demonstrated convincingly, therefore, that manipulation of tiller density *per se.* can increase herbage production.

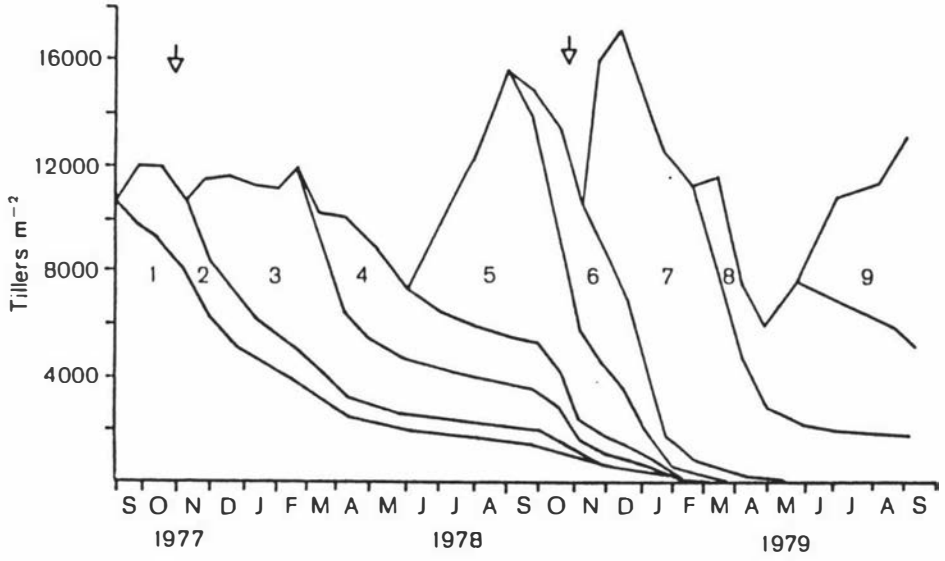
A third avenue of study has been the determination of tiller longevity and of seasonal patterns of tiller natality and mortality, with a view to identifying ways in which the natural cycle of tiller replacement might be enhanced. If such studies are analysed, three levels of complexity can be identified.

First, many authors present graphs of changes in tiller population over a period of time, and frequently an increase in tiller population density in late winter or early spring is the visually striking feature of the data (see e.g. Garwood, 1969; Hunt, 1989). However, this leaves ambiguity as to the relative contributions of tiller natality and mortality to any observed change in population density. Therefore most authors, including Garwood (1969), L'Huillier (1987), and Hunt (1989) also present tiller appearance and death rates obtained by short term monitoring of tagged tillers. This represents a second level of complexity.

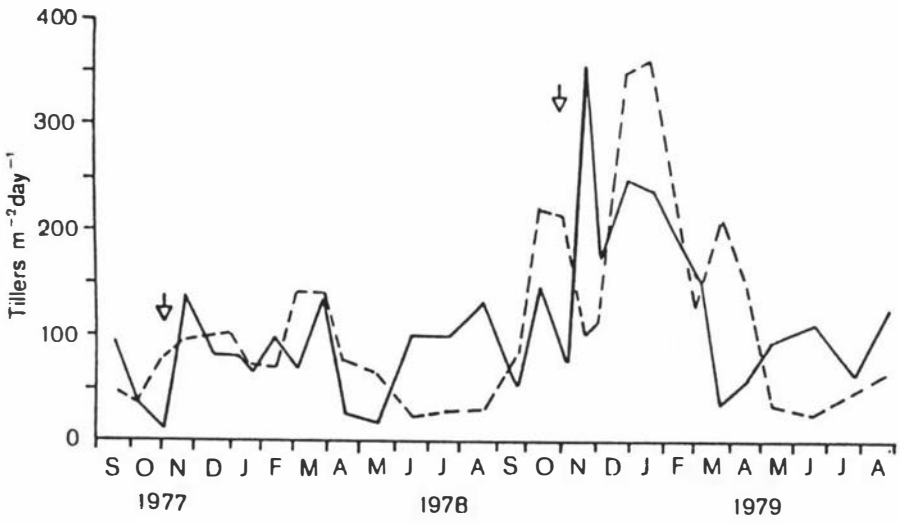
Thirdly, a very few studies have involved the observation of individual tillers for 1 to 2 years in order to establish the longevity of tillers appearing at particular times of the year. Such data can be presented as a tiller age-cohort survivorship diagram (Jewiss, 1966) and relatively few studies have defined tiller dynamics at this third level of complexity. Those that have, however (including Langer, 1956; Jewiss, 1966; Colvill & Marshall, 1984; Korte, 1986), all indicate a substantial turnover of the tiller population in early-summer, coinciding with flowering. The percentage of tillers dying and being replaced in early-summer can be as high as 80-90%, and appears to show both inter- and intra-specific variation (Jewiss 1966).

Garwood's (1969) and Korte's (1986) data illustrate well the way in which this type of information can be used to identify differences in the behaviour of different swards. In Korte's (1986) study, a late-winter rise in tiller density from some 8,000 tillers m^{-2} to more than 15,000 tillers m^{-2} is accounted for by a rather subtle decrease in tiller death during June, July and August (Figures 2.1a,b). Tiller appearance was 2 to 4 times higher in December than in late winter (Figure 2.1b), but there was little change in tiller population density during December because tiller death rates were also high at this time (Figures 2.1a,b). A similar seasonality of tiller natality and mortality was also recorded by L'Huillier (1987).

Figure 2.1. (a) Tiller age-cohort survival diagram showing tiller population density by age categories for a 'Grasslands Nui' ryegrass sward over 2 years at Palmerston North. (Korte, 1986). Arrows indicate defoliation of the main crop of reproductive tillers.



(b) Tiller natality (————) and mortality (— — —) for the same sward.



Garwood's (1969) data is not reproduced here, but for swards of S23 and S24 perennial ryegrasses at Hurley (Britain) he also observed a spring increase in tiller density. However, seasonal patterns of tiller natality and mortality which he recorded do not match those observed by Korte (1986). In particular, tiller appearance rates in Garwood's (1969) study showed peaks

in April and October (Northern hemisphere spring and autumn), and this pattern is quite different from that observed by Korte (1986).

Korte's (1986) use of the fixed quadrat method for determining tiller natality and mortality means that age structures of the tiller population are known (Figure 2.1a), whereas this information is not available from Garwood's (1969) study. On the other hand, a disadvantage of the fixed quadrat method is that the disturbance of marking tillers can stimulate tiller production, and so could bias results (Arosteguy, 1982).

The possibility of manipulating the age structure of a tiller population has been discussed briefly by Tallowin (1981), who concluded that there is a need to quantify the relative contributions of tillers of different age classes to sward production and persistence. It would appear, however that none of the studies published to date has provided information on the contribution of different age classes of tillers to herbage production, or on age-classes of tillers active in producing new tillers, although information on tillering activity of particular age classes of tillers has recently been collected for 'Grasslands Matua' prairie grass (*Bromus willdenowii* Kunth.; C. K. Black, unpublished data).

Although investigation of tiller dynamics is not in itself an objective of the present study (Section 1.2) it is evident from the above discussion that knowledge of certain aspects of tiller dynamics in grass swards is incomplete, and that there could be possibilities for meeting Objective iii (obtaining systematic pasture growth rate advantages, see Section 1.2) through improved knowledge of tiller dynamics of the grass sward.

2.2.4 Control of reproductive growth

The period of intense tillering activity observed by Korte (1986) and L'Huillier (1987) is associated with sward reproductive growth and occurs at a time when pasture supply typically exceeds animal demand, giving farmers some flexibility to vary grazing management. (In winter, grazing regimes are largely determined by feed-rationing constraints.) There has therefore been considerable research in New Zealand aimed at identifying grazing management strategies to "control" or remove reproductive growth, in order to enhance tillering, which is popularly held to be inhibited by the presence of seedheads, due to apical dominance effects (Matthew, 1991).

Because reproductive growth is associated with a decrease in herbage digestibility (Browse *et al.*, 1981), control of reproductive growth is held to optimise both sward performance and animal performance during the early-summer period (Hughes, 1983). Korte *et al.* (1982) concluded that control of reproductive development was more important than management to control leaf area and light interception, and that leafy vegetative swards in summer were obtained by hard grazings which removed reproductive tillers (Korte *et al.*, 1984). The value of control of reproductive growth in this way has also been established for dairy pastures (Hoogendoorn, 1987), however, in hill country the primary reason advanced for control of reproductive growth is usually the need to reduce the level of surplus pasture herbage accumulation in spring (Sheath *et al.*, 1984). The reduction in herbage accumulation rate following control of reproductive growth is important in hill country management because pasture growth rates in hill swards typically show a proportionately greater contrast between winter and late-spring than do pasture growth rates for lowland pastures, with low winter pasture growth being a factor limiting stocking rates of hill swards. The data of Clark *et al.* (1982) demonstrates how the resulting imbalance between herbage accumulation and herbage consumption on a hill country farm in late-spring can result in excessive accumulation of uneaten herbage and consequent reduction in animal performance. Management of late-spring reproductive growth is therefore an important aspect of farm management for both lowland and hill country properties, and is often discussed at farmer conferences (see e.g. Hughes, 1983; Sheath & Bircham, 1983; Roadley, 1985). In Britain, management of reproductive growth is also an issue, but for a different reason, namely that animals are kept indoors over winter. As a result, turnout date has an important bearing on the control of reproductive growth (Carton *et al.*, 1989), as does the need to conserve feed during summer to meet animal requirements in winter (Hodgson, 1990).

One aspect of control of reproductive growth which does need further study, however, is the implementation on a whole farm scale, since the recommended harder grazing regime implies reduced herbage consumption by animals and therefore, paradoxically, decreased removal of seedheads over the farm as a whole. Hoogendoorn's (1987) work was confined to paddock scale trials. Korte *et al.* (1984) noted the difficulty in achieving their recommended close grazing of whole farms at a time of surplus pasture growth and suggested forage conservation and mechanical topping as

possible solutions. Butler (1986) made a significant contribution in demonstrating that faster rotations leaving a higher residual herbage mass could achieve control of reproductive growth without the reduction in animal intake associated with grazing to lower herbage masses and L'Huillier (1987) has pointed out that high stocking rate is a useful means of achieving pasture control. However there is as yet no consensus among researchers or farmers as to how best to control reproductive growth on a whole farm scale.

2.3 Root systems of field swards

2.3.1 Root distribution and seasonal patterns of replacement

The paucity of literature on the behaviour of root systems of field swards has already been mentioned (Section 1.1). This is not to say that root systems of grasses have seldom been studied. A review by Troughton (1957) cites some 800 references, and although there is no recent comprehensive review, research on roots has continued. The great majority of this research, however, has been carried out under very closely controlled conditions, usually in the laboratory. Even where field measurements have been made, early studies often present only qualitative analysis or diagrammatic data on root distribution. Where quantitative data is presented this is usually for root number or root mass, because techniques for measuring root length are relatively recent (Section 2.5.2.3).

For example, Stuckey (1941) classified a number of grasses as having annual (timothy, perennial ryegrass) or perennial (cocksfoot, Kentucky bluegrass) root systems; while Weaver & Zink (1946) demonstrated that individual roots of a number of prairie grass species may live for two years or more, despite death of the tops every year. In New Zealand, Jacques carried out a large amount of research on pasture root systems and published many papers, but his description of root replacement in perennial ryegrass (Jacques, 1956) presents only a conceptualised diagram without data, illustrating an annual cycle in which roots appear in June and July and move down the soil profile during summer, reaching their maximum depth the following winter.

Seasonal variation in root elongation, number of new roots appearing and in root longevity have been presented by Garwood (1967a,b), and are

reproduced in Figure 2.2 (page 17). The numbers of new roots appearing and the rates of elongation were greatest in spring (April), while autumn roots tended to survive the winter, and lived longer than roots produced at other times of the year. Caradus & Evans (1977) found root appearance in New Zealand also to be greatest in spring (September).

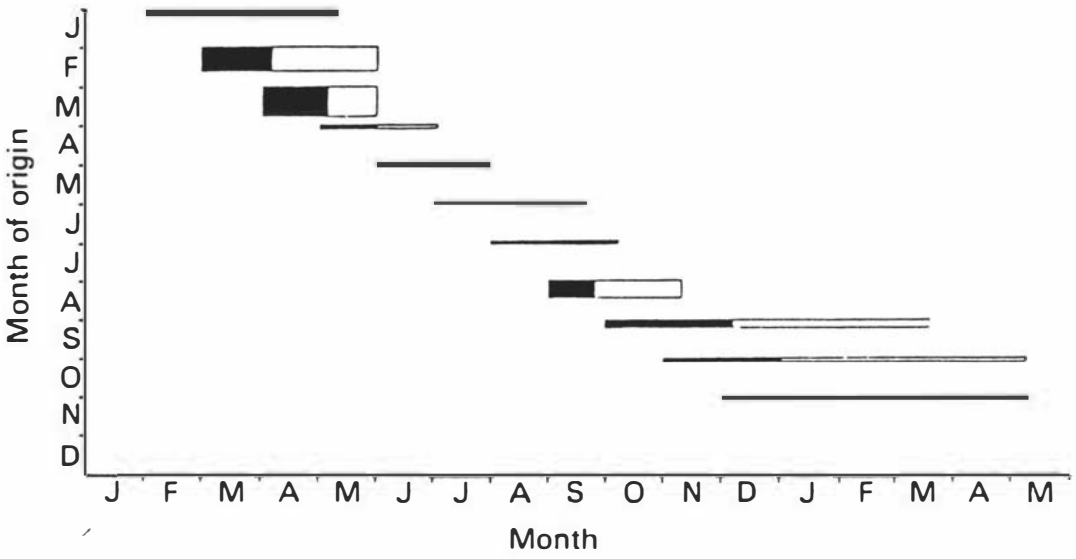
The reported root mass of grass swards varies markedly. Gibbs (1986) summarises 9 studies where root mass for ryegrass swards was reported and values range from 1.94 to 19.84 t DM ha^{-1}. The reason for this wide range is unclear, but variation in sampling depth, age of sward, and in rate of decomposition of dead roots probably all contribute. A consistent finding of all studies, however, is that root mass is concentrated near the soil surface, with typically some 60% to 80% of total root mass in the upper 0 - 150 mm soil depth. (see e.g. Gibbs, 1986; Barker *et al.*, 1988).

Likewise, there is no consensus in the literature about the length of life of grass roots. Jacques & Schwass (1956) estimated from the ratio of white roots to total roots per plant that perennial ryegrass replaces 68% of its root system annually and tall fescue 60%. Troughton (1981a) estimated root longevity by monitoring survival of plants prevented from forming new roots and estimated mean length of life of ryegrass roots to be 365 and 191 days for undefoliated and defoliated plants of perennial ryegrass, respectively. By contrast, Garwood's (1967b) data derived from direct observation of individual roots suggest an average life of approximately 180 days for autumn-formed roots and about 60 days in the case of summer-formed roots. Gibbs (1986) reported root longevities ranging from 29 to 81 days, and these data were also derived from direct observation of individual roots. One thing is clear, however. Those data derived from direct measurement suggest continuous turnover of the root system (as occurs above ground with leaves) rather than an annual replacement of the root system (Stuckey, 1941; Jacques, 1956; Hunt & Easton, 1989).

Root longevity should not be confused with root turnover, which is a function of both root longevity and rate of decomposition. Warembourg & Paul (1977) used ^{14}C to estimate root turnover of native grassland in Canada, and obtained a value of 107 days for the half life of roots. Gibbs (1986) found the half life of perennial ryegrass roots to be 254 days, but this value was derived from the ratio of roots present to cumulative total of roots formed. Therefore half life would be overestimated, because of the confounding

effect of including in the calculations newer roots which had not yet begun to decay.

Figure 2.2. Seasonal patterns of root elongation and longevity (Garwood, 1967b). For roots formed in a particular month, horizontal bars indicate the duration of elongation (solid bar) and the additional period over which roots are deemed live (hollow bar). Vertical thickness of bars is proportional to the number of roots produced in each month.



Related to root turnover, and of considerable interest in understanding the overall carbon economy of grazed swards, is the rate of root production expressed as mass flow. Atkinson (1984) details assumptions required to estimate mass flow from rhizotron observations of root appearance and presents values for apple trees ranging from 3.2 g m^{-3} $week^{-1}$ in early spring to 17.1 g m^{-3} $week^{-1}$ in autumn, however it appears the method has never been applied to measurements on grassland. Deinum (1985) has estimated mass flow based on change in instantaneous root mass over time and suggested that about 80 kg carbohydrate ha^{-1} day^{-1} was translocated to roots in grass swards in Holland. This method, however, is subject to difficulties parallel to those faced by Brougham (Section 2.2.1) in attempting to define dynamics of herbage mass accumulation without independent

measurements of leaf formation and leaf death. In Deinum's (1985) study root decomposition rates were merely estimated based on an assumed turnover of ½ per year. A method of measuring root mass production independently of root decomposition has recently been applied to measurements of root production in grass swards by Steen (1983, 1984; Section 2.5.2.4). Steen found that in a grass ley in Sweden, 4 - 6 t DM ha^{-1} were produced annually below ground. Steen's (1985) data also shows that some roots begin decomposing within a few weeks of formation, again suggesting continual turnover, rather than annual replacement of the root system.

2.3.2 Grazing management effects on roots

The effect of defoliation on root production has received extensive study over many years (see e.g. Roberts & Hunt, 1936; Mitchell, 1954; Jacques & Edmond, 1952; Evans 1973, 1976), and it is a consistent finding that defoliation reduces root growth (Troughton, 1957). Evans found that defoliation to 75, 50 or 25 mm reduced root growth of ryegrass plants to less than 65%, 30% and 10%, respectively, of control plants, and that the time for normal growth to be resumed ranged from 10 days at the laxer defoliation to 15 days with defoliation at 50 or 25 mm. From these observations he suggested (Evans, 1976) that limitation of root growth due to overgrazing might reduce herbage production during subsequent periods of stress, for example through a smaller volume of water being available to plants during dry spells. He also suggested that reduced root system size might account for pulling of plants in autumn when old roots were weakening but the next season's root growth cycle had not commenced. Based on Evans' findings, there appears to be need for more precise information on the way in which reduction in root growth due to untimely grazing or excessive grazing pressure might in turn limit herbage production.

2.4 Root - shoot relationships

There are two distinct aspects of root/shoot interaction. One aspect relates to the fact that there will be a finite number of bud sites for root and shoot formation, and examines initiation of new roots and shoots in terms of developmental morphology - the number of sites available, and the percentage of those sites which later develop into mature roots and shoots. The other aspect of root/shoot interaction concerns the allocation of nutrients

from roots to shoots and of photosynthesis products from shoots to roots. This topic has generated intense theoretical debate, and a number of distinct models describing the interaction between root and shoot systems of plants have been developed (Wilson, 1988).

2.4.1 Developmental morphology

The overwhelming emphasis in studies mentioned in Sections 2.2.1 to 2.2.3 above has been on understanding the behaviour of field swards as a population of individual tillers, or in terms of a response function defining the relationship between sward properties such as leaf area, and the input of external factors such as light. However, such a philosophy overlooks the possibility that a lack of growth sites for expression of responses might conceivably limit sward productivity. For this reason morphological investigation is also useful in understanding the behaviour of field swards.

Evans & Grover (1940) noted that morphology of graminaceous plants is determined by the strict segmental pattern of development, and basic anatomical studies (e.g. Soper & Mitchell, 1956; Hitch & Sharman 1971; Bell, 1976) confirm this. Notable attempts to use such information to analyse the behaviour of grass plants include mapping of nodal structure of plants of Kentucky bluegrass (*Poa pratensis* L.; Etter, 1951), similar maps for tiller hierarchies of perennial ryegrass (Mitchell, 1953), and analysis of leaf growth in ryegrass (Silsbury, 1970).

The segmental structure is also implicit in the concept of 'site filling' (F_s; Davies, 1974; Davies & Thomas 1983), and the analogous ratios for root:leaf (F_{rl}) and root:tiller (F_{rt}) appearance data (Hunt & Thomas, 1985). It is worth noting however, that while the authors who coined these terms were aware of an underlying morphological principle, they did not in fact set out to define behaviour of grass plants in terms of the segmental structure. Davies (1974) used 'tillers per site available' primarily as an index of the effects of cutting in stimulating or inhibiting tillering, while the analysis of Hunt & Thomas (1985) is more concerned to derive a mathematical model of plant growth than to identify underlying morphological relationships.

Davies (1974) calculated a theoretical maximum value for F_s of 0.48, although several authors including Davies (1974), Davies & Thomas (1983) and Simon & Lemaire (1987) report values in excess of this figure. More

recently a rigorous mathematical analysis of the concept of site filling has been published (Neuteboom & Lantinga, 1989) and shows that the theoretical maximum for F_s is in fact 0.69. Davies' (1974) calculation of the value 0.48 had not allowed for the possible formation of tillers from the prophyll axillary bud at the base of each tiller, an event which does commonly occur, at least in studies by Mitchell (1953).

Neuteboom & Lantinga (1989) also state that the calculations of Hunt & Thomas (1985) were incorrect, although the reasons given are stated intuitively rather than in the form of a mathematical proof. What does emerge categorically from Neuteboom & Lantinga's analysis, however, is that the definition of site filling ratio in terms of the segmental morphology of the plant requires the specification of an additional parameter not mentioned by Davies (1974) or Hunt & Thomas (1985). This parameter is the delay (n, number of leaf appearance intervals or phyllochrons) between leaf appearance and tiller appearance at a particular segment or phytomer.

A corollary of Neuteboom & Lantinga's (1989) definition of the parameter n, is that site filling is a complex concept determined by several variables, and therefore may at times be subject to ambiguities of interpretation. First, site filling ratio as defined by Davies (1974) could be reduced by either a higher value of n or by an increased proportion of nodes never producing tillers. Second, it is very possible that tillers of different hierarchical orders could display different values for n, so that results of calculations based on an average value might not reflect the behaviour of individual tillers. Thirdly, it may be that buds produced in certain seasons (for example winter) might have a tendency to remain dormant for several leaf appearance intervals, then develop at a time when environmental conditions were more favourable. Finally, if the concept were to be extended to roots, it would be necessary to not only incorporate a parameter defining the delay between leaf and root production at a particular node, but also to incorporate the possibility of multiple roots per node. In short, because of the delay between leaf appearance and tiller or root appearance at a particular node, and because of the possibility of multiple roots per node, calculation of values for F_s, F_{rl}, and F_{rs} in a field study, even if technically feasible, may not actually provide definitive information about root dynamics. Parameters which allow just one interpretation would be useful.

A more detailed analysis of segmental morphology which also gives a

notation for recording events at a particular segment and incorporates information on root production is given by Klepper *et al.* (1984) for wheat. In the study by Klepper *et al.* (1984) the ratio of roots:leaves was approximately 2 and, allowing for the delay between leaf and root production at a particular segment, this corresponds to 4 roots per node. Although there may not be immediate application in terms of identifying optimum grazing management strategies, it could be worthwhile to have information on the number of sites available for root production and the percentage of such sites in field swards typically developing into nodal roots. Such information does not appear to be currently available for ryegrass.

2.4.2 Root/shoot balance

Wilson (1988) divides the models describing root/shoot balance into 4 categories. The first of these is the allometric model, which assumes a linear relationship between log shoot weight and log root weight, the slope of which, *k*, is a sensitive indicator of logarithmic ratio of root growth to shoot growth (Troughton, 1956). One useful application of the allometric model is to check for inter-specific differences between root-shoot partitioning in plants growing in the same environment. However, *k* changes with environmental factors such as nutrient supply and with stage of plant development (decreased allocation to roots while flowering, for example) (Troughton, 1956). Models which allow for variation in *k* are termed "functional equilibrium" models (Brouwer, 1983; de Willigen & van Noordwijk, 1987; Wilson, 1988). A formal statement of functional equilibrium is that of Davidson (1969):

root mass x rate of absorption α leaf mass x rate of photosynthesis

The third category of model recognised by Wilson (1988) is Thornley's (Thornley, 1972) which is essentially an attempt to re-express Davidson's (1969) empirical statement of functional equilibrium in mechanistic terms. A fourth category is the hormone model which assumes that root/shoot balance is controlled through the action of hormones of root origin on the shoot and vice versa.

In this study, however, the interest in models of root/shoot balance is not to define the theoretical basis of plant behaviour. Rather, a model will be useful here if observed data from a field study are consistent with the model, and

the model then leads to identification of a strategy for manipulation of the sward so as to enhance productivity (Objective iii, Section 1.2).

2.4.3 Need for further study

From the above it is evident that further information on the behaviour of root systems of field swards would be useful. There is conflicting information, for example, as to the longevity and seasonality of replacement of ryegrass roots.

Furthermore, much of the research which has been done was not carried out on field swards, notable exceptions being the work of Garwood (1967a,b), Deinum (1985) and Steen (1985). For New Zealand conditions, the major study by Evans (1971a,b) used seedling plants in pots to avoid problems in distinguishing live from dead roots in established swards. Caradus & Evans (1977) do report seasonality of root replacement for field swards, but their data is for numbers of "new" nodal roots with no quantification of root production in terms of mass or length.

Thus, there is clearly a need for a field study, carried out under New Zealand conditions, to monitor seasonal changes in root production and changes induced by differing grazing managements. Data from such a study could then be used to identify key periods of root formation and the extent to which it is possible to manipulate root growth at such periods through varying the grazing management. With such information, it should be possible to develop specific grazing management recommendations so as to avoid reducing root growth and causing subsequent loss of herbage production (Evans 1976; Section 2.3.2); and also to answer a number of other questions about the behaviour of pasture root systems (Sections 2.4.1 & 2.4.2).

2.5 Techniques for root measurement

2.5.1 Introduction

Bohm's (1979) review of methods of studying root systems cites more than 1,000 references. Despite such a vast literature there have been very few papers reporting study of rates of turnover (dynamics) of root systems. The purpose of the brief review which follows is therefore not to give a

comprehensive description of methods for studying root systems, but rather to background the development of methods to be used in the present study where information such as mass flow of new root production and number of sites available for root production was desired.

2.5.2 Existing methods for root measurement

2.5.2.1 Early methods

Earlier workers, for example Weaver & Voigt (1950), commonly used excavation methods or extracted soil monoliths for study in the laboratory. These methods are extremely time consuming and most of Weaver & Voigt's (1950) results are presented in qualitative form as photographs for visual comparison by the reader. Weaver & Voigt (1950) do present some of their results in terms of root mass for particular soil horizons, but such data are presented so as to describe the vertical distribution of root rather than to draw conclusions about root function or root-shoot relationships.

2.5.2.2 Root mass

Root mass is readily obtained by washing and drying roots from soil core samples. Roberts & Hunt (1936), Jacques (1937), and Jacques (1943) are among early studies which present root mass data. Jacques (1943) presents profile drawings similar to the photographs of Weaver & Voigt (1950) but in addition presents comparative data on the weight of root (g) for unspecified sampling areas of plots which had received different fertiliser treatments. However, in Jacques' (1943) study, other quantitative data such as the ratio of numbers of roots:number of tillers per plant is used instead of the root mass data to make inferences about the relationship between root and shoot systems.

Many subsequent workers have reported root masses under pasture and as mentioned above (Section 2.3.1) Gibbs (1986) summarises 9 studies on ryegrass, all of which report root mass, with values ranging from 1.94 t DM ha^{-1} for 0 - 100 mm soil depth at 12 months after sowing to 19.84 t DM ha^{-1} for mature swards. In the more recent studies a shift in approach is evident, and authors presenting root mass data per unit ground area have tended to to use this root mass data to address directly, the functional relationship between root and shoot. This shift in approach is illustrated by the comment

of Bohm (1979) that root weight can be regarded as a fundamental measure of photosynthate storage in a plant, and is also the basis for calculation of root-shoot ratios. The study by Deinum (1985) referred to in Section 2.3.1 is a good example of the application of the approach implied by Bohm (1979). However, the uncertainties in Deinum's (1985) study with respect to estimation of actual mass fluxes from the net change in root mass over time highlights the limitations of using change of root mass over time as a measure of mass flow of root tissue.

Bohm (1979) recommends that correction for adhering soil be made by drying roots at 650 °C and weighing the ash residue. A refinement of this method is to treat the ash with hydrochloric acid, then dry, before weighing the ash residue. The acid treatment is assumed to redissolve mineral matter from the roots themselves, but not soil mineral matter, although this correction is in fact not recommended by Bohm (1979), and is not useful for calcareous soils in any case.

2.5.2.3 Root length

Root length determination became a practical possibility only as recently as 1966 with the development of the line intersect method (Head, 1966; Newman, 1966), later revised and simplified by Tennant (1975). Since then root length has been increasingly adopted as the preferred measure in root studies (Bohm 1979).

Root length is regarded as the most appropriate parameter for predicting water uptake by plant roots (Taylor & Klepper, 1975). In Fitter's (1976) studies of plant nutrition the ratio root mass:root length provided information on mean root diameter allowing inference to be drawn about the amount of branching and about potential for nutrient uptake; and mass:length ratios have also provided useful information in more general studies of root distribution in maize crops (Barber 1971). Measurement of root length rather than root mass is fundamental to the concept of "root occupancy ratio" proposed by Gandar and Hughes (1988).

2.5.2.4 The "net stocking" or "refilled core" technique

This technique, of which there are several variations, essentially involves making a hole in the soil profile, refilling the hole with soil containing no roots, and harvesting after a specified period to determine rate of growth of roots into the refilled core. Bohm (1979) credits the invention of this technique to Hendrickson & Veihmeyer (1931), and notes that the method has many applications, but is apparently little used as only two references to its use are cited. More recently the method was used for studies of root turnover in Scots pine (*Pinus sylvestris* L.; Person 1980), and has also been used in studies of pasture root turnover (Steen, 1983, 1984). Steen (1984) used 70 mm diameter cores refilled with sieved soil collected from the same plots in which the cores were sited. The cores were installed by placing a 6 mm mesh net stocking over a plastic tube of the same diameter as the core-hole. The mesh was then inserted into the core-hole and the plastic tube withdrawn as soil was packed in, leaving a refilled core marked by the net stocking.

This method has the major advantage that all roots found in the filled core at harvest must have formed since the placement of the core. In this sense the method gives at least an index of the rate of new root formation, a parameter which parallels gross production in tissue turnover studies (Davies, 1981). It must be noted, however, that the placement of the core itself could affect subsequent root behaviour within the core and introduce a possible source of error.

2.5.2.5 Minirhizotron observation tubes

This technique was also invented in the 1930's but little used until comparatively recently (Bohm 1979). The last ten years has seen the advent of miniature television cameras small enough to be lowered into a plastic tube and of image analysis technology. These developments have resulted in an upsurge of interest in the minirhizotron method. Troughton (1981b) notes that minirhizotrons allow for much greater flexibility of trial design at much lower cost than large scale walk-in size chambers (rhizotrons), at least 15 of which exist at various laboratories around the world.

One advantage of the method is that the tube-soil interface can be considered a thin slice of the soil profile under study, and the number of

roots arriving at the tube wall taken as a measure of the rate of root production (McMichael & Taylor, 1987). As mentioned above, Atkinson (1984) also derives root lengths per unit volume of bulk soil from minirhizotron counts, although Troughton (1981b) cautions that there is evidence that roots can concentrate at the interface between soil and window.

2.5.2.6 The "core-break" method

In this method soil cores are broken at specified positions and the number of roots emerging from the broken face counted. The counts can then be calibrated to give an estimate of root mass or root length. This method does save the steps of washing and cleaning the sample, and makes counting itself very rapid compared to direct root length determination. It has therefore been adopted by numerous workers, including Drew & Slaker (1980), Bragg *et al.* (1984) and Fairley (1985). It was not adopted in this study because of a concern that the indirect measurement might lead to systematic error, or at least to higher co-efficients of variation than direct measurement. Indeed, Gibbs (1986) found that values obtained by this method for root mass and root length of ryegrass swards in Canterbury were much lower than "should have been present."

2.5.2.7 Soil moisture extraction

Bohm (1979) reviews the use of soil moisture depletion rate (measured by the gravimetric method or the neutron probe method) as an indirect measure of root activity.

This method, theoretically, measures one aspect of root function, rather than simply quantifying the amount of root present. Given that the presence of dead roots in the profile can be a major problem in root studies (Jacques, 1956; Garwood, 1967a; Evans, 1970), a method which determined root activity, as opposed to total root mass or length, might be a major advantage.

2.5.2.8 Root staining

Carman (1982) found that chlorotriazinyl dyes could be used to stain plant root systems without damage to the roots themselves and that the stain

persisted for up to 30 days. By successive applications of different coloured dyes it was possible to colour-code roots of *Sorghum bicolor* L. according to their date of origin (Carman, 1982). The method is applicable only to porous soil media where infiltration time is rapid, because the stain solution is alkaline (pH > 7.5) and, in order to avoid root damage, must be flushed out with water after staining has occurred.

Gibbs (1986) used a staining technique adapted from Ward *et al.* (1978), and using congo red dye, to distinguish live from dead roots. The precise mode of action of this stain is uncertain, but it appears that the dye binds to certain cell wall components. Roots recently killed by boiling will stain, but roots which have begun to decompose do not stain. Another stain which has been used to attempt to distinguish live roots from dead roots is tetrazolium (Troughton, 1957), however this method, although widely used in the seed industry, appears to be unreliable for root staining because of slow penetration of the stain through suberised cells surrounding the root cortex, to the more actively respiring tissues of the stele.

2.5.2.9 Imaging technology

Recently plant root systems have been imaged non-destructively both by computer assisted tomography (CAT) and nuclear magnetic resonance (NMR).

CAT technology was used by Hainsworth & Aylmore (1986) to quantify soil moisture depletion zones around individual roots of radish (*Raphanus sativus* L.) and the technique is also described by Brown *et al.* (1987).

NMR studies on plant roots in-situ have been published by several authors, including Rogers & Bottomley (1987) and Matyac *et al.* (1987). Using NMR Rogers & Bottomley (1987) were able to detect roots as fine as 0.3 mm diameter within 150 mm diameter pots. However, they found that some soil media were unsuitable for NMR imaging. In some cases the loss of signal could be attributed to a high ferro-magnetic content of the soil and in other cases the cause was uncertain, but was assumed to result from poor RF magnetic field penetration of the sample being imaged. A unique feature of NMR imaging is that it is often possible to distinguish live and dead structures through differences in image intensity (Wolf, 1986; Rogers & Bottomley, 1987).

2.6 Need for technique development

Based on the existing information on techniques for study of root dynamics of field swards (Section 2.5), it was felt that before addressing the questions of root behaviour identified in Section 2.4.3, technique development would be needed. Deinum's (1985) estimates of root mass turnover appear to be subject to considerable uncertainty due to simultaneous growth and death of new roots (Section 2.5.2.2). Although Steen (1983, 1984) had specifically measured root production using a mesh bag technique (Section 2.5.2.4), the installation of mesh bags would likely be too time consuming for a large scale study, and the method had not been tested for measuring the effects of grazing management on root growth. Finally, measurement of the physical quantity of root present begs the question of root activity and root function (Sections 2.4.2, 2.5.2.7). Accordingly it was decided that the first experiment would incorporate an evaluation and comparison of information yielded by some of the above techniques.

CHAPTER 3: INITIAL TECHNIQUE DEVELOPMENT AND ASSESSMENT OF SWARD ROOT PRODUCTION RESPONSES TO DIFFERING DEFOLIATION TREATMENTS DURING REPRODUCTIVE GROWTH.

3.1 Introduction

In commencing this study, some information on methodology was available from DSIR Plant Physiology Division where studies of rooting patterns in kiwifruit (*Actinidia deliciosa* (A. Chev) Liang et A. R. Ferg.) had been in progress (Gandar & Hughes, 1988); and from information supplied by A. D. Mackay (pers. comm.) on his previous work (Barber & Mackay, 1986) with maize (*Zea mays* L.). However, because the objectives of this study (Sections 1.2 & 2.4.3) involved measurements not previously attempted in New Zealand, namely definition of pasture root responses to grazing, and quantification of seasonal variation in pasture root mass and root production in terms of tissue mass flow, new techniques had largely to be developed from first principles.

Moreover, it was realised at the outset that the labour-intensive nature of root measurement and the aim in this study to make simultaneous measurements on root and tiller dynamics (Section 1.2) would require either a large labour input or development of faster root sampling methods than those currently available. Accordingly, this chapter describes a short term field experiment (Experiment 1) set up in November 1985 to evaluate techniques for root measurement, to provide information on logistical questions such as sampling time and on related statistical questions such as coefficients of variation, and to give preliminary data on root responses to grazing management.

3.2 Experimental

3.2.1 Objectives

The first objective of this experiment was to compare the effectiveness of two direct measurement techniques (quantity of roots in "intact" core samples and root growth into refilled cores) and one indirect technique (rate of soil moisture depletion) for measuring root dynamics of pasture.

A second objective was to make preliminary measurements on root mass and root production in field swards. Specifically, it was hoped to examine the effect of manipulation of reproductive stem development on root behaviour of a perennial ryegrass (*Lolium perenne* L.) dominant pasture.

3.2.2 Site

The experiment was conducted during summer 1985/86 at the Pasture and Crop Research Unit, Massey University. The pasture had been sown in March 1983 after approximately 2 years in crops and the seed mixture used was perennial ryegrass (*Lolium perenne* L. cv. 'Ellett') 18 kg ha^{-1}, white clover (*Trifolium repens* L. cv. 'Grasslands Pitau') 2 kg ha^{-1}, and red clover (*Trifolium pratense* L. cv. 'Grasslands Pawera') 2 kg ha^{-1}. The soil at the site was a Tokomaru silt loam (Typic Fragiaqualf)

Long-term average monthly temperatures for the site range from 8.0 °C (July) to 17.6 °C (January). Mean annual rainfall is 995 mm, and over the period of the trial rainfall measured at the DSIR observation centre, approximately 1 km distant, was 307 mm; and pan evaporation 382 mm; indicating that there should not have been substantial soil moisture deficit during the trial.

3.2.3 Defoliation treatments

Manipulation of reproductive growth was achieved by means of six mowing treatments, which were imposed from 15 November 1985 (Day 0); and were arranged in a randomised complete block design with 4 replicates. Plot size was 5 m x 3 m. These treatments and the cutting dates are summarised in Table 3.1. In three treatments the strategy was to allow reproductive growth to proceed to different stages by leaving plots uncut for varying periods, and then resuming cutting. These three treatments were uncut to head emergence (RUHE), uncut to anthesis (RUAN), uncut to seed-set (RUSS), respectively. In the remaining three treatments vegetative growth was stimulated by cutting on 15 November 1985 (Day 0) and periodically thereafter, until the final harvest on 2 February 1986 (Day 79). The vegetative treatments included a hard frequent cutting regime (VEGH), a lax infrequent cutting regime (VEGL) and a regime intended to allow uncut vegetative growth (VEGU) by not cutting after 20 December.

Table 3.1: The six cutting treatments and timing of cutting (Days from start of experiment)

	Cutting Treatment	Abbreviation	Cutting Date 15 Nov	26 Nov	13 Dec	20 Dec	7 Jan	20 Jan
1.	Vegetative: cut frequent hard	VEGH[1]	0	-	28	-	53	66
2.	Vegetative: cut infrequent lax	VEGL	0	-	-	35	53	-
3.	Reproductive: uncut to head emergence	RUHE	-	11	-	35	53	-
4.	Reproductive: uncut to anthesis	RUAN	-	-	28	-	53	-
5.	Reproductive: uncut to seed set	RUSS	-	-	-	-	53	-
6.	Vegetative: uncut	VEGU	0	-	-	35	-	-

1. Cutting height on 15 November was 15 mm and thereafter 40 mm. For all other treatments cutting height on 15 November was 40 mm and thereafter 160 mm.

Plate 3.1: General view of plots shortly after cutting of VEGL, VEGU, and RUHE plots on Day 35.

3.2.4 Root sampling

Intact core samples were obtained by hand driving a 78 mm internal diameter steel tube into the ground and withdrawing the tube by means of a tractor hydraulic system (Plates 3.2a, 3.2b & 3.2c).

For refilled core sampling Steen's (1983) method (Section 2.5.2.4) was modified. Core-holes were drilled through a hole in the bottom of a shallow box using a post-hole borer fitted with a 75 mm diameter auger (Plates 3.3a & 3.3b) This method was fast (approximately 3 minutes per hole) and caused a minimum of disturbance to grass adjacent to the hole. Fine builders sand (sand filled) or Manawatu silt loam B horizon (silt filled) was used to refill the core-holes, and a clay cap of approximately 15 mm thickness pressed onto the top of each core hole to act as a vapour seal. Refilling took about 5 minutes per hole.

It was found that the corer used to harvest the intact core samples would normally run cleanly down the hole left by the post-hole borer and so could be used to extract the refilled core. Therefore no net stocking was used.

Plate 3.2: Intact core sampling; (a) driving corer (b) lifting corer (c) core divided into 3 soil-depth segments.

(a)

(b)

(c)

Plate 3.3: Refilled core sampling; (a) drilling core-hole (b) freshly drilled hole ready for refilling.

(a)

(b)

On occasions when the corer deviated from the core hole, soil collected was discarded and remaining sand or silt fill extracted with a hand trowel.

Six sand filled and six silt filled cores were installed in each of the 4 replicates of VEGL treatment on 14 November 1985 or on 15 November (Day 0), and five of these cores were harvested on Days 26, 32, 55, 62 & 80 to establish the time course of root growth into refilled cores. In all other plots two sand filled cores and two silt filled cores were installed. The two sand filled cores were harvested on Days 56 and 80 and one only of the silt filled cores was harvested on Day 80. One intact core per plot was harvested from all plots on two occasions (Days 26 and 80).

Intact soil cores were divided on collection into three segments (0 - 70 mm, 70 - 300 mm, 300 - 700 mm); and refilled core material into two parts (0 - 300 mm, 300 - 700 mm). After collection cores were stored in airtight plastic bags at 4 °C until they could be processed (up to 21 days).

Change in gravimetric soil moisture over time was evaluated as an indirect measure of root growth. For each of the 24 plots, 5 cores of 21 mm diameter were taken from two depths (0 - 100 mm and 300 - 450 mm) on days 79 and 86, weighed moist, and oven dried at 105 °C for 24 h.

3.2.5 Root extraction and measurement

Roots from refilled cores were recovered by placing the sample on a sieve and spraying with water (Bohm 1979). A 4 mm mesh was used for sand filled cores, and a 1 mm mesh for silt filled cores. By directing the hose into a drum below, the washings could be collected. These washings were poured through a 0.2 mm sieve. If any fine root material was recovered the water pressure was reduced.

Intact cores were broken up and obvious roots picked out by hand. A subsample was then washed and remaining roots collected. These two fractions of roots from the one core sample were later measured separately and summed.

Extracted root samples were stored in 90% ethanol solution to prevent microbial decomposition.

3.2.6 Root mass, root length and mean root diameter determination

Root samples were first squeezed dry and weighed (fresh weight, FW). Root length was determined at this stage and samples were then dried overnight at 70 °C, and weighed to determine dry weight (DW). Finally, samples were ashed for 4 hours at 650 °C and weight of ash subtracted to determine ash-free dry weight (AFDW) (Bohm, 1979). Root mass:length ratio was used to calculate mean root diameter of 'new' roots in sand filled cores. This calculation is described by Barker *et al.* 1988, and for the present study was simplified (Appendix 2.7) to:

$$D = 0.1262 * \sqrt{Wt/Len}$$

Where: D = mean diameter
Wt = plot root mass (g m^{-2})
Len = plot root length (km m^{-2})

Calculation of mean root diameter (Barker *et al.*, 1988) is normally based on FW, assuming a specific density for root material of 1.0 g cc^{-1}. In this study, because roots had often dried somewhat before fresh weight was determined, and to avoid errors arising from differences in quantity of soil adhering in different samples, AFDW was used to calculate mean root diameter. AFDW was converted to a 'corrected' FW by dividing by a constant (0.08), derived from Schuurman & Knot's (1974) finding that AFDW averaged 8% of root FW.

Root lengths were determined using the line intersect method (Section 2.5.2.3). The dimensions of the counting tray were 295 mm x 210 mm and a 10 mm grid was used. Root length was calculated as 7.857 mm per intersect (Tennant, 1975). Subsampling was necessary and subsample size was chosen so as to give approximately 1500 intersects for each sample counted.

In this experiment no attempt was made to distinguish between live and dead roots, or between roots of ryegrass and roots of other pasture species. Differences in root lengths between cutting treatments are assumed to reflect differences in root production of ryegrass, therefore, as ryegrass was the major component of the swards (Section 3.2.2).

Presentation of root density per unit volume or per unit weight of soil is preferred by some authors (e.g. Barber, 1971; Evans, 1978; Barker *et al.*, 1988) but has the disadvantage that, because quantity of roots decreases with soil depth, values change depending on the sampling depth over which they are averaged. Comparison of results between experiments is therefore difficult, unless uniform sampling depths are used. Furthermore, values for successive soil horizons are not additive. For example, a root length density of 300 km m^{-3} for 0 - 100 mm soil depth and 100 km m^{-3} for 100 - 300 mm soil depth would give a value of 167 km m^{-3} for 0 - 300 mm soil depth. One way to avoid both these difficulties is to express root data as cumulative totals per square meter of ground surface for specified soil depths, and this approach is adopted here.

3.2.7 Above-ground measurements

Simultaneous measurement of above- and below-ground sward changes was not an aim in this preliminary experiment, but in order to give some above-ground data as an aid to interpretation of root data, herbage mass was measured on 15 November (Day 0), 12 December (Day 27) and 2 February (Day 79) and herbage dissections carried out to determine sward botanical composition and leaf and stem fractions on these dates. Grass tiller and clover growing point and stolon densities were measured on 5 February to quantify further, composition of swards, but due to lack of time only 18 plots (3 replicates) were counted.

3.2.8 Statistical analysis

All data were initially analysed in accordance with the randomised complete block design of the experiment. The root data showed a non-normal distribution with a positive skew. Log transformation was used to reduce this problem. In one case a refilled core with a very large quantity of root present (standardised residual in analysis of variance = 2.62) was treated as a missing plot.

To test whether patterns of root growth were constant across treatments over time, data from intact cores harvested on Days 26 and 80 were combined and analysed using the "repeated measures" option of the SAS

General Linear Models (GLM) procedure; as were sand filled cores harvested on Days 56 and 80. Similarly, to test for differences in the pattern of treatment effects with soil depth, or between core-types, samples for 0 - 300 mm soil depths of sand and silt filled cores harvested on Day 80; and samples for 0 - 300 mm and 300 - 700 mm soil depths of sand filled cores harvested on Day 80 were analysed as split plot effects.

The validity of split plot designs for analysing repeated observations on the same plots has been much questioned on the grounds that observations from the two sets of measurements are not strictly independent (Rowell & Walters, 1976). The SAS repeated measures procedure effectively calculates an analysis of variance as for a split-plot in time model (Steel & Torrie, 1981), so does not automatically guarantee validity of the analysis. However, tests for treatment effects averaged over split-plot effects **are** valid (Rowell & Walters 1976; Cole & Grizzle, 1966). In the special case where there are only two times in the analysis, the tests for significance of differences between times and of the treatment x time interaction are also valid (LaTour & Miniard, 1983; Appendix 1.1), and this applies to the two split-plot in time analyses described above. For the case of two types of soil cores, or two soil depths, the situation is analogous to that for two times (Appendix 1.1).

Where it was evident that there were trends in the data affecting more than one treatment (for example vegetative treatments versus reproductive treatments) sums of squares for appropriate orthogonal contrasts were extracted from the analyses of variance, and tested for significance.

3.3 Results

3.3.1 Evaluation of techniques

3.3.1.1 Coefficients of variation

As a preliminary step, data from sand filled cores harvested on Day 56 were examined in detail. The various measurements on a particular sample (FW, DW, AFDW, root length) were all rather highly correlated, though correlations between root length and the three root weight measurements were lower than those among the root weight measurements themselves (Table 3.2).

Table 3.2 Correlations (r) between root parameters for sand filled core samples harvested at Day 56.

	FW	DW	AFDW
LENGTH	0.71	0.77	0.74
AFDW	0.89	0.96	
DW	0.87		

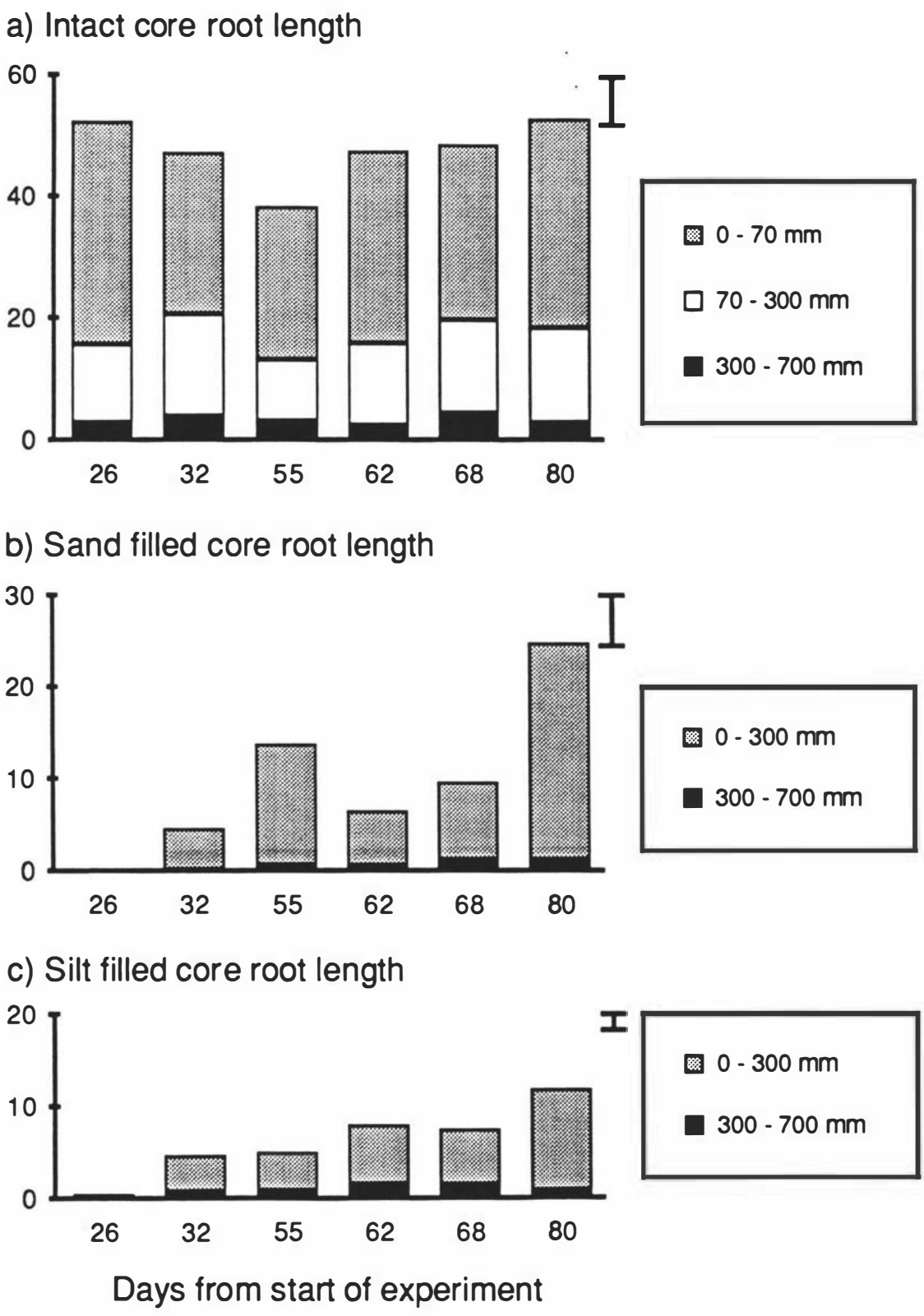
By contrast, measurements which involved different samples from the same plots usually had very low correlations. For example correlation between root length in 0 - 300 mm segments of the sand filled cores harvested on Day 80, and root length in 300 - 700 mm segments of the same cores was 0.08; while correlation between sand filled core samples for Day 56 and those of Day 80 was 0.01. However, for 0 - 300 mm segments of sand and silt filled cores harvested on day 80, r was 0.34.

For the Day 56, 0 - 300 mm soil depth sand filled core samples, coefficients of variation for individual observations of root FW, DW, AFDW, and root length were 51.3%, 59.7%, 59.6% and 50.7% respectively. (By comparison, coefficients of variation for intact core root lengths were 30.8 % and 30.9% for samples from 0 - 70 mm depth harvested on Days 26 and 80 respectively). Because of the correlation between root length and root weight measurements on the same sample, and the lower coefficient of variation for root length, further results in this chapter are presented mainly as km root length per m^2 ground surface.

3.3.1.2 Change in quantity of root with time

Over the 80 days of the experiment there was no statistically significant change in total root length in intact cores (Figure 3.1a). By contrast, data from sand filled cores indicated substantial root formation after 32 days. Root lengths in sand filled cores were lower at Day 62 than at Day 55, but after 80 days they had attained 53% of the values measured in intact cores,

Figure 3.1: Root lengths (km m^{-2}) in (a) intact (b) sand filled and (c) silt filled cores of VEGL plots, over the course of the experiment. Standard error bars shown apply to data from 80 Day harvests. Proportionately smaller S.E.'s (not shown) apply to data from earlier harvests of refilled cores.



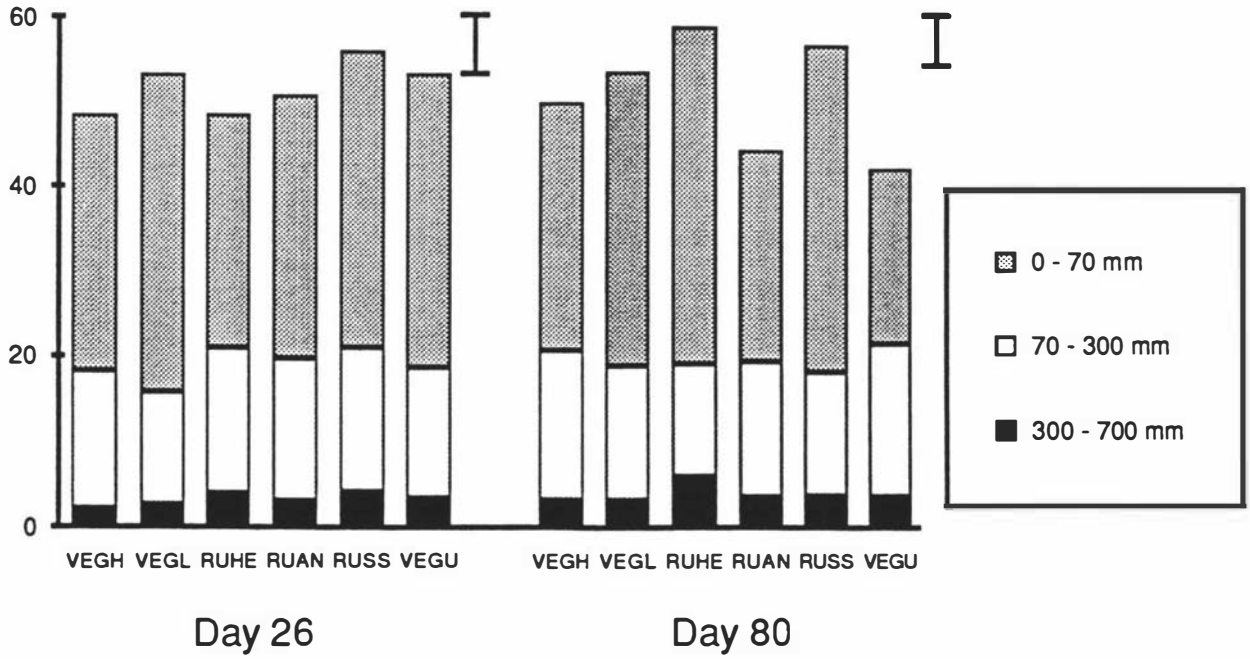
with apparent root growth rates of up to 58 kg DM ha^{-1} day^{-1} (Figure 3.1b; Table 3.5, page 46). Root growth into silt filled cores showed a pattern similar to that found in sand filled cores (Figure 3.1c), although there was no decrease between Days 55 and 62, and total root lengths were significantly less ($P < 0.001$) than in sand filled cores (Figures 3.1b, 3.1c).

3.3.1.3 Measurement of effect of mowing on root behaviour

Three measurement techniques were used to compare root mass, root growth or root activity for the 6 mowing treatments.

Intact core sampling at Days 26 and 80 did not reveal any statistically significant differences between mowing treatments (Figure 3.2), despite lower coefficients of variation than for refilled core data (Section 3.3.1.1).

Figure 3.2: Root lengths (km m^{-2}) in intact cores at Days 26 and 80.



For sand filled cores, no significant differences in root growth were detected at Day 56 (Figure 3.3a), but by Day 80, RUHE and RUAN plots had higher root growth than RUSS plots ($P < 0.05$) with the VEGH and VEGL treatments being intermediate (Figure 3.3b). An orthogonal contrast sum of squares for RUAN and RUHE treatments versus the three VEG treatments was not statistically significant ($F_{1,14} = 2.78$, $P = 0.12$) when sand filled core data was analysed alone, but when sand and silt filled core data were analysed together in a split plot analysis (Section 3.2.8) the result was significant ($F_{1,13} = 6.99$, $P = 0.02$). Root growth in silt filled cores was again less than in sand filled cores ($P < 0.0001$). Differences among mowing treatments for silt filled core data were not significant at $P = 0.05$, but at $P = 0.10$, root length for RUAN plots was significantly higher than for RUSS and VEGU plots (Figure 3.3c).

Split-plot analysis of the refilled core data showed a significant treatment x time interaction for sand filled core data for Days 56 and 80; a significant treatment x soil depth interaction for sand filled cores, Day 80; and no significant treatment by core type interaction when sand and silt filled cores were analysed together (Table 3.3, page 44).

The third technique tested for sensitivity in assessing effects of mowing treatments on root growth was measurement of change in soil moisture between Days 76 and 83. At Day 76 soil moisture levels were high following 24 mm rain on Days 70 and 71. No statistically significant differences in soil moisture levels or in rate of removal of moisture were detected, though values for rate of removal of water at depth were highest on RUAN and RUSS treatments (Table 3.4, page 45).

Figure 3.3: Root lengths (km m^{-2}) in refilled cores for 6 mowing treatments; (a) sand filled cores, Day 56 (b) sand filled cores, Day 80 (c) silt filled cores, Day 80.

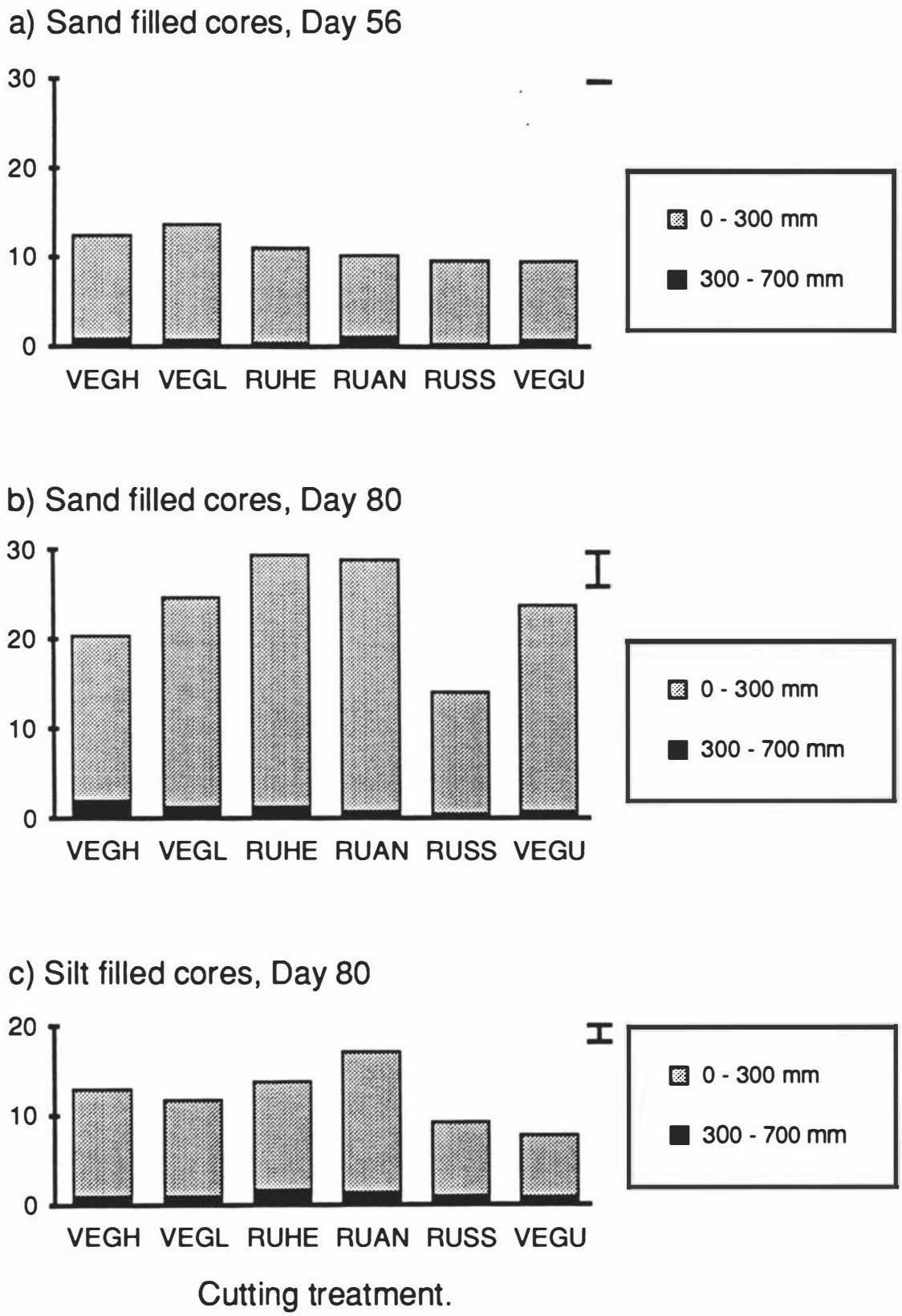


Table 3.3: Probabilities for tests of statistical significance for refilled core data analysed as split plot effects.

Data included in analysis	Main effect	Split plot effect.	Interaction
Sand-filled cores, Day 56, 0 - 300 mm	Mowing	Time	Mowing x Time
Sand-filled cores, Day 80, 0 - 300 mm	NS (P = 0.260)	P < 0.0001	P = 0.038
Sand-filled cores, Day 80, 0 - 300 mm	Mowing	Core type	Mowing x Core type
Silt-filled cores, Day 80, 0 - 300 mm	P = 0.060	P < 0.0001	NS (P = 0.251)
Sand-filled cores, Day 80, 0 - 300 mm	Mowing	Soil depth	Mowing x Soil depth
Sand-filled cores, Day 80, 300 - 700 mm	P = 0.030	P < 0.0001	P = 0.043

Note: all analyses performed on log-transformed data.

Table 3.4 Gravimetric soil moisture contents (%) in two soil depths for the six cutting treatments at Days 76 and 83.

	0 - 100 mm soil depth			300 - 450 mm soil depth.		
Mowing Treatment	Day 76	Day 83	Diff.[1]	Day 76	Day 83	Diff.[1]
1. VEGH	28.6	19.4	9.2	21.4	21.0	0.4
2. VEGL	28.2	20.6	7.6	21.0	19.8	1.2
3. RUHE	28.1	20.2	7.9	21.3	20.1	1.2
4. RUAN	28.2	20.0	8.2	21.4	19.7	1.7
5. RUSS	28.2	19.5	8.7	21.4	19.8	1.6
6. VEGU	29.1	19.5	9.6	21.8	20.9	0.9
S.E.M.	0.7	0.8	1.0	0.6	0.6	0.5

1. Difference between Days 76 and 83.

3.3.2 Effect of mowing treatments

3.3.2.1 Root mass and rate of root growth

Data from Section 3.3.1.3 above were used to derive other information. Apparent root growth rates (kg DM ha^{-1} day^{-1}) were calculated from differences in root mass in sand filled cores between Days 56 and 80, and ratios of root length in refilled cores:root length in intact cores were calculated as a measure of root turnover (Table 3.5, page 46). For sand filled core data, Day 80, mean root radius was calculated, as described above (Section 3.2.6), from mass:length ratio (Table 3.5). Statistically significant differences between the mowing treatments were found only for root radius, the 3 reproductive treatments having coarser diameter roots than the 3 vegetative treatments.

3.3.2.2 Above-ground measurements

Herbage mass data for Days 0 & 27 were used to calculate botanical composition and accumulation rates for VEGH, VEGL, and RUSS treatments (Table 3.6, page 47). Mowing regimes for RUHE and VEGU plots up to this time had been identical to those for RUSS and VEGL plots, respectively.

Herbage accumulation on RUSS plots was more than double that on VEGH or VEGL plots, but comprised mainly grass stem at the expense of grass leaf and clover components of the sward (Table 3.6, page 47).

Table 3.5: Root mass (g m^{-2} for 0 - 300 mm soil depth) for Days 56 & 80; 'apparent' root growth rates (Kg DM ha^{-1} day^{-1}); and estimates of root turnover and mean root radius.

	Mowing Treatment							
	VEGH	VEGL	RUAN	RUHE	RUSS	VEGU	SIGNIF.	SEM.
Root mass (g m^{-2})								
Day 56	58	80	85	86	80	59	NS	22
Day 80	119	176	223	175	136	154	NS	36
Apparent Root growth rate (Kg DM ha^{-1} day^{-1})								
Days 0 - 80	14.9	22.0	27.9	21.9	17.0	19.3	NS	3.9
Days 56 - 80	25.6	40.2	57.6	37.0	23.5	39.6	NS	19.2
Ratio of Root Length at Day 80, sand filled:intact.								
0 - 300 mm depth	0.43	0.53	0.60	0.70	0.27	0.64	NS	0.10
300 - 700 mm depth	0.61	0.52	0.40	0.36	0.21	0.32	NS	0.23
Mean root radius (mm) for sand filled core samples, 0 - 300 mm soil depth.								
Day 56	0.28	0.31	0.35	0.36	0.38	0.32	*	0.02
Day 80	0.31	0.33	0.35	0.37	0.38	0.32	+	0.02

Table 3.6: Herbage mass and herbage accumulation rates under different cutting treatments between Days 0 and 28.

Cutting Treatment	Grass			Clover	Weed	Dead	Total	S.E.[1]
	Leaf	Stem	Total					
Herbage Mass (kg DM ha^{-1})								
Day 0								
VEGH	25	467	492	32	0	233	757	(113)[1]
VEGL	291	771	1062	116	23	580	1781	(184)
RUSS	1122	1701	2823	186	4	711	3724	(377)
Day 28								
VEGH	933	332	1262	387	173	125	1950	(154)
VEGL	976	681	1657	544	310	141	2652	(122)
RUSS	843	4355	5198	340	82	597	6217	(802)
Herbage accumulation rate (kg DM ha^{-1} day^{-1}) Days 0 - 28								
VEGH	32.4	-4.8	27.6	12.7	6.2	-3.9	43.0	(5.3)
VEGL	24.5	-3.2	21.3	15.3	10.2	15.6	31.1	(3.9)
RUSS	-10.0	94.8	84.8	5.5	2.8	-4.1	89.0	(18.8)

1. Standard error of total values in parentheses.

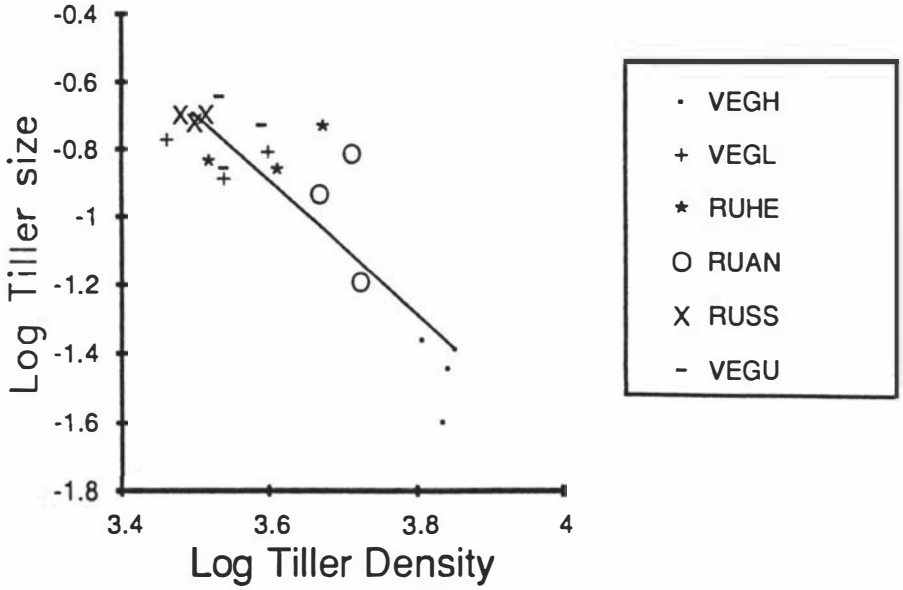
By Day 80 herbage mass exceeded 8000 kg DM ha^{-1} on the reproductive and VEGU plots, and the VEGH plots had higher ryegrass tiller densities and lower clover stolon densities at this time, than other treatments (Table 3.7).

Table 3.7: Herbage mass, ryegrass tiller numbers and clover stolon densities at Day 80

Cutting Treatment	Herbage mass (kg DM/ha) Grass	Clover	Total	Ryegrass tiller density (tillers m^{-2})	Clover Stolon density (m m^{-2})
VEGH	2330	28	2930	4774	32.6
VEGL	5250	840	7030	2327	53.6
RUHE	6180	1510	8230	3052	54.4
RUAN	6250	1130	8120	3233	45.6
RUSS	6560	1380	8260	2372	47.7
VEGU	7050	1040	8720	2455	63.8
S.E.M.	972	485	1010	496	11.0

Numbers of new tillers were noted on RUHE and RUAN plots about 7 days after the first mowing on Days 11 and 28, respectively, and tiller numbers at Day 80 were higher on these two plots than on the VEGU, VEGL or RUSS treatments (Table 3.7). Pairwise comparisons between RUHE or RUAN and RUSS, VEGU or VEGL treatments were not statistically significant ($P > 0.05$), but an orthogonal contrast comparing ryegrass tiller density for RUHE and RUAN treatments with other treatments (excepting VEGH) was significant ($F_{1,10} = 7.13$, $P = 0.024$). To clarify if this effect merely represented a flush of small tillers, tiller densities were adjusted for tiller size as follows. Mean tiller size for each plot was calculated by dividing grass herbage mass (g m^{-2}, Day 80) by grass tiller number per m^2. Log-transformed values for mean tiller size were regressed against those for tiller density (ryegrass + *Poa*), and the residual deviations from the regression line analysed for differences among treatments. This regression line was highly significant, with slope = -1.98 and $r^2 = 0.72$, and with values for the RUHE and RUAN plots concentrated above the line (Figure 3.4).

Figure 3.4: Plot of log mean tiller size vs log tiller density for individual plots of the 6 mowing treatments.



An orthogonal contrast comparing the RUAN and RUHE treatments and the remaining 4 treatments was highly significant ($F_{1,10} = 11.71$, $P < 0.01$) when the calculations were based on total herbage mass, but less significant $F_{1,10} = 4.63$, $P = 0.056$) when calculations were based on grass herbage mass only.

3.4 Discussion

3.4.1 Comparison of techniques

Water extraction patterns were not found to be a sensitive indicator of root growth. No significant differences between treatments for gravimetric soil moisture were detected (Table 3.4). It appeared that attempts to measure root activity indirectly by rates of water extraction were confounded by differences in evaporation and transpiration arising from differences in herbage mass on the different defoliation treatments. Any rainfall occurring during the measurement period would also be a difficulty. Similar problems with this method were reported by Bohm (1979).

Rapid root growth in refilled cores at a time when root mass in intact cores was fairly constant (Figures 3.1a, 3.1b, Table 3.5) implies substantial turnover of root material. Provided it were established that root growth into refilled cores was not unduly enhanced by the technique itself, then values in Table 3.5 would give an estimate of rates of root production in the field, analogous to an above-ground pasture growth rate. Similarly refilled core data in conjunction with intact core data would estimate root turnover. The ratio intact core mass (kg DM ha^{-1}):refilled core root appearance (kg AFDM ha^{-1} day^{-1}) has the dimension days, and is an estimate of root turnover time. There is no doubt, though, that the accuracy of such estimates should be established by some form of comparison with behaviour of roots in undisturbed soil, especially given the unexplained difference in quantity of root recovered from sand and silt filled cores (Figure 3.3b,c).

In fact, when data in Figure 3.3 was presented at a conference (Matthew *et al.,* 1986), concern was expressed by members of the audience that the refilled core technique would greatly overestimate root production. Possible sources of error suggested were (1) lateral branching from severed ends of roots at the core-face (2) lower bulk density or increased pore space arising from textural or compaction differences between refilled cores and background soil and (3) if root tips had a greater propensity to enter the core than to leave it, there could be a "root trapping" effect.

A number of points can be raised against these arguments. First, lateral branching of ryegrass roots is rare. Ryegrass responds to root pruning by

producing new roots from a younger node (Section 6.3.2). Secondly, excavations of spare core holes suggested that roots entered the core obliquely at all levels and had usually not progressed more than 100 - 200 mm down within the core at harvest. Thus, even if root trapping is a potential problem with the method, the 56 or 80 day period between placement and harvest of refilled cores did not allow time for it to occur. Finally, correlation between data for 0 - 300 mm and 300 - 700 mm soil depths of Day 56 sand filled cores was near zero (Section 3.3.1.1), and should have been higher if there had been a root trapping effect. Moreover, even if systematic errors did occur, data from refilled cores could still be assumed to provide a valid comparison between mowing treatments. Also, serial placement and harvest of refilled cores in a controlled experiment would allow measurement of seasonality of root growth, even if some question remained about the calibration of refilled core data.

To clarify whether or not there was a systematic error associated with refilled core sampling, the minirhizotron method was subsequently used to compare numbers of roots arriving at tubes in refilled cores and numbers of roots arriving at tubes in undisturbed soil in the same plots (Appendix 2.6). Although there was some variation with season and soil depth, it was found that numbers of roots arriving at observation tubes at the centre of sand filled cores were normally less than those arriving at tubes in undisturbed soil, so that the indication is that the sand filled core technique used here under-estimated root growth, especially for the 0 - 70 mm soil depth. (Appendix 2.6). This is not surprising in view of the removal of vegetation in order to install refilled cores, but this bias might perhaps have been eliminated if cores had been covered with a turf cap, rather than a clay cap.

In addition to providing information on root production over time, the refilled core sampling technique also detected differences in root growth between mowing treatments (Sections 3.3.1.3; 3.4.2). The fact that refilled core sampling was able to detect differences between defoliation treatments in root growth, and was the only technique to do so, indicates the sensitivity of this technique.

In contrast, the intact core technique appeared to be a very insensitive indicator of root growth or of mowing effects on root growth, although necessary reference information about the total quantity of root at a

particular time was obtained. This enabled calculation of derived parameters such as root turnover time, and in theory root death rate could be calculated from the difference between apparent root growth in refilled cores and change in intact core root mass or length over the same time period. In the light of uncertainty about the calibration of refilled core data, no calculation of root death rate was attempted, however.

Both refilled core and intact core measurements were time consuming. Total processing time was approximately 2 hours per core for sample collection, root extraction and cleaning, and root length determination. Refinement of techniques to overcome these logistical problems continued during subsequent experiments, and is reported in Appendix 2.

3.4.2 Rate of root turnover

Apparent root growth rates (Table 3.5) and rates of root turnover indicated by ratio of root length in refilled cores:root length in intact cores on Day 80 (Table 3.5) were high. Previously published information has, for the most part, suggested that root turnover during the period November - January is low (Section 2.3.1). At the same time it should be noted that the rates of root appearance indicated by increase in root mass in sand filled cores over time (up to 58 kg DM ha^{-1} day^{-1} between Day 56 and Day 80, Table 3.5) are consistent with a value of 80 kg DM ha^{-1} day^{-1} calculated theoretically by Deinum (1985), and are consistent with an average root longevity for ryegrass of 36 to 55 days observed by Gibbs (1986).

The higher apparent root growth between Days 56 and 80 than between Days 0 and 56 (Table 3.5) is similar to results of Steen (1984), who attributed the effect to time taken for dry soil placed in refilled core holes to absorb moisture, and allow root growth to begin. Another explanation might be the delay between colonisation of a refilled core by tips of elongating nodal roots, and development of a crop of lateral branches as those same roots mature. If the latter is true, the results of refilled core sampling might depend partly on the length of time between placement and harvest of refilled cores.

Again, the reason for the fall in root lengths in sand filled cores between Days 55 and 62 is not known. Because the effect was not seen in silt filled

cores as well it is considered unlikely to be due to effects of defoliation on Day 53. Rainfall between Day 38 and Day 44 was 85 mm, and it is possible that high soil moisture levels at this time affected sand and silt filled cores differently. For example, Eccles (1988), using the same sand and silt fill materials, found that soil oxygen levels in silt filled glasshouse pots declined more slowly during soak treatments, and recovered more slowly after soaked pots were removed from water, than did soil oxygen levels in sand filled pots. This is opposite to what would have been predicted on the basis of the difference in texture of the sand and silt materials.

3.4.3 Effect of mowing treatments

3.4.3.1 Effects on root growth

Two features of these results are noteworthy. First, differences in root growth between VEGH and VEGL plots were much smaller than might have been expected. Other studies have reported large effects of defoliation on root growth (Section 2.3.2), and it is generally assumed that similar effects of substantial magnitude would also be observed in field studies (Evans 1976, Hunt & Easton, 1989). In this study, except for silt filled core data, VEGL plots consistently had higher values for root parameters than VEGH plots but the differences were always small and were never significant.

Second, effects which were statistically significant relate mainly to high root lengths in refilled cores on RUHE and RUAN plots on Day 80 (Figures 3.3b, 3.3c). Troughton (1956) showed that partitioning to roots decreased during reproductive growth, and in a later study (Troughton, 1978) reported that root weight of grass plants decreases in mid summer, at the time of ear emergence.

On the other hand, reproductive swards of ryegrass in spring are known to have high rates of photosynthesis (Parsons & Robson, 1981a,b), and from data presented by these authors it can be calculated that, even allowing for a decreased proportion of photosynthesis products allocated to roots, absolute quantity of carbohydrate partitioned to roots would increase during reproductive development. For example, if the herbage accumulation values of vegetative and reproductive swards in Table 3.6 (43 & 89 kg DM ha^{-1}

day^{-1}, respectively) are considered to represent 85% and 90%, respectively, of net assimilation, then allocation to roots would be 7.6 kg DM ha^{-1} day^{-1} for vegetative swards, and 9.9 kg DM ha^{-1} day^{-1} for reproductive swards. This perhaps explains the higher root growth on RUAN and RUHE plots in this experiment. Also, research carried out in Sweden by Steen (1984) showed that cutting stimulated root growth, and that the effect was larger following the first cut in June than after cutting regrowth in August. Steen's (1984) data would therefore seem to parallel the results in Figure 3.3; although ryegrass is not listed as a component of the swards studied by Steen (1984).

The fact that root mass differences were not statistically significant (Table 3.5) does not necessarily discount the root length results, particularly as treatment rankings were similar for root length and mass, but was taken to indicate that more than one refilled core per plot would be needed to control within plot variation in future experiments.

3.4.3.2 Effect on shoot growth

The high herbage accumulation rates during reproductive growth, and changes in composition (Tables 3.6, 3.7) are a well-known feature of the behaviour of reproductive swards. The physiological basis for such changes has been described by Parsons & Robson (1981a,b) and the effects on grass swards under differing managements were reported in detail by Butler (1986), and by Hoogendoorn (1987). Differences in pasture growth rates under different spring rotation lengths (L'Huillier, 1987) are probably also due to this effect.

The analysis of tiller densities in Figure 3.4 effectively compares the observed tiller densities with those expected, given the operation of the -3/2 relationship between tiller size and tiller density (Davies, 1988; Xia, 1991; Section 2.2.3). The evidence that tiller density at Day 80 was higher on RUHE and RUAN plots than expected on the basis of the -3/2 power rule (Table 3.7, Figure 3.4) suggests that at least some new tillers formed after the interruption of reproductive growth (Korte, 1981) persist in the sward for some time. In the situation discussed in Section 2.2.3 where a sward with a high tiller density is released from grazing it would be expected that the increase in tiller size on release from grazing would also move the size-

density combination above the -3/2 power line, and such swards are known to be more productive (Bircham, 1981; Parsons *et al.*, 1984; Grant *et al.*, 1988). Therefore, although herbage accumulation was not measured in the latter part of the experiment, this suggests that RUHE and RUAN plots, in addition to producing more root growth than other plots, might also have had higher rates of herbage accumulation. Also of interest is the duration of any tillering response. In the work of Grant *et. al.* (1988) tiller density increases following a grazing in mid August were short lived, and had disappeared by late September (40 days). In this Experiment tiller density increases following interruption of reproductive growth in mid or late November on RUHE and RUAN swards, respectively, appear to have persisited until February (60 days).

Roots of new tillers appearing after defoliation of RUHE and RUAN plots could well have contributed to root lengths in refilled cores on Day 80, and detection of such roots in the 0 - 300 mm depth at Day 80, but not in the 300 - 700 mm depth due to insufficient time to reach the lower depth, would result in a treatment x depth interaction as seen for root lengths in sand filled cores at Day 80. Similarly detection of such roots at Day 80 but not at Day 56 would result in a treatment x time interaction as seen in sand filled core data (0 - 300 mm depth) for Days 56 and 80.

Taken together, these findings of increased root and tiller production on RUHE and RUAN plots suggest that previous assumptions about the need for early removal of seedheads in field swards (Section 2.2.4) might need revision. Specifically, from these results, a hypothesis was developed that removal of flowering tillers, after a brief period of reproductive growth, had promoted both tiller appearance and root growth. Stimulation of root production in this way would appear to have been much greater than any stimulation of root growth as a result of laxer defoliation.

3.5 Summary

1. The refilled core technique is a suitable method for determining seasonal variation in new root production under pasture, and for detection of defoliation effects on root production this method was also the most sensitive of the techniques tested.

2. Use of the refilled core technique in conjunction with intact core sampling allows estimates of root turnover time and in theory allows the calculation of root death, though the errors associated with such a calculation may be rather large.

3. The preliminary investigation of root dynamics showed that new root formation and root turnover in early summer was high, with root length in sand filled cores after 80 days growth being more than half that recovered from intact cores.

4. New root production was greater if reproductive growth was removed at head emergence or anthesis than if seedheads were removed early or allowed to mature, and swards with high root production also showed evidence of high tillering rates during the experiment.

CHAPTER 4: SEASONALITY OF ROOT GROWTH AND EFFECTS OF HARD OR LAX DEFOLIATION.

4.1 Introduction and overview

This chapter reports results from a further field experiment (Experiment 2, October 1986 - May 1988) designed to provide information on seasonal root dynamics of perennial ryegrass dominant field swards. Supporting measurements on tiller dynamics, tissue turnover and herbage accumulation are presented in Chapter 5; and discussion of the relationship between above- and below-ground measurements is presented in Chapter 7, together with similar discussion relating to a subsequent experiment (Experiment 3, Chapter 6).

In Experiment 2, contrasting lax (LL) and hard (HH) grazing managements were applied for an initial period of 12 months, followed by the introduction of cross-over lax-hard (LH) and hard-lax (HL) grazing managements for a further 6 months. Root and tiller dynamics were measured in detail throughout the experiment but other above-ground parameters, such as herbage accumulation rates, were not measured until later in the experiment when improvement of root sampling techniques and consequent time savings had been achieved, and when complementary above ground measurements were conducted as a part of a separate study (Xia, 1991).

4.2 Experimental

4.2.1. Background and objectives

The strategy of contrasting lax (LL) and hard (HH) grazing managements was adopted because of the small root responses to defoliation in Experiment 1 (Section 3.4.3.1). One objective was to clarify if the insensitivity of root growth to defoliation over the November - February period for VEGH and VEGL plots of Experiment 1 would also apply at other times of the year and over the extremes of grazing normally encountered in farm practice.

Also, these contrasting managements could be expected (Section 2.2.3) to result in contrasting high tiller densities on hard-grazed plots and lower tiller

densities on lax-grazed plots. A second objective was to determine whether manipulation of tiller density in this way could provide a mechanism for the manipulation of root growth.

Finally, the introduction of the cross-over lax-hard (LH) and hard-lax (HL) grazing managements was timed (7 December 1987) to coincide with the period of sward reproductive growth so as to provide information on the repeatability of the increase in root growth and tillering observed when RUHE and RUAN swards had been allowed a period of reproductive growth during Experiment 1 (Section 3.4.3.2).

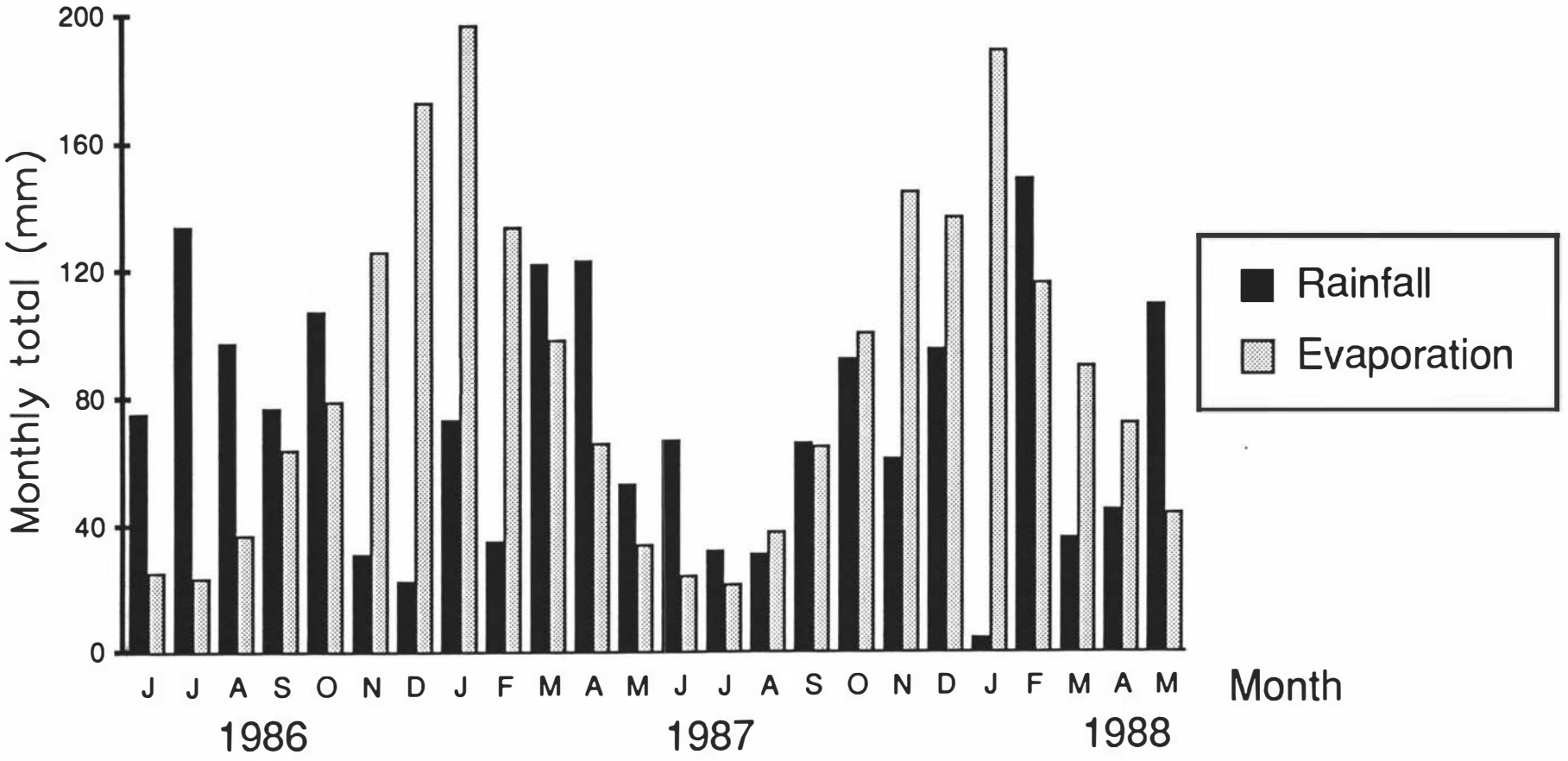
4.2.2 Site

The experimental site was in a paddock adjacent to that used for Experiment 1, with soil type and climate as described in Section 3.2.2, and with a perennial ryegrass (cv. Ellett) based pasture. In July 1986 the site was sprayed with herbicide (active ingredients picloram and 2,4-D) to remove clover. The resulting sward was predominantly ryegrass, but contained approximately 500 to 1000 tillers m^{-2} *Poa trivialis* L. After removal of clover, nitrogen was applied as urea at 15 kg N ha^{-1}, approximately every three weeks.

Rainfall and pan evaporation (monthly totals), recorded at DSIR meteorological station over the course of the experiment, are presented in Figure 4.1 (page 59). Soil moisture levels were excessively high in spring 1986, delaying the start of the experiment, but with only 32 mm rain between late October and mid-December Figure 4.1), soil moisture levels fell quickly, and were measured at 16% (gravimetric soil moisture, MW/MSx100, 150 - 250 mm soil depth) on 5 December 1986. Rainfall of 21 mm in late December 1986 and 74 mm in January 1987 improved soil moisture levels, but not sufficiently to promote vigorous pasture growth until further rain fell in March 1987.

It was decided to use irrigation during summer 1987 - 1988, to prevent excessive soil moisture deficits, such as that which occurred during December 1986. Accordingly, approximately 100 mm irrigation was applied to plots from late December 1987 and through January 1988, when only 5 mm rainfall was recorded (Figure 4.1).

Figure 4.1: Monthly totals for rainfall and pan evaporation, recorded at DSIR Grasslands Division, 1 km distant from the experimental site.



4.2.3 Experimental design and statistical analysis

The four treatments described above (Section 4.1) were applied to 10 m x 10 m plots arranged in a Latin square design (4 replicates) to account for possible fertility gradients away from a race adjacent to the plots (columns), or down the slope of approximately 10° (rows). However, for the first 12 months, when LH and HL plots received grazing management identical to LL and HH plots, respectively, measurements were made only on the 8 plots designated LL and HH. Therefore, for this period a Latin square analysis was not possible and the experiment was analysed as a randomised complete block design with 4 replicates of two treatments.

Data for Harvests 2 to 10 inclusive were analysed using the split-plot in time model, either with the "repeated measures" option of the SAS general linear models procedure, or using MINITAB, which produced sums of squares identical to those from the SAS procedure, for a test set of data. The split-plot in time model is valid for testing grazing effects averaged over times, but can produce optimistic estimates of significance when used for testing differences between times or for testing treatment x time interactions. (Appendix 1.1, Section 3.2.8). Therefore, where preliminary analysis by the split-plot in time model identified time or treatment x time effects of interest, the significance of these was confirmed by pair-wise comparison of two harvests or of plot means for two particular groups of harvests. Significance of treatment x depth effects was established in the same way. Inclusion of only two time periods or two depths in a split-plot in time analysis in this way overcomes problems arising from heterogeneity of error variance or of correlation across times, but would not correct for any systematic differences in technique (Appendix 1.1).

For all data collected after the cross-over LH and HL treatments were introduced in December 1987 (Harvests 10 to 12, inclusive), the Latin square analysis of variance was used and where means from different harvests were compared, this was done by calculating a pooled variance and LSD for the specific pair of means in question.

4.2.4 Grazing strategy

The primary aim of the grazing strategy throughout the experiment was to maintain a contrast between herbage mass on LL and HH plots. To compare

root growth, continuous grazing management would have been preferred as this would have eliminated possible fluctuations in root growth relating to the stage in a grazing cycle, but as the plots were too small to sustain continuous grazing by even one animal, and insufficient land area was available for larger plots, it was necessary to adopt a rotational grazing strategy. However, in order to minimize possible interruption of root growth after grazing, grazings were made as frequently as was conveniently possible. Accordingly, grazing interval was as short as 10 days in periods of high herbage accumulation in spring and summer, but was as long as 40 days on LL plots in winter.

Target herbage masses for LL and HH plots, respectively, were 3500 and 1500 kg DM ha^{-1} pregrazing; and 1800 and 800 kg DM ha^{-1} residual after grazing, although some variation around these targets occurred. In order to achieve desired removal of herbage within a few hours, grazing was with 15 to 30 sheep per 10 m x 10 m plot. This grazing strategy also minimized within plot variation in grazing height. It was initially intended that the grazing frequency for LL and HH plots be identical, and that the differential between LL and HH treatments be attained by varying the numbers of animals. However at some times of the year it proved difficult to maintain the contrast in herbage mass between LL and HH plots with a common grazing interval, and at these times grazing intervals for the LL plots were lengthened.

Prior to implementation of crossover LH and HL managements in early December 1987, grazing of these 8 plots was always paired with the corresponding LL or HH plot in the same replicate or row of plots (equal numbers of sheep put onto and removed from both plots at the same time during each grazing day). As stated above, no measurements were made on these 8 LH and HL plots prior to September 1987, as before this these plots had been merely duplicates of the LL and HH treatments.

Electric fencing was used, and as a result there were behavioural effects on the animals used for grazing. Even with 30 sheep on the 10 m x 10 m plots and grazing times as short as a few hours, sheep would graze preferentially near the fence, then ruminate in the centre of the plot, with consequent dung and urine concentration in the centre of plots. As a result of this grazing behaviour fertility gradients developed within plots during the experiment. Herbage growth at the centre of plots became visibly more vigorous than at the edge and nutrient transfer to the centre areas of plots was confirmed by

measurement of soil total P on a split plot basis (centre areas and edge areas of plots) on 3 August 1987 (Section 5.2.1). Once fertility gradients were noted the centre areas of plots were avoided for root and tiller sampling purposes, but some other measurements at later harvests were made on a split plot basis to gauge effects of soil fertility on sward dynamics (Sections 5.2.1, 5.2.2).

4.2.5 Measurements

Twelve root harvests were made at six-weekly intervals throughout the experiment, and above-ground measurements were co-ordinated with these twelve root harvests. Because of seasonal and treatment variations in grazing frequency, it was not possible to co-ordinate grazings with root harvests, however.

Harvests were normally completed over a 5 day period and root and herbage samples stored under refrigeration until they could be processed. Commencement dates for the 12 harvests were:

Harvest 1	3	December	1986
Harvest 2	16	January	1987
Harvest 3	24	February	1987
Harvest 4	8	April	1987
Harvest 5	26	May	1987
Harvest 6	8	July	1987
Harvest 7	20	August	1987
Harvest 8	19	October	1987
Harvest 9	3	December	1987
Harvest 10	21	January	1988
Harvest 11	24	March	1988
Harvest 12	10	May	1988

For each harvest (excepting Harvests 1 and 11, when not all measurements were made) root mass and root length in intact and refilled cores were measured, as described previously (Section 3.2.4); and tiller densities and tiller appearance and death rates were also measured. For Harvests 1 to 7 herbage mass was measured to ascertain that treatments were within the guidelines established for 'lax' and 'hard' grazing; and for Harvests 8 to 12 herbage accumulation and tissue turnover measurements were also carried out.

Root sampling depths were 0 - 70 mm (zone of high root concentration in pastures), 70 - 250 mm (approximate boundary of A horizon), and 250 - 600 mm; although in harvests 6 to 8 cores from the 250 - 600 mm soil depth could not be extracted because high soil moisture levels resulted in cores themselves remaining behind when the corer was lifted.

Four intact cores (61 mm diameter) per plot were collected and bulked after washing to provide the intact core samples for 0 - 70 mm and 70 - 250 mm soil depths for Harvests 1 to 9. For later harvests, a smaller 21 mm diameter corer was used, and 10 cores per plot collected for these soil depths. For the 250 - 600 mm soil depth, 1 core (61 mm diameter) per plot was collected at all harvests where this soil depth was sampled. For refilled core samples, 4 cores (79 mm diameter) were collected per plot for 0 - 70 mm and 70 - 250 mm soil depths at all harvests, and 1 core per plot for the 250 - 650 mm soil depth. For Harvests 1,2 & 4 additional refilled cores were placed in each plot and harvested 80 days later at Harvests 3,4 & 6. Mean root diameter was calculated as described in Section 3.2.6. For each harvest, approximately three days were required (2 persons) to collect and store core samples and place refilled cores for the following harvest.

Tiller densities were measured by Mitchell and Glenday's (1958) method, and tiller appearance by the fixed quadrat method, using techniques similar to those of Korte (1981). Further details of above-ground measurements are given in Section 5.2; and a schedule of grazing and measurement dates is given in Appendix 3.

4.3 Results

Results for Experiment 2 were analysed in two subsets. Data for LL and HH treatments for Harvests 2 to 10 were analysed in one subset to provide information on seasonal variation in root mass and root production on plots subjected to LL and HH grazing managements over a 12 month period from January 1987 to January 1988 (Section 4.3.1). Data for all four treatments for Harvests 10 and 12 were analysed in a second subset to provide further confirmation of seasonal differences in root production between summer and autumn, and to provide information on the effects manipulation of reproductive growth might have on subsequent root production (Section 4.3.2).

4.3.1 Seasonal variation in root mass, root length, mean root diameter, and new root production under LL and HH grazing managements (Experiment 2)

4.3.1.1 Data from intact core sampling

The intact core data for root mass (AFDW[1] , see section 3.2.6), root length (km root length m^{-2}) and mean root diameter (mm) for the three soil depths and LL and HH grazing managements are shown in Tables 4.1 (page 67), 4.2 (page 68), and 4.3 (page 69), respectively. Corresponding totals for root mass and root length in the 0 - 250 mm soil depth are presented graphically in Figures 4.2a and 4.2b (page 65). Results of analysis of variance of these data (Appendix 4) show that, statistically, seasonal changes in quantity of root are the dominant effects in the data set, with grazing management effects often non-significant, and no significant grazing by season interactions. The various significant F-tests (Appendix 4) result from a number of different effects, however.

Over the 12 month period average percentage distribution by soil depth of total root mass in intact cores was 76.9%, 16.9% and 6.25% for the 0 - 70 mm, 70 - 250 mm, and 250 - 600 mm soil depths respectively (Table 4.1).
Corresponding percentage distribution of root length in the three soil depths was 68.1%, 23.9%, and 8.0% respectively (Table 4.2), the higher percentage distribution of root length at depth reflecting the finer mean diameter of roots from the deeper sampling depths (Table 4.3).

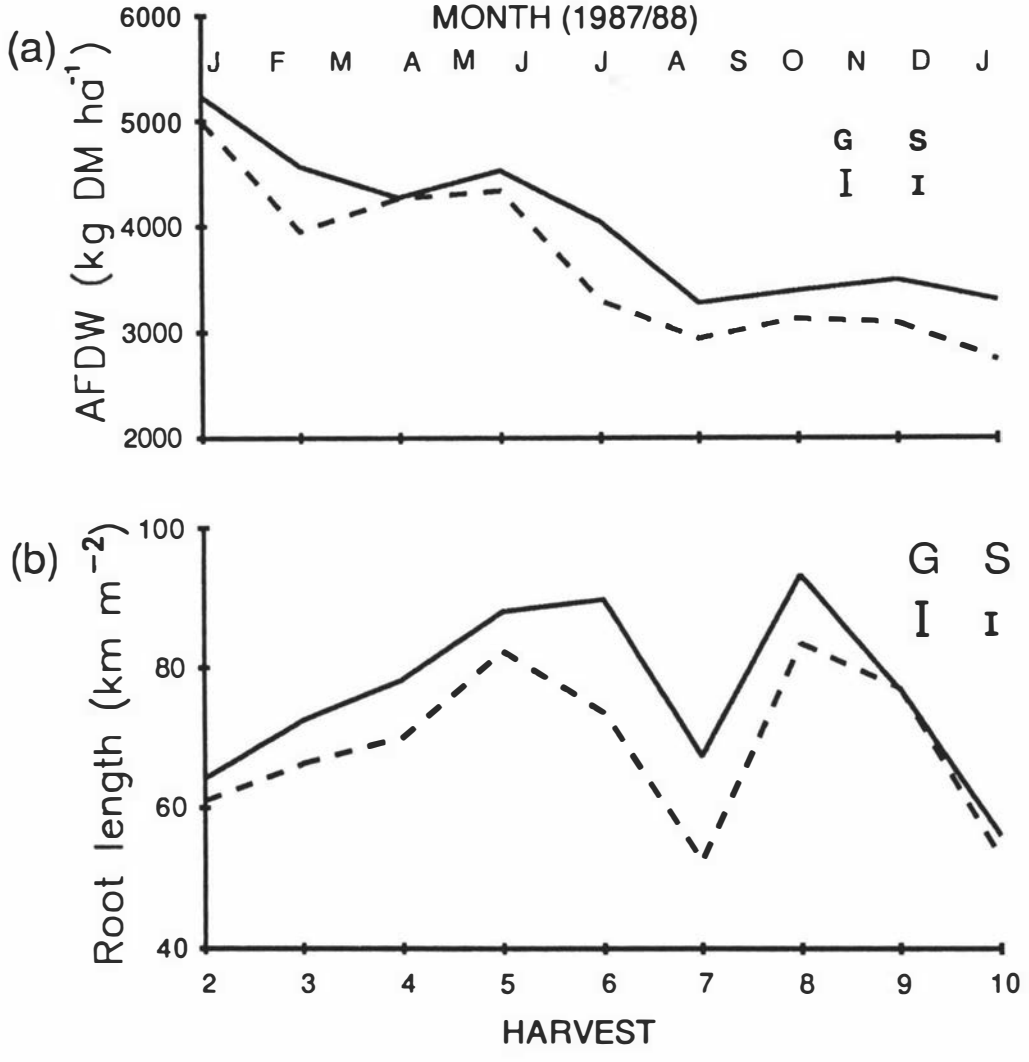
There was a steady decline in total root mass during the experiment (Figure 4.2a), with root mass falling from 5.1 t DM ha^{-1} in January 1987 to 3.2 t DM ha^{-1} (for 0 - 250 mm soil depth, averaged over HH and LL treatments). The statistical significance of this effect was confirmed by split-plot in time analysis of variance of plot means for Harvests 2 & 3 compared to means for Harvests 9 & 10 ($F_{1,6} = 144$, $P < 0.001$).

The LL plots had consistently higher root mass and root length values than HH plots but, as in Experiment 1, the magnitude of these differences induced by grazing management was small, seldom exceeding 15%, and averaging 10.4%

1. AFDW = ash-free dry weight, kg DM ha^{-1}, see Section 3.2.6

(P = 0.05) and 11.3% (P = 0.04), respectively, for root mass and root length values for 0 - 250 mm soil depth averaged over the 12 month period (Figure 4.2a,b; Tables 4.1, 4.2). Values for the 70 - 250 mm soil depth had lower coefficients of variation and showed stronger evidence of grazing effects than did values for the 0-70 mm soil depth (Tables 4.1, 4.2; Appendix 4).

Figure 4.2: Seasonal and grazing management effects on (a) intact core root mass (AFDW, t ha^{-1}) and (b) intact core root length (km m^{-2}); summed for 0 - 250 mm soil depth. (LL ————, HH — — — —; G = standard error for grazing effects averaged over time; S = standard error averaged over time, for mean of LL & HH plots at individual harvests).



There was evidence of soil depth x season interactions for the quantity of root present. This was tested by calculating ratios of root mass or root length for upper:middle or upper:lower soil depths and analysing for differences over time.

The ratio root length in intact cores (0 - 70 mm soil depth):root length (70 - 250 mm soil depth) was highest at Harvest 7 and lowest at Harvest 9 (Figure 4.3, page 66); analysing data for Harvests 7 & 9 only, ($F_{1,6} = 8.1$, $P = 0.03$), but no such event was evident when corresponding ratios for root mass were calculated. Also, the ratio of root mass or length in upper:lower soil depths tended to increase in winter and decrease in summer and this would seem to indicate loss of root from deeper soil horizons in winter, although the evidence for this is rather less conclusive due to lack of samples from the 250 - 600 mm soil depth for Harvests 6 - 8. One example of this effect was that mean ratio of intact core root length (0 - 70 mm soil depth):root length (250 - 600 mm soil depth) was 8, 7, 11, and 22 for Harvests 2, 3, 4, and 5, respectively (comparing means for Harvests 2 & 3 with means for Harvests 4 & 5, $F_{1,6} = 11.51$, $P = 0.02$).

Mean root diameter showed seasonal variation, with the lowest values for the 0 - 70 mm soil depth occurring at Harvest 8 and for the 70 - 250 mm soil depth occurring at Harvest 9 (Table 4.3). For the two lower soil depths mean diameter of roots was slightly greater for LL plots than for HH plots (Table 4.3) but these effects were not statistically significant (Appendix 4).

Figure 4.3: Seasonal variation in ratio of intact core root length for 0 - 70 mm: 70 - 250 mm soil depths, averaged over LL and HH plots.

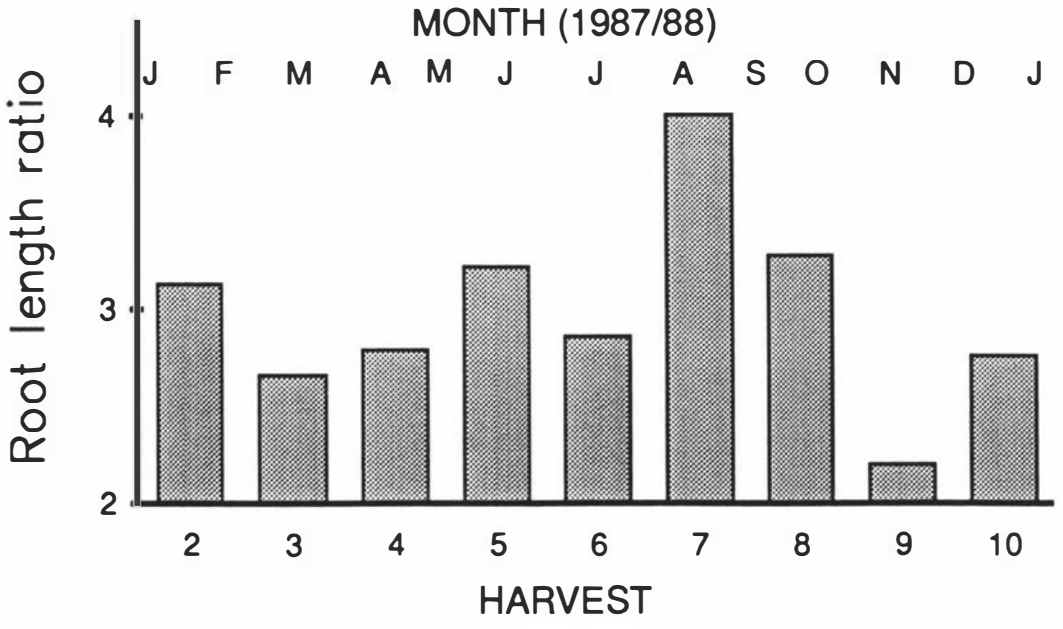


Table 4.1: Root mass (kg ash-free DM ha^{-1}) for three soil depths and two grazing managements determined by intact core sampling for period January 1987 to January 1988 (Experiment 2).

Grazing	Soil depth (mm)	Harvest 2	3	4	5	6	7	8	9	10	MEAN	S.E.[1]	S.E.[2]
LL													
	0 - 70	4366	3656	3411	3755	3322	2739	2791	2903	2612	3284	247	237
	70 - 250	866	911	863	769	723	544	611	600	705	732	40	53
	250 - 600	301	364	201	133	-	-	-	208	253	253	74	42
HH													
	0 - 70	4221	3274	3473	3557	2712	2362	2550	2572	2198	2991	247	237
	70 - 250	774	682	791	782	592	567	583	512	544	647	40	53
	250 - 600	278	321	300	169	-	-	-	188	195	248	74	42
MEAN													
	0 - 70	4293	3465	3442	3656	3017	2551	2670	2737	2404	3137	175	168
	70 - 250	820	796	827	775	657	555	597	556	625	690	28	38
	250 - 600	289	342	251	151	-	-	-	198	224	251	52	30

1. Standard error appropriate for comparing grazing managements averaged over time.
2. Approximate standard error for comparing means of different harvests within the same grazing treatments. This standard error derived from split-plot in time analysis, and subject to confirmation of significance for specific pairs of means, see Appendix 1.1.

Table 4.2: Root length (km m^{-2}) for three soil depths and two grazing managements determined by intact core sampling for period January 1987 to January 1988 (Experiment 2).

Grazing	Soil Depth (mm)	Harvest 2	3	4	5	6	7	8	9	10	MEAN	S.E.[1]	S.E.[2]
LL													
	0 - 70	49.2	52.0	58.4	67.0	68.8	54.5	72.4	52.0	37.9	56.9	4.8	4.7
	70 - 250	15.1	20.6	20.0	21.1	21.0	12.9	21.1	25.2	18.2	19.5	0.5	1.8
	250 - 600	6.3	8.1	5.7	2.6	-	-	-	6.0	6.9	6.2	1.8	1.0
HH													
	0 - 70	45.3	49.0	51.0	63.0	52.3	40.8	63.1	53.1	37.7	50.6	4.8	4.7
	70 - 250	15.7	17.4	19.0	20.5	21.4	11.2	20.5	24.2	15.2	19.5	0.5	1.8
	250 - 600	7.0	8.8	6.2	4.1	-	-	-	5.3	5.9	6.4	1.8	1.0
MEAN													
	0 - 70	47.3	50.5	54.7	65.0	60.5	47.7	67.8	52.5	37.8	53.4	3.4	3.4
	70 - 250	15.4	19.0	19.5	20.9	21.2	12.0	20.8	24.7	16.7	18.9	0.4	1.3
	250 - 600	6.7	8.4	5.9	3.3	-	-	-	6.6	6.4	6.3	1.3	0.7

1. Standard error appropriate for comparing grazing managements averaged over time.
2. Approximate standard error for comparing means of different harvests within the same grazing treatments. This standard error derived from split-plot in time analysis, and subject to confirmation of significance for specific pairs of means, see Appendix 1.1.

Table 4.3: Mean root diameter (mm) for intact core samples from three soil depths and two grazing managements for period January 1987 to January 1988 (Experiment 2).

Grazing	Soil Depth (mm)	Harvest 2	3	4	5	6	7	8	9	10	MEAN	S.E.[1]	S.E.[2]
LL													
	0 - 70	0.378	0.334	0.305	0.299	0.281	0.282	0.250	0.297	0.332	0.302	0.017	0.010
	70 - 250	0.303	0.265	0.262	0.242	0.235	0.262	0.214	0.195	0.250	0.247	0.010	0.009
	250 - 600	0.276	0.275	0.229	0.287	-	-	-	0.241	0.244	0.256	0.009	0.017
HH													
	0 - 70	0.385	0.327	0.332	0.300	0.289	0.302	0.258	0.279	0.301	0.302	0.017	0.010
	70 - 250	0.282	0.250	0.258	0.252	0.210	0.286	0.214	0.184	0.238	0.241	0.010	0.009
	250 - 600	0.248	0.240	0.284	0.259	-	-	-	0.226	0.227	0.246	0.009	0.017
MEAN													
	0 - 70	0.381	0.331	0.319	0.299	0.285	0.292	0.253	0.288	0.317	0.302	0.013	0.008
	70 - 250	0.292	0.258	0.260	0.246	0.222	0.274	0.214	0.189	0.244	0.244	0.007	0.006
	250 - 600	0.262	0.258	0.257	0.273	-	-	-	0.234	0.236	0.251	0.006	0.012

1. Standard error appropriate for comparing grazing managements averaged over time.
2. Approximate standard error for comparing means of different harvests within the same grazing treatments. This standard error derived from split-plot in time analysis, and subject to confirmation of significance for specific pairs of means, see Appendix 1.1.

4.3.1.2 Data from refilled core sampling

For refilled core results, only apparent root growth rates (kg ash-free root DM ha^{-1} day^{-1}; Figure 4.4, page 71; Table 4.4, page 72) and mean root diameter (Table 4.5, page 71; Figure 4.5, page 71), are presented, together with some data from refilled cores left for two harvest intervals. Refilled core root length data have been omitted because they rather closely mirror root mass (Table 4.4), and do not add anything to discussion of the results. A summary of results of analysis of variance for refilled core root length data appears in Appendix 4, however.

Seasonal variation in apparent root growth rate calculated from refilled core data (Figure 4.4, Table 4.4) resembled a seasonal pasture growth curve, but with values about 10% - 20% of those expected for above ground herbage accumulation, and ranging from 1.5 kg DM ha^{-1} day^{-1} in June/July (Harvest 6) to 10.2 kg DM ha^{-1} day^{-1} in February (Harvest 3). These seasonal changes were highly significant ($F_{1,6} = 72$, $P < 0.001$) when plot means for apparent root production for Harvests 5 & 6 were analysed with corresponding means for Harvests 7 - 10). The relationship between these values for root production and above ground tissue turnover is examined and discussed in Section 7.2.2.

Differences in root growth due to grazing management were again small. Averaged over the 9 harvests for 0 - 250 mm soil depth, values for root production were 6.8 kg DM ha^{-1} day^{-1} ($F_{1,3} = 0.04$, NS) for both LL and HH plots (Table 4.4). As with intact core samples (Section 4.3.1.1), there were no significant grazing management x time interactions for apparent root production.

Seasonal changes in diameter of the new roots were seen even more strongly in refilled cores than in intact cores (Figure 4.5), with values greatest in mid winter and falling sharply in spring (Mean values for new roots in 0 - 70 mm soil depth were 0.336 mm and 0.182 mm for Harvests 6 & 9 respectively, Table 4.5; $F_{1,6} = 103$, $P < 0.001$). There was no evidence that grazing management influenced diameter of new roots in refilled cores.

Figure 4.4: Seasonal and grazing management variation in apparent root growth rates (kg DM ha^{-1} day^{-1} for 0 - 250 mm soil depth; LL ————————, HH — — — —). G = standard error for grazing effects averaged over time; S = standard error averaged over time, for mean of LL & HH plots at individual harvests.

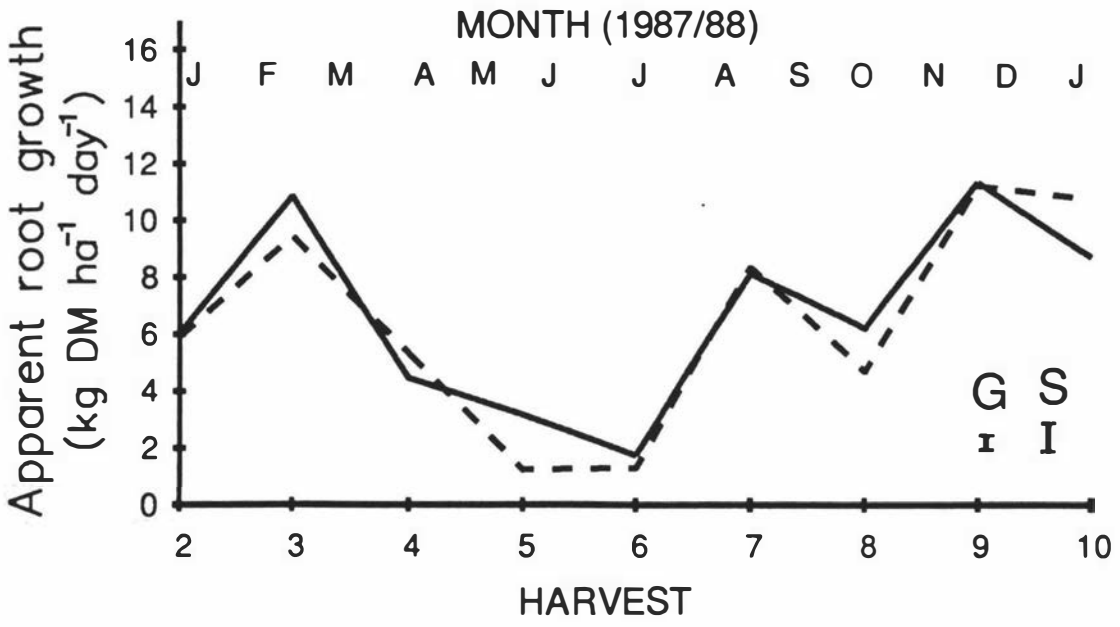


Figure 4.5: Seasonal change in refilled core root diameter for LL (————————) and HH (— — — —) grazing managements (Values calculated from root mass and length totals over 0 - 250 mm soil depth). G = standard error for grazing effects averaged over time; S = standard error averaged over time, for mean of LL & HH plots at individual harvests.

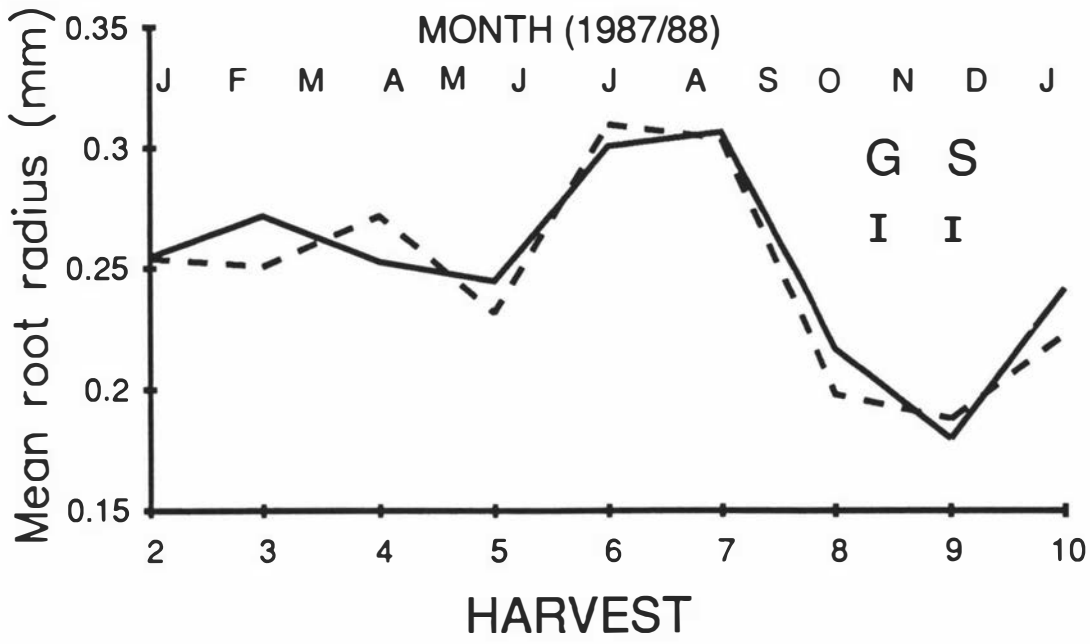


Table 4.4: Apparent root production (kg DM ha^{-1} day^{-1} ash-free DM) for three soil depths and two grazing managements determined from refilled core samples for period January 1987 to January 1988 (Experiment 2)

Grazing	Soil Depth (mm)	Harvest 2	3	4	5	6	7	8	9	10	MEAN	S.E.[1]	S.E.[2]
LL													
	0 - 70	4.3	7.6	3.1	2.3	1.2	6.1	4.2	6.8	5.9	4.6	0.4	0.9
	70 - 250	1.8	3.3	1.4	0.9	0.6	2.0	2.0	4.6	2.8	2.2	0.3	0.6
	250 - 600	1.1	0.6	0.4	0.1	-	-	-	2.4	1.4	1.1	0.3	0.4
HH													
	0 - 70	3.8	6.0	3.6	0.9	0.9	5.8	4.8	7.8	7.7	4.6	0.4	0.9
	70 - 250	2.1	3.4	1.8	0.4	0.4	2.6	2.8	3.5	3.1	2.2	0.3	0.6
	250 - 600	1.9	0.8	0.6	0.1	-	-	-	1.8	3.0	1.4	0.3	0.4
MEAN													
	0 - 70	4.0	6.8	3.3	1.6	1.1	6.0	4.5	7.3	6.8	4.6	0.3	0.6
	70 - 250	1.9	3.4	1.6	0.6	0.5	2.3	2.4	4.0	2.9	2.2	0.2	0.4
	250 - 600	1.3	0.6	0.5	0.1	-	-	-	2.2	2.2	1.2	0.2	0.3

1. Standard error appropriate for comparing grazing managements averaged over time.
2. Approximate standard error for comparing means of different harvests within the same grazing treatments. This standard error derived from split-plot in time analysis, and subject to confirmation of significance for specific pairs of means, see Appendix 1.1.

Table 4.5: Mean root diameter (mm) for refilled core samples from two soil depths and two grazing managements for period January 1987 to January 1988 (Experiment 2).

Grazing	Soil Depth (mm)	Harvest 2	3	4	5	6	7	8	9	10	MEAN	S.E.[1]	S.E.[2]
LL													
	0 - 70	0.277	0.261	0.242	0.241	0.347	0.307	0.228	0.189	0.260	0.261	0.017	0.014
	70 - 250	0.216	0.285	0.292	0.256	0.250	0.313	0.199	0.175	0.220	0.245	0.010	0.014
	250 - 600	0.237	0.242	0.289	0.202	-	-	-	0.187	0.164	0.218	0.019	0.019
HH													
	0 - 70	0.277	0.250	0.285	0.228	0.325	0.301	0.196	0.195	0.238	0.255	0.017	0.014
	70 - 250	0.223	0.250	0.259	0.255	0.282	0.313	0.201	0.170	0.211	0.241	0.010	0.014
	250 - 600	0.236	0.220	0.266	0.129	-	-	-	0.176	0.204	0.215	0.019	0.019
MEAN													
	0 - 70	0.277	0.255	0.264	0.235	0.336	0.303	0.212	0.195	0.246	0.258	0.012	0.010
	70 - 250	0.220	0.268	0.275	0.258	0.266	0.313	0.200	0.172	0.216	0.243	0.007	0.010
	250 - 600	0.237	0.231	0.276	0.166	-	-	-	0.183	0.181	0.217	0.013	0.013

1. Standard error appropriate for comparing grazing managements averaged over time.
2. Approximate standard error for comparing means of different harvests within the same grazing treatments. This standard error derived from split-plot in time analysis, and subject to confirmation of significance for specific pairs of means, see Appendix 1.1.

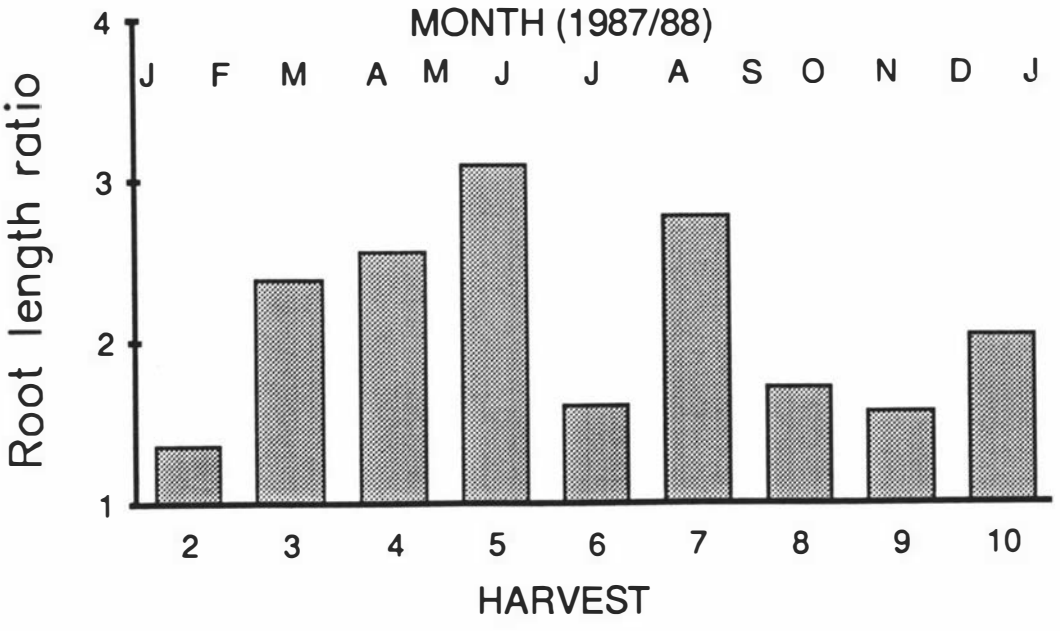
The tendency for an increase during winter in the proportion of total root mass or length found in the upper soil horizon was again evident. For example, the ratio apparent root production (0 - 70 mm soil depth): apparent root production (250 - 600 mm soil depth) increased from 6 at Harvest 2 to 22 at Harvest 4 (Table 4.6). Again, the ratio refilled core root length (0 - 70 mm soil depth):root length (70 - 250 mm soil depth) showed a clear winter maximum (Figure 4.6), except for Harvest 6 where the ratio is lower reflecting low root production values (Table 4.4) and larger diameter roots (Table 4.5) for the 0 - 70 mm soil depth at this time.

Table 4.6: Ratio apparent root production for 0 - 70 mm soil depth: apparent root production for 250 - 600 mm soil depth.

	Harvest					
Grazing Management	2	3	4	5	9	10
LL	8	15	15	18	3	9
HH	4	17	27	15	4	3
Mean	6	16	22	16	4	6
LSR of Mean	1.7	1.7	2.4	1.5	1.5	1.3

1. LSR = least significant ratio (Steel and Torrie, 1981) derived from standard error of log-transformed data.

Figure 4.6: Seasonal change in ratio of refilled core root length for upper:middle soil depths (0 - 70 mm:70 - 250 mm).



Estimated root turnover time (Section 3.4.1) was calculated for the 70 - 250 mm soil depth, and showed pronounced seasonal variation, but no statistically significant differences between grazing management treatments (Table 4.7). The calculation was based on values for the 70 - 250 mm soil depth, because intact core results for this depth had lower co-efficients of variation and showed clearer evidence of treatment effects, than did values for the 0 - 70 mm soil depth (Table 4.1, Appendix 4). Also there was evidence (Appendix 2.6) that refilled core data for the 0 - 70 mm soil depth underestimated actual root production more than data for the 70 - 250 mm soil depth.

Table 4.7: Estimate of root turnover time (Days) derived from ratio intact core root mass: refilled core root mass (70 - 250 mm soil depth).

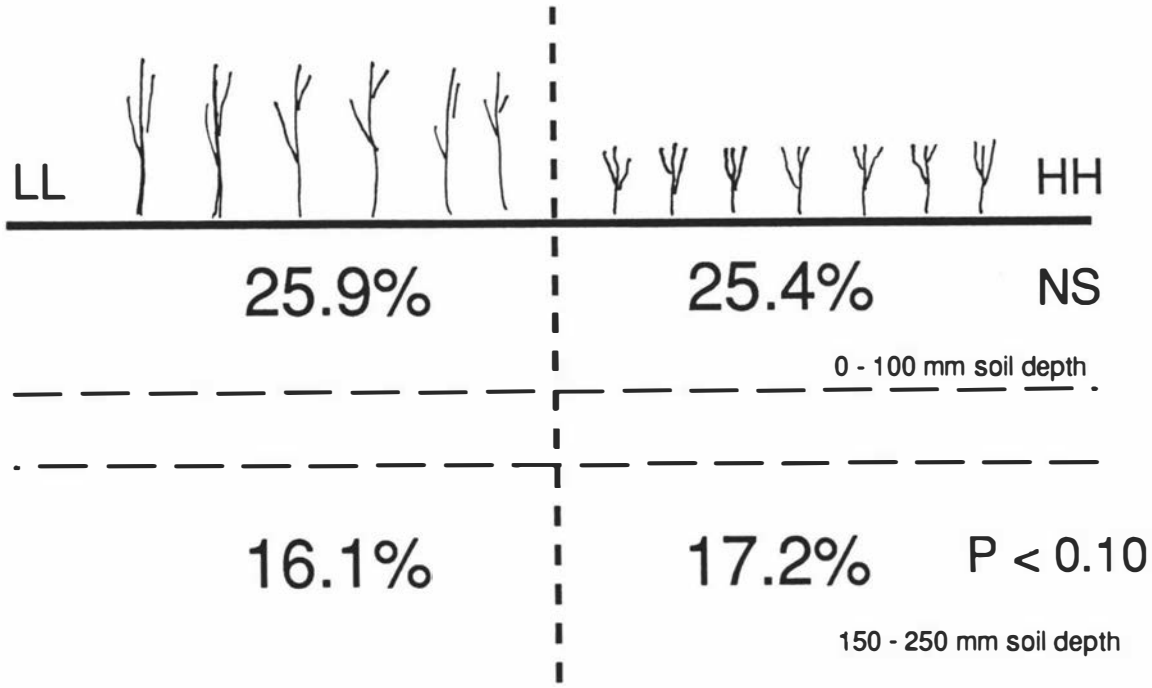
	Harvest								
Grazing	2	3	4	5	6	7	8	9	10
LL	555	311	660	984	1326	269	311	169	342
HH	392	227	475	2741	1911	226	231	160	196
Mean	473	269	568	1862	1618	247	271	165	269
SEM.	96	17	93	622	399	27	38	42	59

In order to obtain a weighted mean value for replacement time, the 72 data used to compile the means presented in Table 4.7 were log-transformed, a Fourier equation fitted (Lambert *et al.*, 1986), and the constant back-transformed. Since all sine and cosine terms in the Fourier equation sum to zero when the curve is integrated over a 360 degree (annual) cycle, the constant represents the mean root replacement time. The value so obtained was 650 days (SE $\pm$ 72 Days).

When placing refilled cores for Harvests 1, 2, and 4; additional refilled cores were placed and harvested approximately 80 days later at Harvests 2, 3, and 5, respectively. Mean apparent root growth rates for the 8 LL and HH plots were 3.8, 5.9 and 1.5 kg DM ha^{-1} day^{-1} for 80 day periods preceding Harvests 2, 3, and 5, respectively. These values are only 60% to 70% of mean values for corresponding 40 day periods (Table 4.4), suggesting that by the time the refilled cores had been in place 80 days, much of the root formed in the first 40 days had already died and decayed.

Finally, there was possible evidence that root growth into refilled cores for Harvests 2 & 3 exhibited a grazing management x soil depth interaction. Refilled core data for Harvests 2 & 3 appeared to indicate a higher proportion of total new root growth in the 0 - 70 mm soil depth for the LL-grazed plots, but in the 70 -250 mm soil depth for the HH-grazed plots. This interaction was not statistically significant when data for Harvest 2 or 3 were analysed individually, but approached significance when values for the two harvests were averaged ($F_{1,6}$ = 4.9, P = 0.07). It was thought this interaction might be due to differences between LL & HH plots in soil moisture distribution with depth. Some gravimetric soil moisture measurements had been made on 3 December 1986, and these are diagrammatically represented in Figure 4.7.

Figure 4.7. Gravimetric soil moisture levels for LL and HH plots on 3 December 1986. Data are for 0 - 100 mm and 150 - 250 mm soil depths.



4.3.2 Introduction of cross-over LH and HL grazing managements

Intact core root mass, length and diameter for Harvests 10 and 12 are presented in Table 4.8 (page 78), and refilled core root mass and mean diameter in Table 4.9 (page 79).

For this period, there was evidence of a continued decline in intact core root mass. For 0 - 600 mm soil depths, intact core root mass for Harvests 10 & 12 was 3108 and 2642 kg AFDW ha^{-1}, respectively (S.E. = 108, $F_{1,12} = 9.4$, P = 0.01) and the tendency for roots in 70 - 250 mm and 250 - 600 mm soil depths to have finer mean diameter than those for the 0 - 70 mm soil depth (Section 4.3.1.1) was confirmed. There were no statistically significant differences between grazing managements for intact core root mass, root length or mean diameter (Table 4.8) although there were significant grazing management effects on apparent root production in this period (Table 4.9).

At Harvest 10, root production for HL plots was higher than for other treatments and this observation coincided with the appearance of numbers of seed heads in late December 1987/January 1988, after these plots were switched from hard to lax grazing on 7 December 1987 (Section 5.3.3.2). At Harvest 12 hard-grazed plots (HH and LH) had significantly less root production than lax-grazed (LL and HL) treatments, and values for LH plots averaged only 79% of those for HH plots (Table 4.9), although this difference between LH and HH plots was not statistically significant.

Table 4.8: Root mass (AFDW, kg DM ha^{-1}), root length (km m^{-2}) and mean root diameter for intact core samples at Harvests 10 & 12.

	Grazing management											
	LL		HH		LH		HL		S.E.M.		Signif.	
	H.10	H.12	H.10	H.12	H.10	H.12	H.10	H.12	H. 10	H.12	H.10	H.12
Root mass												
Soil depth (mm)												
0 - 70	2612	1779	2198	2258	1841	1741	2173	1905	341	173	NS	NS
70 - 250	705	417	544	321	623	377	715	572	108	70	NS	NS
250 - 600	253	335	195	331	352	233	219	300	51	98	NS	NS
Root length												
Soil depth (mm)												
0 - 70	37.9	33.5	37.7	36.1	36.1	28.2	36.3	33.5	6.1	2.9	NS	NS
70 - 250	18.2	9.3	15.2	6.6	18.9	9.2	15.7	11.8	3.3	1.3	NS	NS
250 - 600	6.9	7.7	5.9	8.5	9.9	5.5	6.3	6.9	1.6	2.0	NS	NS
Mean root Diameter												
Soil depth (mm)												
0 - 70	0.332	0.293	0.301	0.315	0.292	0.315	0.309	0.301	0.016	0.014	NS	NS
70 - 250	0.250	0.275	0.238	0.275	0.231	0.257	0.268	0.280	0.010	0.012	NS	NS
250 - 600	0.244	0.264	0.227	0.256	0.242	0.274	0.237	0.255	0.010	0.017	NS	NS

Table 4.9: Apparent root production (kg DM ha^{-1} day^{-1}) and mean root diameter for refilled core samples at Harvests 10 & 12.

	Grazing management											
	LL		HH		LH		HL		S.E.		Signif.	
	H.10	H.12	H.10	H.12	H.10	H.12	H.10	H.12	H.10	H.12	H.10	H.12
Root production												
Soil depth (mm)												
0 - 70	5.9	2.3	7.7	1.3	7.2	1.0	9.5	2.7	0.4	0.4	*	*
70 - 250	2.8	1.4	3.1	0.7	3.3	0.5	3.7	2.0	0.5	0.3	NS	*
250 - 600	1.4	2.7	3.0	1.2	1.2	1.1	1.5	1.9	0.4	0.7	NS	NS
Mean root Diameter												
Soil depth (mm)												
0 - 70	0.260	0.329	0.238	0.340	0.240	0.330	0.248	0.338	0.005	0.014	*	NS
70 - 250	0.220	0.349	0.211	0.352	0.305	0.309	0.227	0.307	0.029	0.037	NS	NS
250 - 600	0.164	0.273	0.204	0.280	0.179	0.253	0.169	0.308	0.015	0.014	NS	NS

4.4 Discussion

4.4.1 Seasonal variation in sward root mass, root length, and root diameter; and seasonality of root replacement

There is evidence from other studies that quantity of root under (Baker, 1957) or produced by (Atkinson, 1984) a grass sward increases in summer and declines in winter, so the reduction in root AFDW (Tables 4.1 & 4.9, Figure 4.2a) over the course of the experiment is unexpected and there is no obvious explanation for it. The decline is unlikely to be due to the decay and disappearance of dead clover roots as experimental measurements did not begin until 5 months after clover had been sprayed, and dead roots should have largely disappeared in that time (Gibbs, 1986). One possibility is that continual addition of mineral nitrogen as urea (Appendix 3) affected root dynamics, but there is no way to confirm this. A summer peak in the quantity of root from intact core samples might have been expected given the high values for apparent root production in refilled cores in summer (Table 4.4; Figure 4.4) and could have been masked by the gradual decline in total root mass over the course of the experiment. For example, the fall in total root mass (Fig 4.1a) is most marked in autumn, and in spring root mass tended to remain constant.

Apart from the decline in intact core root mass during the experiment, the features of root production and turnover identified in this study are:

1. A winter minimum and spring-summer maximum for root production (Table 4.4, Figure 4.4), with corresponding changes in turnover time (Table 4.7).

2. A loss of root from and a reduction of root growth at lower soil depths in winter (Figure 4.6, Table 4.6).

3) Changes in ratio of root for different soil depths (Figure 4.3) and in mean diameter of roots from refilled cores (Figure 4.5, Table 4.5) indicative of a pulse of root replacement in late winter.

4) Evidence from 80-day refilled core data (Section 4.3.1.2), and from estimates of turnover time that ryegrass root replacement is probably much faster than the 68% per year calculated by Jacques & Schwass (1956).

Also, for Harvests 7 to 10 of Figures 4.2b & 4.4, some data differ from those published by Matthew *et al.* (1991), as a result of adjustments to the earlier calculations. The differences do not materially affect any discussion presented in the earlier paper, however.

The sharp rise in apparent root production in July (Figure 4.4) and the observation that roots produced at this time were of larger mean diameter than roots produced at other times of the year (Table 4.5) is evidence for an annual flush of nodal root initiation, consistent with patterns reported in the two earlier New Zealand studies (Jacques, 1956; Caradus & Evans, 1977) and a major British study (Garwood, 1967a). Indeed these large diameter new "white" roots were visibly conspicuous in refilled cores, particularly in the 0 - 70 mm soil depth at Harvest 6 and in 0 - 250 mm soil depths at Harvest 7, where the presence of these roots is indicated by greater mean diameter (Table 4.5).

However, previous New Zealand research (Jacques, 1956; Caradus & Evans, 1977) has suggested that new root formation in perennial ryegrass does not occur in summer. Jacques' (1956) schematic diagram (which is based on unpresented data) shows almost no root activity at all in the 0 - 300 mm soil depth between December and March, and Caradus & Evans' (1977) data shows no root production after October. In contrast, measurement using the refilled core technique showed continued high root production after October with a second peak in December (Figure 4.4). The view that an annual crop of roots commences growth in late autumn, peaks in late winter, and continues growth at depth over the summer and into the following winter (Jacques, 1956; Caradus & Evans, 1977) is therefore not supported by the data. Rather, it would appear that root production in a ryegrass sward is normally continuous, but modified by soil conditions at particular depths in the soil profile.

Thus, as soil conditions became moister in winter, and presumably also hypoxic, it appears that many roots died back (Tables 4.1, 4.2), and such new root growth as did occur, was concentrated in the upper portion of the soil profile (Table 4.6). Then following this annual winter loss of root there was an accelerated replacement in early spring, which saw root mass in the 250 - 600 mm soil depth restored by early December (Table 4.1).

Jacques (1956) also noted that deeper roots are in danger of being killed when the water table rises in winter, although he presents no data, and his

schematic diagram shows root tips at depth remaining active until at least July. On the other hand, the pattern of root growth reported above (Section 4.3) appears similar to that observed in Britain by Garwood (1967b). Although Garwood (1967b, 1968) did record peak root production in spring, and a summer reduction in numbers of new roots elongating near the soil surface; the summer reduction was associated with dry soil conditions when gravimetric soil moisture fell to below 10% on unirrigated plots and to approximately 11% on irrigated plots (Garwood, 1965). He did, however, observe at least some new roots at all times of the year.

This growth strategy of continuous root turnover, modified by impact of seasonal factors, would confer on grass swards an ability to exploit different zones of the soil profile at different times of the year, in response to changing conditions of moisture and nutrient supply.

This study did not closely examine the factors which might be influencing the rate of root production at different times of the year, although the similarity to a pasture growth curve has been mentioned above (Section 4.3.1.2), and this suggests that temperature and moisture are important. It is also evident that high rates of apparent root production at Harvest 9 (Table 4.4), together with low mean diameters for new roots (Table 4.5), coincide with a period of high tiller appearance (Section 5.3.4.3.2), possibly reflecting a crop of roots from the newly formed tillers. This would be consistent with enhanced root production on RUHE and RUAN plots in Experiment 1 (Section 3.3.1.3), where there was also evidence that high root production was linked to high tillering rates (Section 3.4.3.2). Similarly, an apparent correspondence between seasonal patterns of root appearance is evident if seasonal tiller appearance patterns for an S23 perennial ryegrass sward (Garwood, 1969), are compared with seasonal root appearance patterns (Garwood, 1967a), and this link between seasonal patterns of root and tiller appearance has been noted by Garwood himself (Garwood, 1967b).

It is likely that the value for mean root replacement time of 650 days estimated from intact core:refilled core sample ratios (Section 4.3.1.2), substantially overestimates root turnover time. Turnover would be overestimated if intact core samples contained dead roots in the process of decomposition, and this would almost certainly have been the case. Turnover time would also have been overestimated if refilled core data underestimated root production, and

calibration of root growth in refilled cores showed that mean numbers of roots arriving at viewing tubes in refilled cores in Experiment 3 were approximately half those arriving at paired viewing tubes in undisturbed soil. Troughton (1981) estimated maximum root longevity for defoliated ryegrass plants at 191 days, and for two studies involving direct observation, reported longevities of ryegrass roots were 61 - 188 days (Garwood, 1967a), and 29 - 81 days (Gibbs, 1986); and this would seem to confirm that the 650 day value for turnover time can be considered to be an overestimate.

Garwood (1968) reported that the degree of root branching increases and mean diameter decreases with increasing temperature. This effect observed by Garwood (1968) could explain the increase in mean diameter of new roots in refilled cores in winter (Harvests 6 & 7, Figure 4.5); but not the sudden decrease in mean diameter of refilled core roots at Harvests 8 & 9, which, as noted above might be related to production of smaller diameter roots from new tillers in early spring. Similarly the gradual decline in root diameter of intact core samples over winter (Table 4.3) would not be explained by the temperature effect observed by Garwood (1968). The seasonal change in mean diameter of roots from intact core samples resulted in root lengths remaining relatively constant against a background of declining root mass (Figures 4.2a,b). Further study to confirm such effects and examine their functional significance could be worthwhile.

4.4.2 Effect of grazing management on root mass, root length, and root production

The concentration of root near the top of the soil profile (Section 4.3.1.1) agrees well with many other studies (e.g. Evans, 1978; Gibbs, 1986; Mackie Dawson & Atkinson, 1991). Since earlier studies have shown that increased frequency or severity of defoliation decreases root growth (Evans, 1971a), grazing management induced differences in the proportion of root in the various depths might have been expected, but no such differences were found. In general the evidence from this study is that any reduction in quantity of root under more severe grazing occurs throughout the soil profile in proportion to the quantity of root at a given depth.

Previous studies of effects of defoliation on root systems of grasses have found effects of defoliation to be large (Troughton, 1957; Evans, 1973) and

important in determining herbage productivity (Evans, 1971a,b 1976). It is suggested that the relatively small difference in root parameters between LL and HH plots in this study (Sections 4.3.1.1 & 4.3.1.2) can be explained in two ways.

First, although LL and HH plots were consistently grazed to differing residual herbage masses, there was compensation within the sward so that the difference in mass was found largely in the stubble component, and both LL and HH grazing managements developed very similar values for leaf mass and LAI (Section 5.3.3.1). Secondly, many of the earlier studies used seedling plants and/or combinations of frequency and intensity of defoliation much more severe than that applying to grazed swards in the field. Both these points are of critical importance when considering the likely impact of defoliation on root growth. Partitioning of assimilate to the root system in establishing seedlings can be of the order of 50% of total assimilate, whereas in established swards this decreases to 7% (Ryle, 1970) but ranging from 5% to 15% on a seasonal basis (Parsons & Robson, 1981b). It therefore follows that defoliation in seedling plants would cause a much more dramatic reduction in root growth than would defoliation in established swards, and this should be borne in mind when interpreting implications for farm practice of earlier results from studies on young plants.

Similarly, in grazed swards defoliation is normally rather less severe than that often applied during indoor studies. For example Evans (1972) defoliated plants every two days to heights of 25, 50 and 75 mm, whereas on farms using rotational management typical grazing intervals are 15 - 20 days in periods of rapid pasture growth and as long as 60 - 100 days in winter; and stubble height would seldom be less than 50 mm and often longer. Alternatively, under more continuous management regimes grazing interval for any one tiller is likely to be around 10 days (Hodgson & Ollerenshaw, 1969; Arosteguy, 1982) and only a proportion of the total leaf area is removed at any one grazing. This situation more nearly equates to Evans' (1971 a,b) defoliation to 100 mm every two days, where relatively minor reductions in root growth were observed.

This study therefore shows that major effects on root growth as a result of grazing management predicted by Evans (1976) could only be expected if defoliation were much more severe than normally occurs in farm practice. Thus, while increased grazing pressure in HH plots (relative to LL plots) did

consistently reduce root growth in this study, that effect does not appear to be large enough to offer significant opportunity to manipulate root growth through differential grazing management.

The reason for the higher root production on HL plots in conjunction with seed head formation after release from hard grazing (Section 4.3.2) is unclear. This is consistent with a similar increase on RUHE and RUAN plots in Experiment 3 (Sections 3.3.1.3 & 3.4.3.1) which was thought to be possibly due to increased supply of assimilate associated with high photosynthetic uptake reproductive swards (Parsons & Robson, 1981b). On the other hand, if increased root growth of reproductive swards is driven by increased assimilate, then high root growth might also have been expected on LL plots during the main flowering period in November (Harvest 9), and no such effect is evident in the results.

4.4.3 Effects of cross-over grazing management treatments

Data from this phase of Experiment 2 largely confirm the findings (Sections 4.4.1, 4.4.2) from the first phase of the experiment. Root production was high in summer (Harvest 10) and fell to very low values by May (Harvest 12) and grazing management effects on root mass were relatively small.

The low root production on hard-grazed plots may relate to more severe grazing pressure inadvertently applied when sheep removed more herbage than expected, reducing herbage mass to approximately 500 kg DM ha^{-1}, during two consecutive grazings immediately prior to Harvest 12. This may indicate that more substantial reductions in root growth predicted by Evans (1976) could occur in field swards subjected to more frequent and or severe grazing pressure than that applied to HH plots in the current study.

The very low rate of root production at Harvest 12 on the LH plots is hard to understand, given the high rates of new tiller formation and herbage accumulation observed on this treatment (Sections 5.3.3, 5.3.4). It is possible that Harvest 10 (January 1988) might have been too early, and Harvest 12 (May 1988) too late to detect roots formed by new tillers on the LH treatment. Increased root formation following reproductive growth was detected in Experiment 1 in an early February harvest, but not in a January harvest. Another possibility is that roots on decapitated, flowering, parent tillers might remain alive for a period, supplying their daughter tillers and allowing a higher

percentage of assimilate to be partitioned to leaf production on the new tillers. Such an effect does appear to occur with prairie grass (*Bromus willdenowii* Kunth; C. K. Black, personal communication), and has also been suggested as an explanation for behaviour of perennial ryegrass by Garwood (1969). If such an effect does occur, this would indicate a possible way to alter temporarily the allocation between root and shoot, and effectively shift some allocation from below ground to above ground, where it might be expressed as herbage production.

4.4.4 Influence of soil moisture level on root growth

Conditions were dry enough in December 1986 for pastures to begin to turn brown, and it is likely that low soil moisture levels at this time explain the fact that root growth in refilled cores at Harvest 2 (6.0 kg DM ha^{-1} day^{-1} averaged over HH and LL plots, Figure 4.4) was lower than at either Harvest 1 (7.9 kg DM ha^{-1} day^{-1} averaged over LL and HH plots) or Harvest 3 (10.2 kg DM ha^{-1} day^{-1} averaged over LL and HH plots, Figure 4.4).

In addition it has been well documented (Haylock, 1987) that soil moisture levels decrease more quickly at depth under unmown swards than under swards mown short. Haylock (1987) estimated evapotranspiration on a loessial soil similar that used in the current experiment as being 2.7, 3.4 and 3.5 mm day^{-1} for short, medium and long grass respectively, over the period November 1985 to April 1986. He also showed that a differential soil moisture deficit developed gradually over a summer measurement period, and that by late summer volumetric soil moisture at 400 mm soil depth was more than 10% higher on plots mown short than on unmown plots.

In this experiment, gravimetric soil moisture data for early December 1986 (Section 4.3.1.2) appears to indicate the development of differential soil moisture levels under LL and HH plots similar to those recorded by Haylock (1987), with HH plots having higher soil moisture levels at depth. The evidence from some further soil moisture measurements in summer of 1987/88 was that in addition to the lower soil moisture levels at depth, LL plots had higher soil moisture levels in the 0 - 70 mm soil depth than HH plots. This effect was not detected in the December 1986 measurements, however, probably because 10.7 mm rain fell 4 days prior to determination of soil moisture content.

Although the evidence for a difference in soil moisture levels on LL and HH plots is not conclusive, it seems reasonable to assume that soil moisture differences similar to those documented by Haylock (1987) did exist in early December 1986, and continued to develop through December 1986 and January 1987, when rainfall was much less than evapotranspiration (Figure 4.1). If root growth is sensitive to differences in soil moisture level, such differences could therefore explain the grazing x soil depth interaction whereby apparent root growth for the 70 - 250 mm soil depth was higher on HH plots than on LL plots, despite lower total root growth on HH plots (Table 4.4, Section 4.3.1.2). However, a further study designed specifically to substantiate this would be desirable.

4.5 Summary

1. This experiment provides more comprehensive data on variation in ryegrass root mass and root production with season than any previous New Zealand study, and provides the first field data on effects of grazing management on root growth.

2. The annual flush of root production in late winter (August - September) reported by Jacques (1956), and by Caradus and Evans (1977) is confirmed, but contrary to those previous studies, root production remained high over summer, except in periods of soil moisture deficit.

3. Over the range of grazing management regimes studied, effects of grazing management on the root system were found to be relatively small. Because normal farm practice is unlikely to involve either laxer or more severe grazing than the LL or HH regimes used in this study, it is concluded that use of grazing management to manipulate the root system of grass swards is unlikely to lead to any effective strategy to systematically increase herbage accumulation.

4. Further study of effects of grazing on vertical distribution of soil moisture and consequent effects on root growth, of the causes and implications of seasonal changes in root diameter, could be rewarding.

CHAPTER 5: ABOVE-GROUND MEASUREMENTS - TILLER DYNAMICS, HERBAGE MASS & HERBAGE ACCUMULATION, AND OTHER RESULTS.

5.1 Introduction and overview

The second objective of this study (Section 1.2) was to monitor above-ground behaviour of swards in conjunction with the root measurements reported in Chapter 4, and to examine the relationship between above- and below-ground dynamics.

This chapter reports tiller population densities, tiller appearance and death rates, and herbage accumulation measurements carried out during Experiment 2. In the course of the experiment it was realised that stolon formation is an important aspect of ryegrass ecology, so measurements on quantity of stolon present were commenced. These are also reported in this chapter, as are other non-root data collected during Experiment 2, such as information on the extent of soil fertility transfer within plots by grazing animals.

The question of root/shoot relations is addressed in Chapter 7, in conjunction with root and tiller dynamics data from a follow-up experiment reported in Chapter 6.

5.2 Experimental

Details of experimental site and design have been reported in a previous chapter (Sections 4.2.2, 4.2.3). Methods used to provide above-ground and other data supporting root measurements presented in Chapter 4 are now described briefly.

5.2.1 Soil fertility transfer through grazing behaviour

When fertility transfer to the centre of plots became evident (Section 4.2.4) soil samples were collected from both centres and edges of the 16 plots on a split plot basis and analysed for Olsen P, K (meq%), Ca (meq%), Mg (meq%), and pH. Sampling date was 3 August 1987. Olsen P was analysed

as an indicator of soil fertility transfer through dung deposition, and soil K as an indicator of fertility transfer through urine deposition. It is reasonable to assume that gradients in soil N would also have existed (Matthew *et al.*, 1988), though no attempt to measure these was made. Other cations (Ca, Mg) and pH were analysed as part of a standard laboratory procedure for processing soil samples, rather than because differences were expected. Data were analysed using the ANOVA command of MINITAB, as a split-plot randomised complete block design (column effects in the Latin square were not significant and were pooled with the error). Plot position (centre/edge) was entered into the analysis as a random effect.

5.2.2 Stolon development

The term "stolon" is used here to describe underground stem segments of ryegrass tillers, from which adventitious roots and new tillers arise. The term stolon is normally applied to horizontal stems formed by internode elongation above ground, and certain grasses, for example creeping bent (*Agrostis stolonifera* L.) are well known for this growth habit. Though formation of stolons in ryegrass has been described by a number of authors including Harris *et al.* (1979) and Korte & Harris (1987); ryegrass is not usually listed among species with a stoloniferous habit, probably because the stolons are located underground, and tend to be orientated vertically and are relatively short (20 - 30 mm) and therefore easily overlooked.

During the planning of the experiment no provision was made for measurements of stolon formation because few previous studies mention this aspect of ryegrass ecology. However, during measurements at Harvest 1 it quickly became apparent that root samples for the 0 - 70 mm soil depth contained appreciable quantities of underground stem or stolon. For Harvests 1 to 4 this material, much of which was dry and dead, was dissected out from root samples and discarded. Then, at Harvest **5** active formation of new stolons was clearly evident after growing points of most tillers became buried by as much as 10 - **15** mm by earthworm activity following autumn rains. In view of this active stolon formation it was decided to make measurements on stolon length.

For harvest **5** total stolon length was measured, and at future harvests two sub-categories of stolon, smooth internode (I) segments and rough segments with numbers of nodes and associated leaf scars and roots

closely spaced ("vascular" - V) segments were determined. Korte and Harris (1987) note that these stems are stolons rather than rhizomes, because of their morphology, even though they typically are found underground. For the same reason they are reported here with above-ground measurements, rather than with root measurements in Chapter 4. Stolon data for successive harvests were analysed statistically as split plot in time sub-treatments of LL and HH main-treatments. In addition to stolon length measurements, on 16 October 1987 the distance of the growing point above or below ground level was measured for 3 tillers in each of 3 locations on each plot (9 tillers per plot). The 3 locations were centre (C) and edge (E) (Section 5.2.1) of each plot, and (M) mid way between centre and edge. As with soil fertility tests (Section 5.2.1), C, M, and E locations were treated as random, split-plot effects for statistical purposes. Tillers for determination of growing point height were randomly chosen using a point analysis frame.

5.2.3 Herbage mass and herbage accumulation

For Harvests 1 - 6 herbage mass was determined at each harvest to assist in keeping LL and HH grazing managements within specified target values. Ryegrass leaf, vegetative stem, reproductive stem (if present) and other grass components of total herbage mass were determined by herbage dissection. Specific leaf area was also determined at Harvest 2 and subsequently, and sward leaf area index (LAI) calculated. From Harvest 7 onwards, herbage mass was determined before and after each grazing to enable calculation of herbage accumulation rates. For this latter period leaf production and senescence and daughter tiller production were also determined independently at strategic intervals by tissue turnover techniques (Davies, 1981) as part of an independent study (Xia, 1991).

Intensive measurements were made for the period between Harvests 9 and 10. Grazing dates for LL, HH, LH and HL plots over this period are shown in Table 5.1, and herbage mass was measured before and after each grazing to allow calculation of herbage accumulation. Tissue turnover measurements were made on all plots during each interval between grazings. This data formed part of a separate study and has been presented in detail by Xia (1991), however a summary is included here, also.

Table 5.1: Grazing dates (|) for LL, HH, LH and HL plots, December 1987 to January 1988.

Grazing	Date 7 Dec	21 Dec	28 Dec	6 Jan	18 Jan
LL	|		|		|
HH	|		|		|
LH	|	|		|	|
HL				|	|

Herbage mass measurements to enable calculation of herbage accumulation were again made between Harvests 11 and 12. In conjunction with these measurements, tissue turnover measurements were made on tagged tillers in fixed quadrats. This enabled comparison of herbage production potentials of old (appeared before 14 October 1987) and young (appeared after 3 January, 1988) tillers in the sward.

5.2.4 Tiller population densities and tiller appearance and death rates

For each harvest date in Experiment 2 (Section 4.2.5), tiller population densities were measured by collecting and counting thirty 53 mm diameter tiller plugs per plot (Mitchell & Glenday, 1958). Categories determined were (i) vegetative ryegrass tillers, (ii) reproductive ryegrass tillers (if present), (iii) *Poa* sp. (mainly *P. trivialis* L.), and (iv) other grass species.

Sampling of LH and HL treatments commenced in September 1987 (Harvest 7). As with root measurements (Chapter 4), data for successive harvests on LL and HH plots over the period December 1986 to January 1988 were analysed statistically using a split-plot in time analysis. Data for the 4 treatments from September 1987 were analysed for individual harvest dates using the Latin square analysis as described in Section 4.2.3.

Tiller appearance and death rates were determined by monitoring tagged tillers in fixed quadrats (Korte, 1986). Quadrats used were circular, 103 mm diameter, and there were 3 per plot. Total numbers of tillers in fixed quadrats gave a second estimate of tiller population densities for comparison with values obtained using the tiller plug method.

Fixed quadrats were replaced twice during the course of the experiment (Series 2 placed May 1987 and Series 3 placed September 1987) when, as a result of the 6-8 week interval between harvests, quadrats had become buried by earthworm activity and stock trampling. On these two occasions data could be recovered only by destructive means (removal of the fixed quadrats to the laboratory for washing of soil from around tiller bases). Diameter of the replacement quadrats (Series 2 & 3) was reduced from 103 mm to 65 mm with a view to reducing the very high labour requirement for the full set of root and tiller measurements. Dates when tillers in fixed quadrats were retagged are shown in Appendix 3. Tiller appearance and death between each tagging were expressed as tillers m^{-2} day^{-1}, and standard errors determined by analysis of variance of plot mean values.

When fixed quadrats on LL and HH plots were destructively harvested in May 1987, those on plots reserved for LH and HL grazing were considered redundant and were abandoned. However, it was later realised that these buried quadrats could provide information on stolon formation since placement of the tiller tags, and on the identity of parents of spring-formed tillers, an aspect of tiller dynamics for which there is currently little information (Section 2.2.3). Consequently, these quadrats were relocated and destructively sampled in December 1987. For statistical analysis of data from these buried quadrats, individual tagged tillers were treated as independent observations, and parameters such as the number of daughter tillers per tagged tiller analysed for differences between age categories of parent tiller.

The measurements of tiller dynamics required 2-4 days (2 persons) at each tagging, depending on the numbers of daughter tillers to be tagged, and were normally carried out 1-2 weeks after collection of root samples.

5.3 Results and discussion

In this chapter, because of the diversity of measurements reported, results and discussion sections are combined, and data are discussed as they are presented.

5.3.1 Soil fertility transfer

For LL and HH main effects, no statistically significant differences were detected for any of the soil tests. However, the centre/edge split plot effect

attributed to fertility transfer associated with grazing behaviour was significant for Olsen P, meq% K, and meq% Mg (P = 0.008, 0.012 and 0.020, respectively; Table 5.2).

Table 5.2: Soil test values for centre and edges of plots (mean of 4 grazing management treatments) indicating fertility transfer due to grazing behaviour.

Plot position.	Olsen P	meq% K	meq% Ca	meq% Mg	pH
Edge	7.8	0.30	6.98	0.74	5.7
Centre	12.3	0.63	7.61	0.83	5.7
SE	1.15	0.097	0.86	0.022	0.7

The extent to which the fertility gradients had developed (more than 50% difference in Olsen P and more than 100% difference in soil K) is interesting in its own right, considering that plots had been set up less than a year at the time soil tests were taken. Fertility transfer through dung and urine effects is well known on hill country (Suckling, 1975; Gillingham, 1982) or after long periods of transfer on paddocks of flat topography (Hilder, 1964; Matthew *et al.*, 1988); but it is not generally realised that transfer of this magnitude can occur in so short a time.

It is felt that other measurements in the current experiment would not have been seriously affected, however, because the areas of high fertility were avoided for sampling purposes, except where there was a deliberate intention to examine on a split-plot basis the effect of soil fertility on stolon formation (Section 5.3.2).

5.3.2 Stolon formation

Even though stolon measurement did not commence until later in the experiment, enough data was collected to give a picture of an annual cycle of stolon formation and death. At Harvest 5, total stolon length was 96 m and 58 m stolon m^{-2} ground for LL and HH plots respectively, and these values increased steadily until December, then declined again (Table 5.3). Lengths of stolon in these ryegrass swards (Table 5.3) were similar to values of 48 to 150 m m^{-2} which are obtained for white clover swards if stolon mass figures of Hay

& Chapman (1984) are divided by weight per unit length. The major difference is that white clover stolon is orientated horizontally, and ryegrass stolon vertically in the soil, creating a binding effect where the two species grow together.

Table 5.3: Stolon lengths (m stolon m^{-2} ground) for period July 1987 to August 1988 (Harvests 6 to 12).

Stolon category.		Harvest H6	H7	H8	H9	H10	H12	SEM	
	Grazing								
V	LL	36	35	50	44	46	25	}8.5	NS
	HH	48	31	45	49	50	36		
I	LL	66	80	116	120	127	49	}16	**
	HH	41	31	70	88	66	9		
Total	LL	102	115	165	164	173	74	}22	**
	HH	90	61	114	137	116	45		

It appears that data on stolon formation in ryegrass has appeared only twice before in the New Zealand literature. Harris *et al.*, (1979) used gel electrophoresis to show that individual ryegrass plants in a lawn had spread by means of stolon formation to occupy areas up to 1 m diameter. Korte & Harris (1987) in a study published after the present study commenced, describe the morphology of stolon formation and present data on stolon numbers in a 'Grasslands Nui' ryegrass sward, but give no information on stolon lengths or mass.

Stolon formation appeared to be a response to burial of tiller bases. The burial appeared to be partly due to casting by earthworms which were very active after the first autumn rain, and partly due to the effects of trampling by animals when the bearing strength of soil was reduced by high soil moisture levels in winter. These observations agree with those made earlier by Korte and Harris (1987) who also suggested that shading of tiller bases or burial by earthworm casting and animal treading promoted stolon formation in 'Grasslands Nui' ryegrass. Similarly (Hay *et al.*, 1987)), studying seasonal burial of white clover stolon, found that earthworm casting and animal treading were probable causes of this burial in early and late winter, respectively.

In this study none of a population of more than 2,000 tillers tagged survived the winter if internode elongation failed to occur and the growing point remained buried. Burial of fixed quadrat rings averaged 18 mm and 15 mm ($P<0.1$) for LL and HH plots, respectively; and for the sample of 396 tillers analysed in Section 5.3.4.6.2, the mean distance between the tiller tag position and the current soil surface was 21.5 mm. The discrepancy between these two values probably indicates the extent to which tiller axes are angled obliquely below the soil surface. In an informal trial, when tillers on potted plants in a glasshouse had sheaves of 3 mm diameter black plastic tube placed over them, internode formation began within 1 - 2 weeks and stolons up to 150 mm long could be induced. Another point is that underground stolon formation in late winter and above-ground stem elongation at flowering appeared to be two phases of the same event. Most tillers which overwintered flowered, and the continuity between stolon formation and stem elongation is evident from the differing growing point height for tillers of differing status (Table 5.4).

Stolon mass was approximately 1.15 mg DM mm^{-1}, so that the increase in stolon density of approximately 50 m m^{-2} on LL and HH plots during winter (Table 5.3) amounted to about 0.6 t. DM ha^{-1}. Assuming a 90 day period for this stolon formation, this amounts to approximately 7 kg DM ha^{-1} day^{-1}, which would be a substantial proportion of net photosynthesis over the winter period. In fact, growing points of tillers measured in LL plots were 143 mm higher than on HH plots on 16 October, and there was also a soil fertility effect, with a gradation from edges to centres of plots in growing point height above ground on the same date (Table 5.4).

Table 5.4: Height of growing points (mm) above or below (-) soil surface on 16 October, 1987. Data are for 3 positions along soil fertility gradients on LL and HH plots.

Grazing	Fertility status Low E	M	High C	Mean	SEM Grazing
LL	81	181	204	155	13
HH	-14	0	36	7	
Mean	33	90	120		
SEM Fertility		40			

Significance: Grazing effects, $P=0.004$; Fertility effects $P=0.065$.
E = edge of plots, C = centre of plots, M = intermediate between centre and edge of plots.

Greater total length of stolon on LL plots than on HH plots (Table 5.3) and more elevated growing points on 16 October for both LL plots (compared to HH plots) and high fertility (compared to low fertility) portions of plots (Table 5.4) may indicate that plants under stress of closer defoliation or lower soil nutrient status lack substrate for rapid stolon formation after burial. This could have important implications for tillering, as presumably tillering on buried growing points is suppressed. A separate study to quantify in more detail effects of grazing management, soil fertility and other factors on stolon formation, and to determine implications for tillering responses, could be rewarding.

A summary of the seasonal cycle of stolon formation observed in this experiment is as follows:

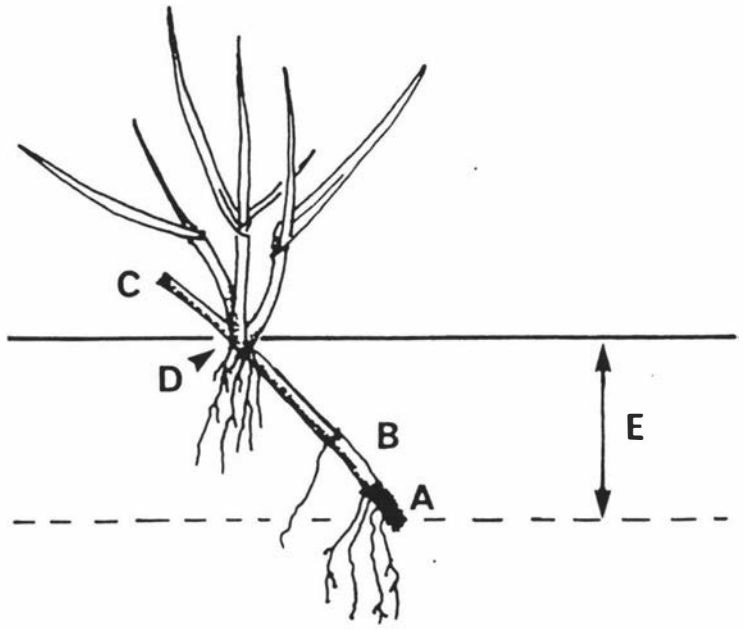
1. Over summer, nodes formed in conjunction with each new leaf produced resulted in a vascular segment of stolon on each tiller, located at or just below ground level and usually about 10 mm long by autumn (A, Figure 5.1). Quantities of this category of stolon formed were similar on LL and HH plots (Table 5.3).

2. With burial, internodes began to elongate, forming internode segments (B, Figure 5.1) and raising the growing point back to ground level. There was evidence that more I stolon was formed on LL plots than on HH plots (Table 5.3).

3. Most tillers which had formed stolons over the winter turned reproductive in spring and flowered. A stub of a flowering tiller decapitated by grazing is shown (C, Figure 5.1). In this sense stolon formation and reproductive stem elongation were consecutive events on individual tiller axes, and both increased soil nutrient status and laxer grazing management advanced the stem elongation phase (Table 5.4).

4. Finally, in November or December, daughter tillers (D, Figure 5.1) formed at the base of flowering tillers. Secondary tillers from these daughter tillers rapidly appeared. A new vegetative plantlet was established, and stolons formed the previous winter disintegrated over summer. The total movement of the soil surface, relative to tiller position, is shown by the distance E (Figure 5.1).

Figure 5.1: Diagram illustrating annual cycle of stolon formation: (a) V segment of stolon formed over summer, (b) I segment of stolon formed after burial, (c) remains of decapitated flowering tiller, (d) daughter tiller hierarchy comprising one primary daughter and 2 secondaries (e) extent of burial over winter.



This process is illustrated in Plate 5.1 which shows a tiller hierarchy excavated from an LH plot in May 1988. Stubbs of two dead flowering tillers are visible (A,B, Plate 5.1), each with a cohort of daughter tillers. A skeleton axis connecting both tillers to a common parent is also visible and indicates that the two tillers flowering in the current season were themselves the sole surviving daughters of one previous season's tiller (C, Plate 5.1).

It is likely that this cycle would vary from season to season and between localities. For example, burial might commence at any time between February and May, depending on rainfall patterns, and in some localities there may be little burial at all (Hay *et al.*, 1987).

The cycle of stolon formation, flowering, and daughter tiller formation represents a pathway for perennation of grass swards. It is often presumed that survival of perennial grass swards is primarily through tillers which remain vegetative and do not flower (see e.g. Korte, 1986). The cycle described above is an alternative pathway for perennation through the appearance of young daughter tillers at the base of flowering tillers. This process is analogous to coppicing of tree crops.

Plate 5.1: Photograph of tiller cohort excavated in May 1988, showing stubs of flowering tillers (arrowed, A & B), associated daughter tiller hierarchies, and previous season's stolon connecting both hierarchies to a common parent tiller (arrowed, C).

Such a perennation pathway has previously been described for timothy (*Phleum pratense* L.) by Langer (1979) and by Jewiss (1966, 1981). The pathway was also described for ryegrass by Colvill & Marshall (1984), who stated that the daughter tillers from flowering tillers are long lived and form the bulk of the next season's flowering tillers, thus ensuring the perenniality of the plant. The existence of such tillers was noted by Korte (1986) who suggested that they might ensure perenniality where few vegetative tillers survive.

It was these observations on the propensity of tillers forming stolons in winter to later flower and form daughter tillers which resulted in the author relocating buried fixed quadrats which had been abandoned the previous summer (Section 5.2.4). These quadrats were analysed so as to give information on the proportions of non-flowering older tillers and of recently formed daughter tillers from flowering tillers in an early-summer sward (Section 5.3.4.6).

5.3.3 Herbage mass and herbage accumulation

5.3.3.1 January to November 1987, prior to introduction of crossover grazing managements

Herbage mass data for Harvests 1 to 6 (Table 5.5) show that the intended contrast in herbage mass between LL and HH plots was maintained. These values do not indicate the highest or lowest levels of herbage mass attained, however, as root harvests and associated above-ground measurements were not synchronised with grazing. The data indicate that the proportion of total herbage mass as leaf was higher, and the proportion as dead material lower, for HH plots than for LL plots, except at Harvest 1 (Table 5.5), and this would have at least partially offset any tendency for harder grazing on HH plots to reduce net assimilation.

Herbage data for Harvests 7 & 8 prior to introduction of crossover grazing managements (Tables 5.6, 5.7) show that leaf area index and herbage accumulation was similar for LL and HH grazing treatments and for the two sets of plots reserved for LH and HL grazing. The similar herbage accumulation is not unexpected, as the range of herbage masses observed over the interval between grazings (Table 5.6) would put LL and HH treatments within the upper and lower boundaries respectively for optimum herbage accumulation (Bircham & Hodgson, 1983); assuming that herbage

Table 5.5: Herbage mass (kg DM ha^{-1}) on LL and HH plots at Harvests 1 to 6.

Harvest	Date	Grazing	Total Herbage	Vegetative Leaf	Vegetative Stem	Rep.	*Poa* sp.	Dead	Leaf Area Index
1	5-12-86	LL	2470	650	340	500	290	680	ND
		HH	990	270	170	110	130	310	
		SEM	180	80	30	50	50	80	
		P	0.010	0.040	0.015	0.012	NS	0.049	
2	17-1-87	LL	3230	1000	140	1090	40	100	0.79
		HH	990	410	40	120	30	400	0.32
		SEM	160	140	40	220	10	110	0.31
		P	0.002	0.054	NS	0.051	NS	0.035	NS
3	24-2-87	LL	2810	1050	330	Trace	20	1400	1.51
		HH	990	580	190	Trace	0	220	0.79
		SEM	200	100	40	-	10	80	0.16
		P	0.007	0.048	0.089	-	n/a	0.002	0.048
5	2-5-85	LL	2210	1280	350	Nil	Trace	580	1.95
		HH	770	620	110	Nil	Trace	40	0.96
		SEM	220	120	40	-	-	100	0.40
		P	0.019	0.030	0.017	-	-	0.031	NS
6	9-7-87	LL	2820	1760	520	Nil	Trace	540	1.98
		HH	980	760	180	Nil	Trace	50	0.81
		SEM	200	80	80	-	-	70	0.23
		P	0.008	0.004	0.062	-	-	0.016	NS

Note: P = statistical probability. n/a - SEM not available, too many zeros in raw data. NS = P > 0.10. Harvest 4 - missing data.

accumulation on rotationally grazed swards at a given average herbage mass is not greatly different from that on continuously grazed swards of the same herbage mass (Parsons *et al.*, 1988a).

Table 5.6: Herbage mass (kg DM ha^{-1}) on LL, HH, LH and HL plots for period 9 September to 20 October (Harvest 7 to Harvest 8).

Harvest	Date	Grazing	Total Herbage	Vegetative Leaf	Stem	*Poa* sp.	Dead	Leaf area Index
7a	9-9-87	LL	2370	680	940	Trace	740	1.62
		HH	900	440	350	Trace	110	1.16
		LH	1880	630	740	Trace	510	1.76
		HL	930	450	350	Trace	130	0.98
		SEM	290	130	140	-	100	0.34
		P	0.026	NS	0.050	-	0.009	NS
7b	25-9-87	LL	3200	1170	900	220	910	2.03
		HH	1830	1100	460	10	270	1.77
		LH	3060	1090	840	490	640	1.86
		HL	1980	1020	520	100	340	1.65
		SEM	230	70	60	100	140	0.20
		P	0.010	NS	0.004	0.062	0.054	NS
8a	2-10-87	LL	1760	230	770	70	680	0.59
		HH	570	110	240	20	190	0.52
		LH	2060	310	890	0	870	0.63
		HL	780	170	360	Trace	250	0.51
		SEM	130	50	70	20	70	0.12
		P	<0.001	NS	0.011	NS	0.028	NS
8b	22-10-87	LL	3430	1060	1030	270	1080	3.36
		HH	1840	1070	490	90	190	3.43
		LH	3360	1010	1060	370	930	4.18
		HL	2080	1120	530	150	270	5.21
		SEM	340	30	110	130	180	0.61
		P	0.053	0.035	0.029	NS	0.042	NS

Note: P = statistical probability. NS = P > 0.10.

Table 5.7: Herbage accumulation for the four grazing managements over two periods prior to the institution of the crossover treatments.

Date	Grazing Management LL	HH	LH	HL	SEM
9-9-87 to 25-9-87	52	59	73	66	13 (NS)
2-10-87 to 22-10-87	84	64	65	70	12 (NS)

5.3.3.2 Summer-autumn 1987/88, following introduction of crossover grazing managements

For the December - January period (Harvest 10), accumulation of components of herbage mass was calculated by summing increases between grazings (for grazing dates see Table 5.1) over the period. It should be noted that this method of determination ignores fluxes occurring during the measurement period, and that total herbage accumulation (Table 5.8, page 102) includes a large negative component arising from decomposition of reproductive stem. For "green" herbage accumulation (leaf, vegetative stem, daughter tillers and *Poa*) values for the grazing management treatments ranked in the order LH>LL>HL>HH (Table 5.8), and parallel tissue turnover measurements resulted in a similar ranking (Figure 5.2). It was also clear that there were highly significant differences between grazing managements for particular herbage components. In particular there was high daughter tiller production on LH plots (Table 5.8), though this was less strongly evident in the tissue turnover data (Figure 5.2).

Figure 5.2 Herbage accumulation rates (kg DM ha^{-1} day^{-1}) for the four grazing treatments (a) 9 - 27 December 1987, (b) 30 December 1987 - 14 January 1988; determined by tissue turnover (Adapted from Xia, 1991).

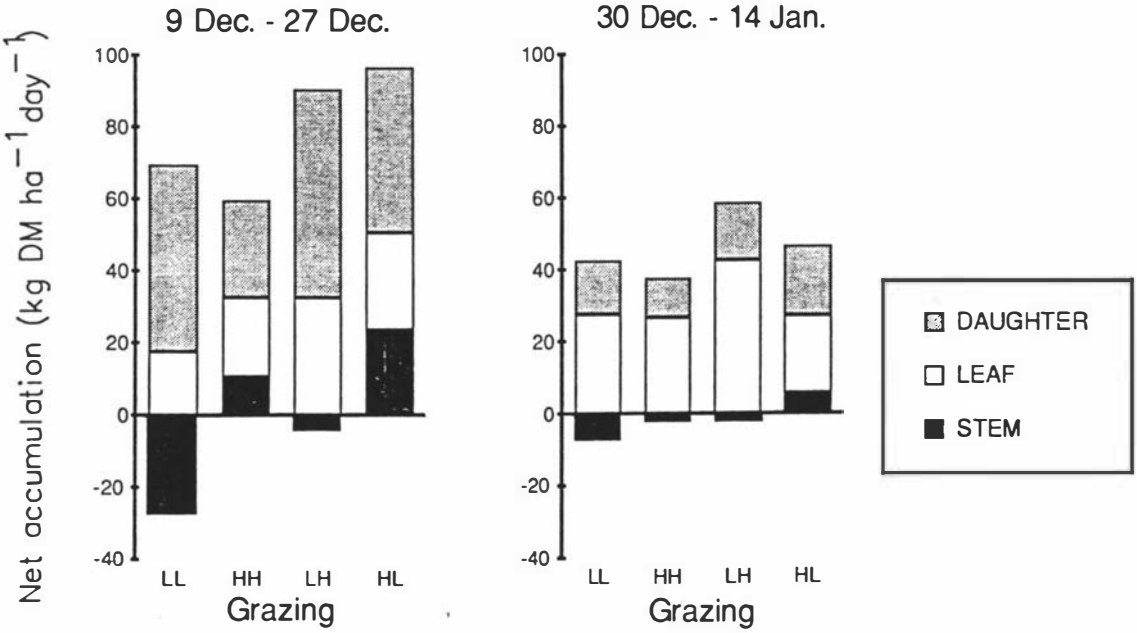


Table 5.8: Total herbage accumulation (kg DM ha^{-1}) determined by quadrat cuts (cutting dates given in Table 5.1) for LL, HH, LH, and HL plots for period 9-12-87 to 18-1-88.

Grazing	Total Herbage	'Green' Herbage	Mature Leaf	Immature Leaf	Daughter Tiller	Veget. Stem	Herbage component Reprod. Stem	*Poa* sp.	Dead
LL	2920	3230	2130	680	130	450	-810	-160	510
HH	1990	2070	1250	520	110	150	10	40	-80
LH	2090	3620	1810	660	530	440	-820	190	-710
HL	3280	2310	1140	550	120	490	210	10	750
SEM	650	380	210	100	50	160	310	130	350
P	NS	0.051	0.026	NS	0.001	NS	0.081	NS	0.065

'Green' herbage = sum of mature & immature leaf, vegetative stem, *Poa*, and daughter tiller components.

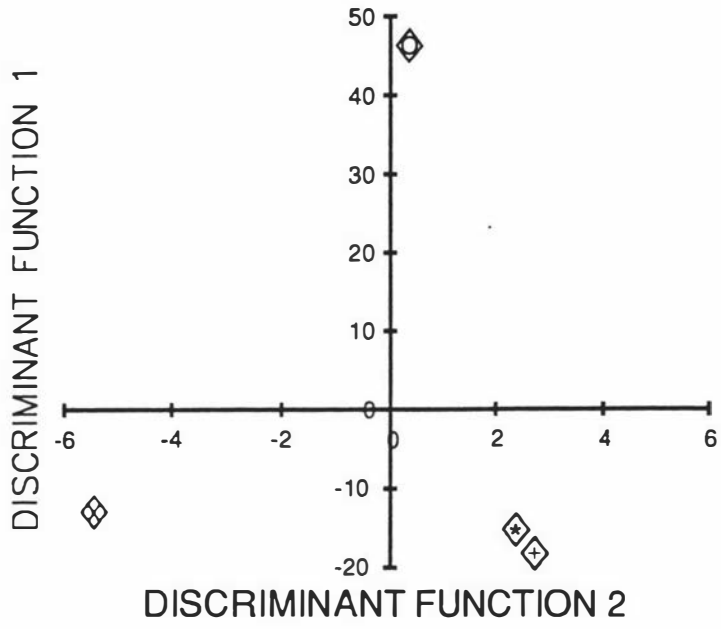
These differences in components of herbage accumulation were analysed using multiple discriminant analysis (MDA) as explained in Appendix 1.3. This MDA of the components of herbage accumulation yielded a first discriminant function which accounted for a very high 98.5% of the multivariate dispersion (Table 5.9). Examination of the canonical structure shows that this variate can be interpreted as indicating herbage accumulation based on daughter tiller, leaf, and *Poa* components, but not on stem or dead components. A second discriminant function was also statistically significant, and a plot of these first two discriminant functions (Figure 5.3), using the format of Chatfield & Collins (1980), shows that the first picks out the LH treatment as being very different from the other treatments, while the second indicates differences in components of herbage accumulation for the LL treatment.

Table 5.9: Canonical structure and summary statistics for multiple discriminant analysis of components of herbage accumulation for the period 9-12-87 to 18-1-88.

Herbage mass component.	Discriminant 1	Discriminant 2
Vegetative stem.	0.23	-0.35
Reproductive stem	-0.63	0.77
Mature leaf	0.38	-0.92
Immature leaf	0.55	-0.80
Daughter tiller	0.99	0.03
Poa	0.75	0.66
Dead	-0.85	-0.15
Canonical r^2	0.999	0.950
P	<0.001	0.048
Proportion of dispersion (%)	98.5	1.4
Cumulative proportion	98.5	99.9

Herbage accumulation was again monitored over the autumn period between Harvests 11 & 12 (23 March to 5 May, 1988). By this stage plots had resumed vegetative growth so that differences in components of herbage accumulation were not evident as they had been after manipulation of reproductive growth in late spring. LH and HL plots produced significantly more herbage over this period than did LL & HH plots, with average herbage accumulation (kg DM ha^{-1} day^{-1}) being 61, 58, 42, & 44 (S.E $\pm$ 6.5) for LH, HL, LL, & HH treatments, respectively.

Figure 5.3: Grazing management means for discriminant functions 1 & 2, obtained by multiple discriminant analysis of components of herbage accumulation. Symbols, (x) = LL, (*) = HH, (o) = LH, (+) = HL.



These herbage mass and tissue turnover results are very much as might have been predicted from established principles of sward dynamics. For example it is well known that gross herbage production is usually higher at high herbage mass, but that the high herbage production is largely offset by high senescence losses (Parsons *et al.*, 1983a,b; Bircham & Hodgson, 1983), and the observations for LL- & HH-grazed plots largely support this (Tables 5.7, 5.8, Figure 5.2). What is perhaps noteworthy about the present results, is that while grazing pressure on HH plots was severe enough to reduce herbage accumulation on these plots to approximately 60% of that on LL plots (Table 5.8), compensations within the plant were such that root mass for the 0 - 250 mm soil depth was reduced by only 20% on HH plots at Harvest 10, root length was reduced by less than 10% (Table 4.8), and values for apparent root production were actually higher on HH plots (Table 4.9).

Also, enhanced net herbage production might have been expected for HL plots after release of a tiller population from grazing (Grant *et al.*, 1988), especially where reproductive growth was likely to occur in the released sward (Table 3.6, Figure 3.4, Section 3.3.4.2). Both tissue turnover data (Figure 5.2, Xia, 1991) and total herbage accumulation data from quadrat cuts (Table 5.8) indicate high total accumulation on the HL plots, although the data also indicate high values for senescence and for reproductive stem on these plots.

On the other hand, some features of the results were not as expected. For example, while an increase in tillering on LH plots after hard grazing was expected (Section 2.2.3), the extent to which this event dominated the MDA was not expected and was the salient feature of the herbage mass and herbage accumulation data for this second period in the experiment. Also, LH plots ranked highly in terms of overall herbage accumulation, both in quadrat cuts, and tissue turnover measurements, and for two different measurement periods. There is good evidence that this high growth is based on the appearance of the new tillers produced after hard grazing of these plots on 7 December (Tables 5.8, 5.9, Figure 5.2).

It has been suggested (Tallowin, 1981) that differences in tiller-age profiles can be induced by grazing management, and might account for differences in sward productivity in summer. It is possible that such an effect might have occurred in the current experiment, but there is little or no data in the literature about the relative productive capacities of old and young tillers. In order to test Tallowin's (1981) hypothesis, a limited series of tissue turnover measurements was commenced in March 1988 for old and young tillers in fixed quadrats, and results from these measurements are included with other tiller dynamics measurements (Section 5.3.4.5).

5.3.4 Tiller dynamics

5.3.4.1 Tiller population densities and size/density relations

Tiller population densities for LL and HH plots for the period January 1987 to January 1988 are presented in Figure 5.4; and for LL, HH, LH and HL plots for the period October 1987 to May 1988 in Figure 5.5. Tiller densities were higher in summer than in winter ($F_{1,6}$ = 96.6, P<0.001 when plot means for Harvests 2, 3 & 10 were compared with plot means for Harvests 5, 6, 7, & 8) and throughout the experiment were higher for HH plots than for LL plots (Figure 5.5; P = 0.002).

Adjustment of tiller densities in response to cross-over LH and HL grazing managements took some months. Tiller densities for LH and HL plots remained intermediate between those of LL and HH plots even at the final harvest in early May 1988 (Figure 5.5). However, when tiller size is taken into account by means of a tiller size/density diagram, it is evident that size/density

combinations for the 16 plots have an underlying common relationship. Figure 5.6 shows tiller size/density combinations (logarithmic scale) for the 16 plots on 9 December 1987 (immediately post-grazing); 18 January 1988 (pre-grazing, but at the end of a regrowth cycle) and on 8 April 1988 (15 days into a regrowth cycle). In each case the slope of the tiller size/density line is approximately -2.5, and the line shows seasonal shift (Figure 5.6). For the plots in Figure 5.6, mean tiller size was estimated on the basis of total herbage mass including dead and stubble components. This was done in order to reduce co-efficents of variation for the data, but needs to be taken account of in interpreting the diagrams.

Seasonal and grazing management effects on the tiller population density of the other grass species present, *Poa trivialis* L., were also evident. The data suggested that the peak tiller density for *Poa* occurs earlier in the summer than does the peak tiller density for ryegrass (Appendix 5). Also tiller population densities for *Poa* were significantly lower on HH than on LL plots (Appendix 5), probably because of sensitivity of the shallow rooted *Poa* to lower soil moisture levels near the soil surface on HH plots in summer (Section 4.4.4).

Figure 5.4: Ryegrass tiller densities on LL (————) and HH (— — —) plots from January 1987 to January 1988. Values shown include reproductive tillers where present. Bars represent SEM for grazing managements averaged over time (G) and approximate SEM for preliminary assessment of seasonal differences (S).

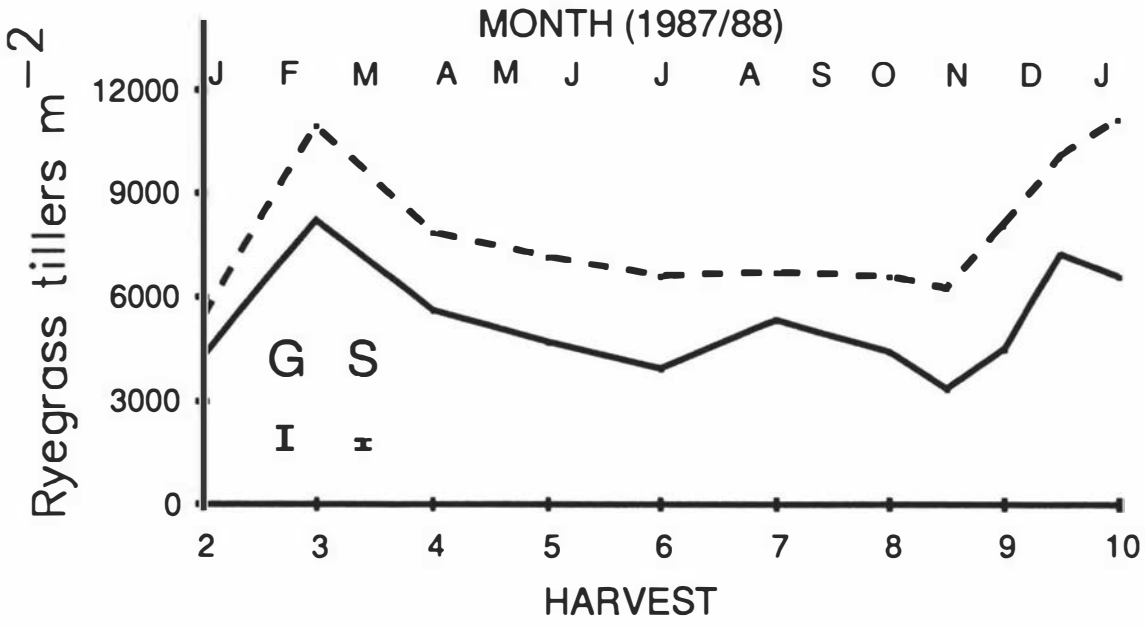


Figure 5.5: Ryegrass tiller densities on LL, HH, LH and HL plots from September 1987 to May 1988. Bars represent SEM for grazing management means at particular dates.

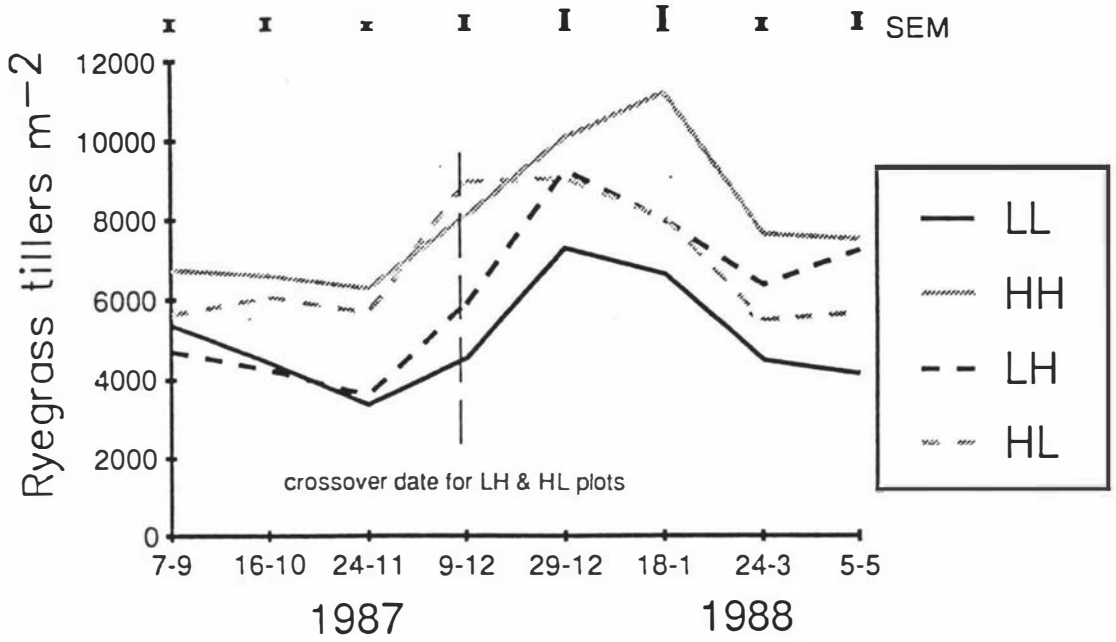
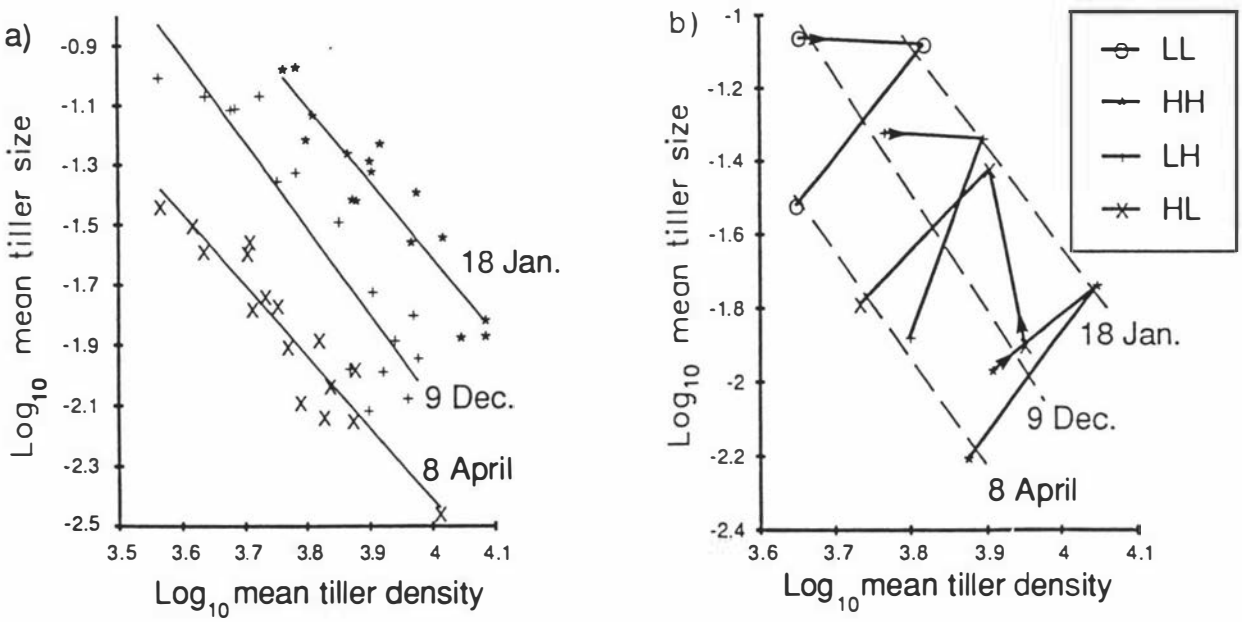


Figure 5.6: Seasonal and grazing management effects on tiller size/density relationships (logarithmic scale) for (a) 16 plots, (b) mean of 4 grazing managements, summer 1987/88.



9 December (+):	Log Tiller weight = 9.45 - 2.88 Log tiller density	$r^2 = 0.845$
18 January (•):	Log Tiller weight = 8.57 - 2.55 Log tiller density	$r^2 = 0.874$
8 April (X):	Log Tiller weight = 7.05 - 2.36 Log tiller density	$r^2 = 0.886$

Variation in tiller population density in response to grazing management and other stimuli such as seasonal changes in incident light levels is now well documented by a number of authors including Grant *et al.* (1981), Lonsdale & Watkinson (1982), Bircham & Hodgson (1983), and Davies (1988). The increased tiller density on HH plots compared to LL plots was expected (Sections 2..2.3 & 4.2.1), and the seasonal movement in the position of the size/density equilibrium line is consistent with a response to increase light levels in summer and declining levels in autumn (Grant *et al.*, 1981; Davies, 1988). The decline may also be partly related to tiller death in dry conditions in late January 1988, as irrigation probably did not fully compensate for low rainfall at this time (Figure 4.1).

Factors affecting the slope of the size/density line, and the movement towards or away from it are less well understood, however. Tiller size/density adjustment is normally assumed to obey the -3/2 power rule (Kays & Harper, 1974; Lonsdale & Watkinson, 1982; Davies 1988). However the proof of the -3/2 power rule requires that plant units of different size have identical morphology (Yoda *et al.*, 1963), whereas in grass swards the proportion of pseudostem tends to increase and the proportion of leaf tends to decrease with increasing herbage mass (see e.g. Section 5.3.3.1, above). Again, it is easily verified by simple arithmetic calculation that adherence to the -3/2 power rule dictates increasing herbage mass with decreasing tiller density, whereas in grass swards at high herbage mass tiller density is expected to decrease due to base shading (Davies, 1988) while herbage mass remains constant due to a high rate of senescence (Bircham & Hodgson, 1983). Under such conditions, if self thinning occurred, a self thinning line would be constrained to follow a slope of -1, not -3/2. Finally, the -3/2 power rule was originally proposed by Yoda *et al.* (1963) to describe the behaviour of plant populations increasing in size at a predetermined density; while grass tillers may be subjected to size reduction due to grazing and respond by initiation of new daughter tillers, so increasing the population density. In this case the -3/2 power rule is actually working in reverse, and there have been few, if any, studies which have monitored plant populations following a trajectory *down* a -3/2 power line.

At the introduction of crossover LH & HL grazing managements on 7 December 1987 tiller densities were already higher than on the corresponding LL and HH plots (Figure 5.5), indicating a possible disturbance effect (Section 5.3.4.4). Because of this, tiller size/density combinations for LH plots after

grazing on 9 December do not fall below the regression line (Figure 5.6), as might have been expected immediately after a hard grazing. It is not felt that this unexpectedly high tiller size/density would have contributed to the high herbage accumulation on LH plots, however, as no such effects were evident prior to hard grazing (Tables 5.6, 5.7) and because mean tiller size on 9 December would have been considerably inflated by dead herbage and stubs of reproductive tillers present after grazing.

On the other hand, by 12 April, size/density values for LL & LH plots were slightly above the regression line, and values for HH and HL plots were slightly below the line (Figure 5.6). When residual deviations around the size/density line for 12 April were subjected to analysis of variance, mean values for LL, LH, HL and HH plots were +0.05, +0.05, -0.09, & -0.01, respectively (SE $\pm$ 02; $P < 0.01$). This indicates that LL- and LH-grazed plots had more than maintained their position relative to HH and HL grazing managements. That this should occur, despite high senescence of reproductive stem on the LL and LH plots (Table 5.8), seems surprising. In this respect the high size/density values for LH plots at a time of high herbage accumulation appears to parallel the high size/density values for RUHE & RUAN plots (Figure 3.4, Sections 3.3.2.2, 3.4.3.2).

The rather high slopes of approximately -2.5 for the size/density relationships in Figure 5.6 probably arise from a combination of factors. These would include the presence of dead herbage which would have inflated mean tiller size values, especially on LL and HL plots; the higher proportion of pseudostem on LL plots; and possibly limitation of expression of potential tillering responses on HH plots due to insufficient substrate supply.

One difference between this study and previous studies is that the seasonal increase in tiller density did not begin until early summer (November, Figure 5.4). By contrast the timing of the increase in tiller density in Korte's 1986 study was July - September (Figure 2.1a, Section 2.2.3) and other New Zealand studies also show evidence of tiller density increase earlier than November (Hunt & Field, 1978; Hunt, 1989). Again, in a British study (Garwood 1969); where seasonal patterns of flowering are expected to be later relative to the longest day than in New Zealand (Korte, 1986), seasonal peaks in tiller density had occurred by April. The reason for the delay in tiller appearance in spring in this study is not known, but differences between seasons in tiller dynamics need to be better understood if reliable pasture management guidelines for

farmers are to be developed.

Ryegrass tiller densities for Harvest 4 (April 1987) include counts of up to 460 and 85 tillers m-2 on LL and HH plots respectively, considered to be young seedlings. The fact that numbers of seedlings were so low indicates that recruitment of seedlings was not important to maintenance of tiller populations in this experiment, unlike the situation in another recent study where large numbers of seedlings were observed (L'Huillier & Aislabie, 1988).

5.3.4.2 Proportion of reproductive tillers

Reproductive tillers were present at Harvests 1 - 3, and at Harvests 8 - 10 the following summer, and the percentage of reproductive tillers for each harvest is given in Table 5.10. The peak of reproductive development had probably passed by the time measurements for the first season commenced in December 1986. For the second season, percentage of reproductive tillers exceeded 30% for LL plots in November 1987, while values for HH plots were never greater than 7% (Table 5.10).

Table 5.10: Percentage of live ryegrass tillers (including grazed stubs) classified as reproductive, for the 4 grazing managements.

Grazing	Harvest and sampling date.						
	1 2 Dec.	2 14 Jan.	3 24 Feb.	8a 24 Nov.	9 9 Dec.	9a 29 Dec.	10 18 Jan.
LL	9.8	13.7	10.1	31.2	20.9	4.4	3.2
HH	5.9	4.4	4.4	6.2	7.0	1.6	0.9
LH	-	-	-	36.4	23.8	3.1	0.5
HL	-	-	-	8.2	6.9	2.8	3.4
SEM	0.7	1.7	0.5	2.5	0.9	0.6	0.6

Harvests 1 -3, 1986/87; Harvests 8a - 10 1987/88.

It is probable that determination of reproductive tiller numbers based on examination of cut stubs in cores underestimated true values. For example, numbers of emerged seedheads on plots were counted in mid-January 1988, and values obtained for HL plots were 451 seedheads m^{-2} (SE $\pm$ 28). This converts to 5.6% as compared to the value of 3.4% in Table 5.10.

A point to note is the reduction in percentage (and in absolute numbers) of reproductive tillers on HH plots (as compared to LL plots) in both seasons. This difference does not appear to be attributable to greater removal of reproductive

tillers on hard grazed plots by grazing, as the count of reproductive tillers included grazed stubs until these were clearly dead. Grazing effects on reproductive tiller density have previously been reported by Butler (1986) who considered that reproductive tiller appearance was reduced if swards were grazed to a low residual from late September, and increased under more frequent grazing, but cautioned that the standard error associated with his data was high.

In the context of this study, however, if grazing management can change percentages of tillers flowering, then the cycle of early-summer tiller formation from winter-formed stolons after flowering (Section 5.3.2) might be influenced by grazing management. Again recovery of buried fixed quadrats was able to provide information on this point (Section 5.3.4.6).

5.3.4.3 Tiller appearance and death

Tiller appearance rate (TAR) and tiller death rate (TDR), for LL & HH plots for the first 12 months of the experiment, is shown in Figures 5.7a and 5.7b, respectively. Data for all four grazing managements, from September 1987 to the end of the experiment, appears in Table 5.11. Co-efficients of variation for TAR and TDR (Figures 5.7a,b) were much higher than for tiller population density and there were indications that there may have been increased tiller appearance and reduced tiller death in the first measurement period after placing fixed quadrats. For example TAR was higher (Figure 5.7a) and TDR lower in May than in March/April. In this time the tiller population density as measured by tiller plugs had fallen substantially (Figure 5.4), but the May measurement was the first measurement made after placing the second series of quadrats. Thus, the data from fixed quadrats on TAR and TDR needs to be interpreted with caution. Even so, there is evidence of both grazing management and seasonal effects.

5.3.4.3.1 Grazing management effects

There was evidence (Table 5.11) that TAR increased and TDR decreased on LH plots, relative to other plots, after the crossover, and that the reverse occurred on HL plots. Such changes were expected in view of the changes in tiller population (Section 5.3.4.1), and the high TAR for LH plots after December 7 is consistent with herbage mass data indicating high new tiller production on LH plots (Table 5.8). For period 3, Table 5.11, tiller appearance

was divided by (tiller density x 100) to give approximate relative tiller appearance rate (tillers tiller^{-1} day^{-1}) and values obtained were 2.3%, 1.6%, 2.4%, & 1.7% (SE $\pm$ 0.3%) for LL, HH, LH & HL grazing managements, respectively.

Tiller appearance rates were higher on HH plots than on LL plots (78 and 58 tillers m^{-2} day^{-1}, respectively, averaged over all harvests; P = 0.04). This probably reflects higher tiller densities on HH plots (Section 5.3.4.1). Where tiller density is higher, it follows that either tillers must be longer lived, or appearance and death rates higher for such a difference to be maintained.

5.3.4.3.2 Seasonal effects

It is also clear from the data (Figures 5.7 a,b) that the highest rates of TAR occurred in summer, at a time of high root production, and approximately

Figure 5.7a: Tiller appearance rate on LL (————) and HH (— — —) plots, December 1986 to March 1988. Plotted data represent the midpoint for a total of ten measurement periods for 3 series of fixed quadrats - see Appendix 3. (‖) denotes new series of rings. G, S, standard errors as for Figure 5.4.

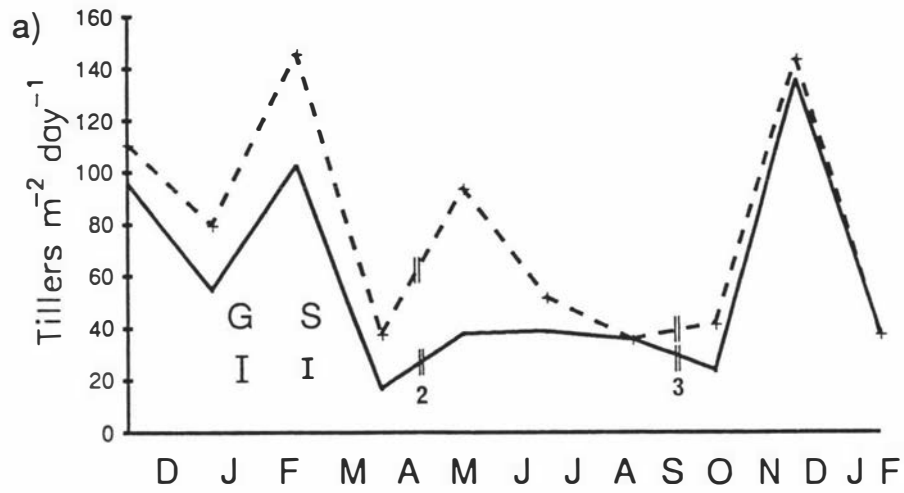
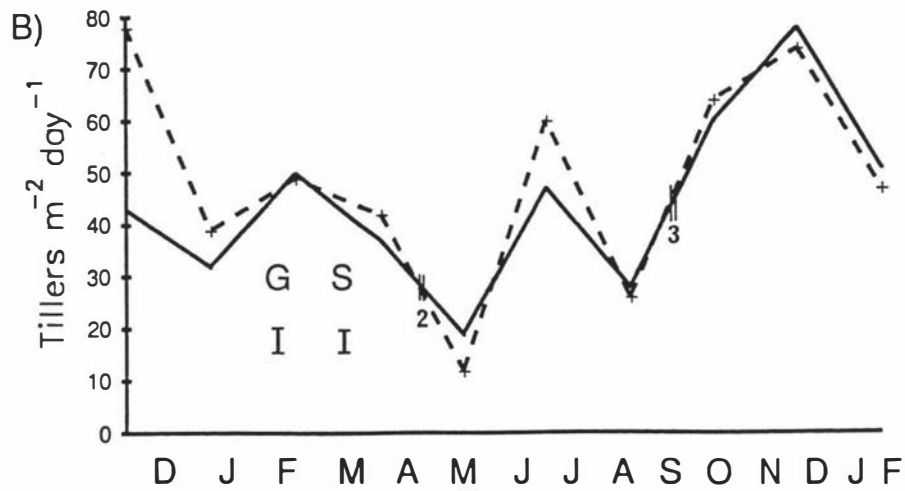


Figure 5.7b: Tiller death rate on LL (————) and HH (— — —) plots, December 1986 to March 1988. For details of measurement intervals see Appendix 3.



coinciding with peak reproductive development (Table 5.10). This is also consistent with evidence from Experiment 1 (Sections 3.4.3.1 & 3.4.3.2) that highest summer root production occurred on RUHE & RUAN plots, and may have been linked to high tillering activity.

In summer 1986/87 the seasonal peak of tiller appearance was interrupted by dry conditions in December/January but resumed in February/March, while in summer 1987/88 there appears to have been a single peak of tiller appearance, with no evidence of a further tiller appearance event when a final measurement of fixed quadrats was made in early April 1988. Tiller size/density plots for 12 April (Figure 5.6a) also indicate that TAR must have been low for the preceding period.

Table 5.11: Tiller appearance and death rates (tillers m^{-2} day^{-1})for 4 grazing managements for third series of fixed quadrats, summer 1987/88.

Period		Grazing Management LL	HH	LH	HL	SEM
1. 18-9 to 14-10-87	TAR	36	36	64	94	15
	TDR	28	26	69	14	16
2. 14-10 to 14-11-87	TAR	24	42	26	88	6
	TDR	60	64	41	61	12
3. 14-11-87 to 3-1-88	TAR	136	144	178	154	20
	TDR	78	74	68	93	10
4. 3-1 to 4-4-88	TAR	39	39	49	18	4
	TDR	51	47	52	66	7

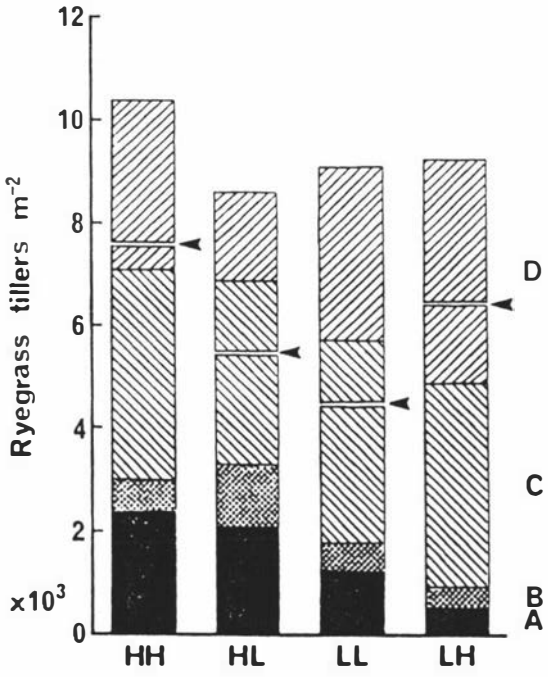
Highest rates of tiller death recorded coincided with high rates of tiller appearance in November/December, although the seasonal fluctuation for TDR was less pronounced than for TAR (Figure 5.7a,b).

In section 2.2.3 it was noted that in order to define tiller population dynamics it is necessary to progress through a number of levels of definition: first, identification of seasonal changes in tiller population density; second, determination of relative contributions of changes in natality and mortality to change in population density; and thirdly, information on the longevity of particular age-classes of tiller. The above data indicating high TAR and TDR

coincident with flowering is consistent with the substantial replacement of tillers at this time observed by Jewiss (1966) for timothy, by Colvill & Marshall (1984) for infrequently cut swards of S23 perennial ryegrass, and by Korte (1986) for 'Grasslands Nui' ryegrass in the second season after establishment.

To determine the extent of turnover at flowering of the tiller population in this study, tiller population age-structures for series 3 fixed quadrats were determined (Figure 5.8) in early April 1988, at the conclusion of measurements. By this date only a small proportion of the tillers were pre-flowering tillers and in addition hard grazing pre-flowering had reduced survival of pre-flowering tillers. Tillers formed before 14 November 1987 accounted for 14% of the total population on LL & LH plots and 31% of total tillers on HH & HL plots (SE $\pm$ 3%, P = 0.002; Figure 5.8). Similarly hard grazing post flowering increased the percentage of the youngest category from 33% (LL & HL plots) to 42% (HH & LH plots, SE $\pm$ 3%, P = 0.03). November-December tillering was not affected by grazing management.

Figure 5.8: Tiller population age-structures for fixed quadrats for the 4 grazing managements, as at 4 April 1988. A, tillers formed before 8-9-87; B, tillers formed between 8- 9 -87 and 14-11-87; C, tillers formed between 14-11-87 and 3-1-88; D, tillers formed between 3-1-88 and 4-4-88. (◂) denotes tiller density outside fixed quadrats and determined by the tiller-core method.



While the data confirms that replacement of the over-wintering tiller population at flowering was a feature of the tiller population dynamics, this also raises the question of which age-classes of parent tillers had produced the replacement daughter tillers. This question represents a fourth level of complexity in defining tiller population dynamics and is dealt with in Section 5.3.4.6.

5.3.4.4 Disturbance effects on tiller density and tiller appearance

Since light levels at the base of the sward are a major determinant of tiller appearance and phytochrome effects appear to be involved in control of tillering (Section 2.2.3), it might be expected that only brief exposures to light would be needed to initiate axillary bud development, and that disturbance such as placement of tiller tags at the base of a tiller may in itself stimulate tiller appearance. Such disturbance effects have been referred to by a number of previous authors, including Davies (1981), Korte (1981) & Arosteguy (1982).

In this study there was also evidence that sward disturbance can enhance tiller initiation. Firstly, tiller densities determined from total numbers of tillers in fixed quadrats were consistently greater than those determined by the tiller-core method (Figure 5.8). Initial tiller densities for fixed quadrats tended to be higher than for the surrounding sward, indicating a tendency to place fixed quadrats around clusters of ryegrass tillers; but these differences usually increased over the first measurement period for fixed quadrats (Sections 5.3.4.3 & 5.3.4.4). Further divergence between population densities, beyond that initially present, is thought to be a disturbance effect. Such disturbance effects would not be of concern in the present study however, so long as after an initial adjustment period, tillers within fixed quadrats settled down to a new equilibrium (c.f. Figure 5.6) where relativities between grazing managements, and between seasonal TAR and TDR were similar to those in the undisturbed population.

Secondly, when monitoring of tiller density on LH and HL plots began in September 1987 prior to the introduction of cross-over treatments, tiller densities for LH plots were lower than those on LL plots; similarly densities on HL plots were lower than those on HH plots ($P = 0.006$; Figure 5.5). Because LL and LH plots, and HH and HL plots had each been grazed in pairs within replicates (Section 4.2.4), these differences cannot be explained by differences in grazing. The only other systematic difference between these pairs of plots was the trampling and other activity which occurred on HH and LL plots, but not on LH and HL plots, during root harvesting and tiller sampling. The only

remaining explanation for the lower tiller densities on the un-harvested plots would therefore seem to be that the disturbance of trampling during collection of root, tiller plug, and herbage mass samples at Harvests 1 to 6 was sufficient to change the balance between tiller appearance and death, causing lower tiller densities on the un-sampled plots. This would seem to be confirmed by the fact that once sampling began on LH & HL plots, tiller density differences between these plots and corresponding LL and HH plots began to disappear (Figure 5.5). There is even some evidence of classic ecological oscillation around an equilibrium in that by the time crossover treatments were introduced in early December the previous differences had reversed, and densities on the crossover plots were higher than on the corresponding HH and LL plots (Figure 5.5; $P<0.01$). In view of these data, it would be interesting to study the role of trampling effects, as distinct from differences in herbage mass, in producing the tiller density differences which exist between rotationally grazed and continuously grazed swards (Hodgson & Wade, 1978).

5.3.4.5 Effect of tiller age on tiller productivity.

Herbage mass data had shown evidence of high daughter tiller production on LH plots and it was of interest to know if an increased proportion of young tillers might explain higher than expected herbage accumulation on these plots (Section 5.3.3.2). Accordingly tissue turnover determinations were carried out for two age classes to determine whether young tillers were more or less productive than older tillers (Table 5.12). Due to lack of time these measurements were not performed for LL plots.

The data show that young tillers tended to be smaller than old tillers, but no evidence that young tillers were more productive. Instead, it appears that tillers of both age classes were more productive on LH plots. Thus it is concluded that increased productivity of LH plots cannot be attributed to different age-structures of tiller populations on these plots. Some other mechanism must have been involved, for example transfer of substrate from decapitated flowering tillers (Colvill & Marshall, 1984; Davies, 1988).

Table 5.12: Herbage accumulation and tissue turnover for old (appeared before 14 October 1987) and young (appeared between 14 November 1987 and 8 January 1988) tillers.

	Grazing treatment.				
	LL	HH	LH	HL	SEM
Net herbage accumulation (24.3 to 5.5.88; kg DM ha^{-1} day^{-1}), determined by quadrat cut.					
	42	44	61	58	6.5
Herbage gross production (mg $tiller^{-1}$ day^{-1})					
Old tillers	-	0.36	0.51	0.75	}0.017
Young tillers	-	0.32	0.69	0.74	
Herbage net production (mg $tiller^{-1}$ day^{-1})					
Old tillers	-	0.32	0.47	0.41	}0.017
Young tillers	-	0.30	0.54	0.42	
Tiller weight (mg)					
Old tillers	-	18	38	45	}14.9
Young tillers.	-	16	28	36	

- denotes LL plots not measured due to lack of time.

5.3.4.6 Tiller demography

It emerged during the course of measurements that it would be of interest to know the age-class of tillers most likely to produce particular categories of new tillers. In particular, it would be of interest to know demographic details for tillers produced between mid-November 1987 and early January 1988 (Figure 5.8). These tillers were probably equivalent to Korte's (1986) category 7 tillers (see Figure 2.1, page 12), and also to the tillers which Colvill & Marshall (1984) considered "especially important because they are long lived, and therefore ensure the perenniality of the plant." The impression from observations on stolon formation (Section 5.3.2) was that these tillers were predominantly daughter tillers from flowering tillers, although Korte (1986) stated that surviving winter (formed) tillers provided numerous sites for tillering after interruption of reproductive growth. In order to provide some preliminary data on these points, 24 fixed quadrats (100 mm diameter) tagged in summer 1986/87 and abandoned in May 1987 were relocated for analysis in December 1987.

5.3.4.6.1 Tiller population structure of a post-flowering sward

As a first step, tillers in the 24 fixed quadrats were classified as to whether they were surviving tagged tillers, daughter tillers of surviving vegetative tillers, or daughter tillers attached to stubs of reproductive tillers (Table 5.13).

Table 5.13: Tiller classification (% total live tillers) for a post-flowering ryegrass sward (December 10, 1987).

	Tagged tillers[1] (current status)		Tillers emerged post tagging		
	Rep.	Veg.	Attached to Veg.	Rep.	Detached: Origin Uncertain
Grazing					
LL	7	11	21	48	12
HH	3	12	19	52	14
SED	2.2	5.1	6.0	12.7	9.6
Signif.	NS	NS	NS	NS	NS

1. Tillers tagged prior to 3 March 1987. Most daughters of reproductive tillers judged to have appeared since flowering.

The data (Table 5.13) is consistent with the pattern illustrated in Figure 5.1 and Plate 5.1 (Section 5.3.2) and shows that a majority of the tillers in these December swards were daughter tillers from flowering tillers. These tillers readily detached from parent tillers during dissection of fixed quadrats, so it is likely that most of the detached tillers of uncertain origin belonged to this category also. It is noteworthy that there were no differences between grazing managements for any of the tiller categories, and also that daughter tillers initiated below ground were morphologically identical to aerial tillers, the formation of which has been described by Korte *et al.* (1987).

In a British study of tiller dynamics in post-flowering swards (Davies *et al.*, 1981) the number of daughter tillers from reproductive tillers was also high, with 82% of tillers present 11 days after cutting derived from reproductive tillers. The similar proportion of daughter tillers from flowering tillers for LL & HH plots may mean that the tiller-core method (Table 5.10) underestimated the proportion of flowering tillers on HH plots.

Inspection of the published tiller age-cohort survival diagrams (Jewiss, 1966; Colvill & Marshall, 1984; Korte, 1986 - see Figure 2.1) shows varying degrees

of turnover in the tiller population for different grass species or for different years (Section 2.2.3). In Jewiss' (1966) study, patterns were consistent over two years, but differed between species. For timothy, there was almost total replacement of tillers, including recently formed spring tillers, at flowering. Many tillers formed immediately post-flowering survived 12 months, and presumably flowered themselves at that time. For meadow fescue the replacement of tillers at flowering was approximately 50% (compared to approximately 90% for timothy) with tillers formed in the first spring normally surviving 15 months, presumably flowering in their second season. Both Colvill & Marshall (1984) and Korte (1986) observed relatively low replacement in the establishment year and virtual complete replacement in the second year for ryegrass swards. This similarity between Colvill & Marshall's (1984) and Korte's (1986) results raises a very interesting point. It appears that it may be a consistent feature of tiller dynamics of grass swards that behaviour differs between first and subsequent years in this way. These data, and also the information on tiller population age-structures suggest that the pattern of tiller demography in these established swards was essentially the same as that for established swards in the two earlier studies (Colvill & Marshall, 1984; Korte, 1986).

5.3.4.6.2 Tiller longevity and propensity to produce daughter tillers

For 12 of the fixed quadrats (approximately 1400 tagged tillers of which 396 had survived at least to flowering), the underground connections between tillers were examined so as to provide information on the propensity of previously tagged tillers (tagged before 3 March 1987) to produce daughter tillers. Also, the current season's recent daughter tillers were categorised according to the type of tiller which had produced them.

Tagged tillers which flowered were more likely to have produced daughter tillers than those which remained vegetative ($P < 0.001$; Table 5.14) and tillers tagged before 8 December 1986 which had survived to flower in spring 1987 had a greater likelihood of producing daughter tillers than tillers tagged in February or March 1987 which also survived to flowering. ($P = 0.017$; Table 5.14).

The high rate of daughter tiller production by older tillers which flowered was further compounded by the fact that this category of parent tillers was large in number at the start of winter, and had higher survival rates over winter than

younger, later formed tillers. This is illustrated by the classification of daughter tillers present in December 1987 according to categories of tagged tiller which produced them (Table 5.15).

Table 5.14: Mean number of live vegetative tillers (December 1987) produced by various categories of previously tagged tillers.

	Date tagged		
Status	15 Nov 86	30 Jan 87	3 March 87
Flowered	2.7 (0.18)	1.7 (0.31)	2.1 (0.33)
Vegetative	1.6 (0.31)	0.83 (0.37)	1.2 (0.46)

SEM in parentheses.

Table 5.15: Numbers of daughter tillers present in December 1987 formed from 4 categories of tagged tiller.

Tagged tiller	Formed before 8 December 1986	Formed between 30 January 1987 and 3 March 1987	Total
Flowered in spring 1987.	443	91	534
Remained vegetative.	32	110	142
Total	475	201	676

A number of authors including Jewiss (1966, 1981), Langer (1979) and Colvill & Marshall (1984) have commented that they believed from their observations that daughter tillers from flowering tillers are important to perennation in perennial ryegrass, and these data confirm those views. However none of the earlier authors produced supporting data. This would be because standard fixed quadrat techniques do not provide such data, and the further analysis of parentage of tillers in fixed quadrats requires extremely time consuming destructive sampling to examine underground connections between tillers. Given that the fixed quadrat method was considered by Davies (1981) to be so time consuming as to be impractical in most circumstances, even without destructive sampling of fixed quadrats, it is clear that provision of more complete information on parentage of new tillers at various times of the year will present major logistical problems.

On the other hand, it is suggested that such information will be needed before it is possible to model tiller population dynamics realistically. Korte (1981) considered that modelling of tiller populations will be possible if it is confirmed that decay curves for particular age cohorts are exponential and the half lives and likelihood of flowering of tillers formed at different times of the year known.

By contrast the above data suggest that models of tiller population dynamics will need to take account of two distinct types of processes operating concurrently and in parallel.

Firstly tiller populations are in equilibrium with the environment, and the balance between natality and mortality is influenced by a number of factors such as grazing management, seasonal variation in light levels, and probably also variation in soil fertility status and soil moisture status (Figure 5.6; Section 5.3.4.1). It is suggested that this equilibrium operates so as to optimise leaf area for particular combinations of environmental factors. If this is the case, it follows logically that sward productivity is normally determined by the environment, rather than by sward state, unless particular managements result in tiller size/density balance either moving above or falling below the equilibrium position. It appears that in the current study there may have been an event which saw assimilate fixed during reproductive growth expressed as enhanced tiller appearance after interruption of reproductive growth.

Other observations in this study which could be explained by the operation of an internal sward compensation of this type include (i) The lower tiller density on LL than on HH plots (Section 5.3.4.1), (ii) the seasonal fluctuation in tiller density (Section 5.3.4.1), (iii) reduced numbers of young tillers on HL and LL plots in April 1988 and increased tiller densities in fixed quadrats (Figure 5.8, Section 5.3.4.3.2), (iv) reduced tiller densities on unsampled plots (Section 5.3.4.4).

Secondly, there appear to be demographic effects which are probably primarily genetic. In the present study these effects were seen as a high TAR and TDR in early summer, which resulted in 58% replacement of the tiller population over a 1½ month period in November/December on HH & HL plots, and 71% (SE $\pm$ 4%) replacement on LL & LH plots, with secondary daughters from the primary daughters increasing the level of replacement to 70 - 85% by early April (Figure 5.8).

5.3.4.6.3 Implications for sward productivity

The high tillering on LH plots seems to have resulted in tiller size/density combinations above the equilibrium (Figure 5.6), and a temporary increase in sward productivity similar to that observed when swards at high tiller density are released from grazing (Grant *et al.,* 1988). In the study by Davies *et al.* (1981) manipulation which increased the degree of reproductive development also increased daughter tiller formation from stubs of flowering tillers, as did leaving longer rather than shorter stubs after cutting. These authors also suggested transfer of metabolites might explain differences in daughter tiller formation, citing Awopetu (1979). A difference between their study and the current study was that herbage production was reduced, not enhanced, when more reproductive swards were cut, although their measurements for herbage production were only for the first 20 days after cutting.

The replacement tillers formed immediately post-flowering were believed to be equivalent to Korte's (1986) category 7 tillers (see Figure 2.1, page 12). These replacement tillers were mostly formed from older flowering tillers, rather than from younger vegetative tillers, and the tendency for an annual cycle based on the flowering and branching of tillers in this category which had survived, as depicted in Plate 5.1, was so strong (Table 5.15) that it was felt that these tillers might be colloquially termed "king tillers."

These demographic effects are quite distinct from the size/density equilibrium effects and recognition of them is important, because there appear to be differences between grass species (Jewiss, 1966) in demographic patterns for effecting tiller replacement, and it may be possible to vary grazing management so as to favour particular demographic patterns. For example, it appears that LH grazing management favoured demographically determined production of daughter tillers from flowering tillers, and that this in turn enabled full utilisation of the environmental potential for increased tillering in summer.

Conversely, poor persistence of ryegrass in New Zealand dairy pastures is a well known problem, and it may be that particular management practices are not entirely compatible with the demographic behaviour of the ryegrass cultivars currently in use. Demographic processes can be modelled by matrix algebra techniques (Lewis, 1977) and a another possibility for future study would be to carry out a sensitivity analysis to see how variation in the

propensity to produce daughter tillers (Table 5.14) for particular age-categories would affect the annual replacement event, and hence stability of tiller populations over time.

5.4 Summary

1. High root production in summer for the ryegrass swards studied in this experiment (Tables 3.2, 4.2) coincides with, and is probably largely explained by, the appearance of large numbers of new tillers.

2. The new tillers were formed from bud sites at underground nodes on stolons of flowering tillers. Stolon formation occurred in winter prior to flowering and appeared to be a response to burial of tiller axes. Rate of stolon formation in winter was found to be sensitive to grazing management and soil fertility status. Stolon formation would have resulted in a substrate cost to the plant of more than 0.5 t DM ha^{-1} $year^{-1}$.

3. There was evidence that the LH grazing management stimulated daughter tiller production, possibly through transfer of substrate from decapitated flowering tillers. This resulted in high herbage accumulation and there might well be an improvement in pasture persistence in such swards also.

4. Daughter tillers produced at the base of flowering tillers in this way appear to play a key role in sward perennation and persistence. Tillers in this category which survive to flower in the following season have a high propensity to contribute to production of that season's replacement daughter tillers. These demographic considerations are quite distinct from questions of tiller size/density equilibrium.

CHAPTER 6: FOLLOW-UP FIELD STUDY.

6.1 Introduction and overview

Data from Experiment 2 raised a number of questions relating to root behaviour. For example, the high root production in November and the accompanying fall in mean root diameter was assumed to be due to new root production by new tillers produced at the same time (Section 4.4.2) but there could also be other possible explanations, such as secondary branching of existing roots produced earlier in the season. Again, there are questions on the behaviour of root systems of perennial ryegrass which remain unanswered (Section 2.4.1), and the question of why high root production in summer had not been reported in earlier New Zealand studies was not fully resolved (Section 4.4.1).

Also, the objective of simultaneous measurement of above-ground parameters in parallel with root measurements (Section 1.2) was not fully met in Experiment 2. Tiller population densities and tiller natality and mortality were recorded throughout Experiment 2, but fixed quadrats had to be moved twice during the experiment so that individual tillers were not followed throughout their lifespan, and herbage accumulation and tissue turnover measurements were not commenced until the experiment had been in progress for some 9 months.

For these reasons, it was decided to carry out a follow-up experiment (Experiment 3) to provide data on the origin of new roots formed at different times of the year, to provide confirmation of the seasonality of root and tiller appearance observed in Experiment 2 and to provide above-ground tissue turnover data in conjunction with refilled core data for a full 12 month period.

Questions of tiller dynamics arising from Experiment 2 were investigated further in follow-up experiments reported in Chapter 8.

6.2 Experimental

The experimental strategy was to make periodic sets of measurements as in Experiment 2. In order to allow for similar measurements to be conducted,

but without the very high labour input which had been required in Experiment 2, the interval between harvests was increased from 6 weeks to 2 months, root measurements were limited to 0 - 250 mm soil depth, and measurements were restricted to 3 plots (replicates) under common grazing management. It was felt that these changes would still allow the objectives stated above to be met, while greatly reducing labour requirements.

The site chosen was 400 m distant from that used in Experiment 1, and details of soil and climate were therefore as given in Section 3.2.2, but the cultivar of ryegrass was 'Grasslands Ruanui', sown in 1979, and clover and other grasses present in the sward were not sprayed out as they had been in Experiment 2. The three plots (replicates), were each approximately 10 m x 10 m and were established in November 1988.

6.2.1 Field measurements

Measurements commenced in December 1988, and were repeated in February, April, June, August, and October 1989 for 7 variables. These variables were: root mass (2 subsamples of eight 21 mm cores, 0 - 70 mm and 70 - 250 mm soil depths, for each plot at each harvest); apparent root production (3 refilled core samples per plot at each harvest); tiller appearance and death rates (TAR, TDR); tiller population density (30 plugs per plot at each harvest); and tissue turnover determination of ryegrass leaf elongation and leaf appearance interval.

Initially 20 fixed quadrats (65 mm diameter) per plot were set up, but at each harvest two fixed quadrats per plot were used for transplanted cores (see below), leaving 8 fixed quadrats per plot maintained throughout the experiment to allow the compilation of a tiller age-cohort survival diagram. Tillers in fixed quadrats were tagged 10 times between November 1988 and February 1990. The more frequent tiller tagging was to prevent loss of fixed quadrats by burial, as happened in Experiment 2, and tiller appearance and death values corresponding to the 6 measurement dates were interpolated by fitting Fourier equations to the raw tiller data (Appendix 6).

The tissue turnover measurements comprised 3 measurements (1 week interval between measurements) of leaf length for all leaves on randomly selected tagged tillers. Sixteen tillers per plot were so measured at each harvest. In Experiment 2 it was evident that herbage production data for

different grazing treatments had largely reflected differences in senescence resulting from differences in herbage mass (Section 5.3.4). Therefore, the tissue turnover measurements were primarily to give data on gross leaf production, which would be unconfounded by any seasonal differences in senescence, and would be a shoot measurement analogous to the apparent root production data from refilled cores.

6.2.2 Transplanted cores

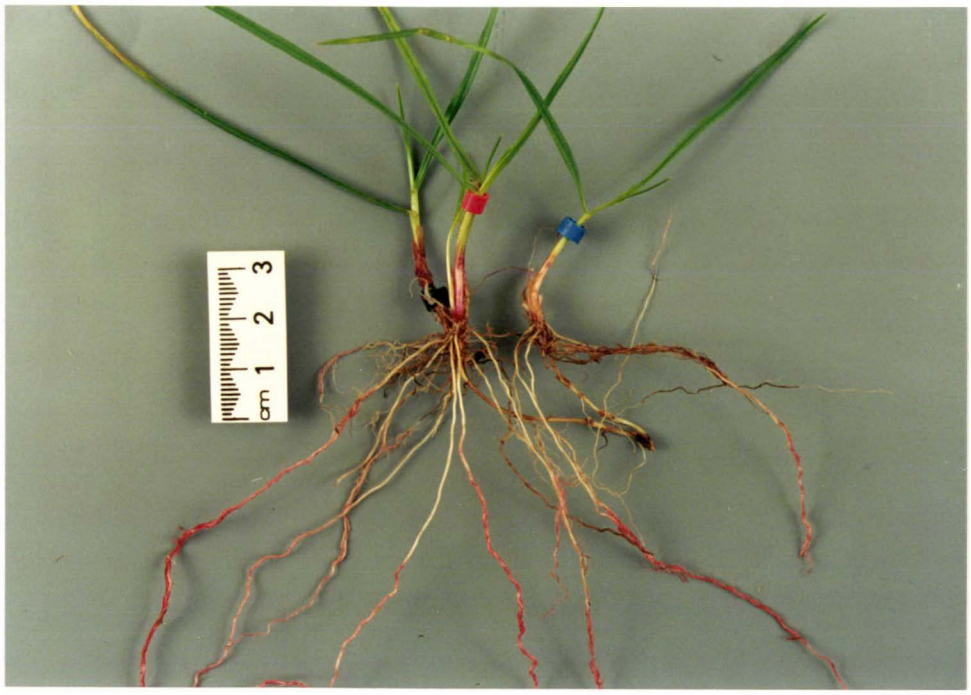
The transplanted core technique was devised specifically to obtain information on the origin of roots produced in summer, with a view to clarifying reasons for the seasonal changes in root diameter (Sections 4.3.1.1, 4.3.1.2, 4.4.1). However, data was also collected on the location on the tiller axis of root initiation and on the number of live roots at different times of the year.

Fixed quadrats of which tillers had previously been colour coded by date of origin were collected with the 78 mm diameter corer described in Section 3.2.5, severed at 100 mm depth, and transplanted into a sandbox. Cores were transplanted at each of the 6 measurement periods of Experiment 3, and grown on in the sandbox for a further 6 weeks. At the end of each period the box was dismantled and sand washed out, taking care not to break off new roots. After "transplanted cores" had been recovered in this way (Plate 6.1a), one of the two cores from each plot was clipped and the new roots (grass and clover roots which had grown out of the soil matrix of the transplanted core into the surrounding sand) retained for determination of root mass and root length. For these samples, root length by diameter categories was also determined by image analysis (Cochrane *et al.*, 1990). For the remaining cores, protruding new roots were stained red by immersing the core in congo red dye (1% w/v; Gibbs, 1986). Finally the soil matrix of the transplanted core itself was washed away to expose the unstained older roots inside. Cohorts of tillers connected by stolons, complete with roots, were then extracted using tweezers (Plate 6.1b).

After washing and dissection of cores as described above, 5 adult tillers per core were selected, and each root measured in detail, working basipetally from the youngest root. Measurements made on each root included length (mm), a score for vigour and degree of branching, and diameter at point of attachment to the tiller axis (mm).

Plate 6.1a: Transplanted core after washing out from sandbox, showing root development after 6 weeks growth into sand.

Plate 6.1b: Cluster of tillers and inter-connecting stolons dissected from a transplanted core.



Root diameter was measured to the nearest 0.1 mm, using a binocular microscope and graduated eyepiece. Similar measurements were made for any daughter tillers on the selected tiller axes. Where possible the nodal position of each root was also recorded, but after the first 3-4 nodes, recognition of individual nodes often became difficult so these records were confined mainly to younger roots.

6.2.3 Statistical analysis of results

The design is technically a split plot in time and as such, repeat observations on the same plots at different times can not be treated as independent observations and analysed for seasonal effects as if they had come from a randomised complete block design.

There is no easy answer to this problem. However, as with other split-plot in time experiments (Appendix 1.1), pairwise comparison of any *two* means using a pooled standard error is valid. Accordingly standard errors for each of the 42 means obtained in the experiment are given in Figure 6.1, and probabilities for T-tests between specific pairs of means are given in the text as appropriate to particular points of discussion. For simplicity, standard errors shown in Figure 6.1 are averaged over time, however.

6.3 Results and discussion

6.3.1 Field measurements

The six harvests made on the three plots provided 18 observations for each of seven variables (root mass, kg ha^{-1}; apparent root production, kg ha^{-1} day^{-1}; leaf extension rate, mm $tiller^{-1}$ day^{-1}; ryegrass leaf appearance rate, leaves $tiller^{-1}$ day^{-1}; ryegrass tiller density, tillers m^{-2}; and ryegrass tiller appearance and death rates, tillers m^{-2} day^{-1}), and the seasonal means and SE's for these are presented in Figure 6.1 (page 130). In addition to providing values of TAR & TDR, data from fixed quadrats were used to compile a tiller age-cohort survival diagram, for the period December 1988 - February 1990 (Figure 6.2, page 131)

Figure 6.1: Seasonal means and standard errors for variables representing above- and below-ground activity in Experiment 3. SEM in parentheses below bars for each mean.

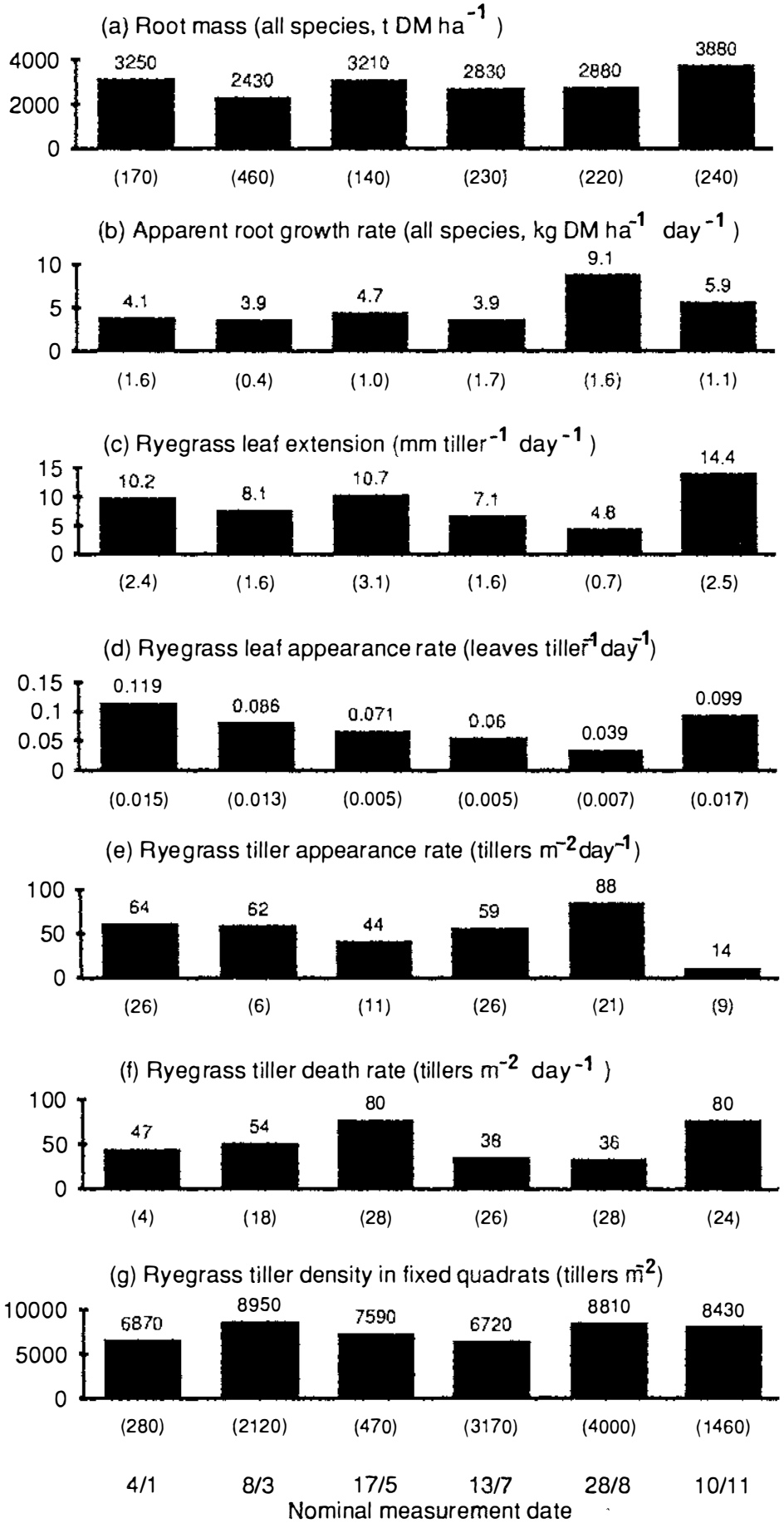
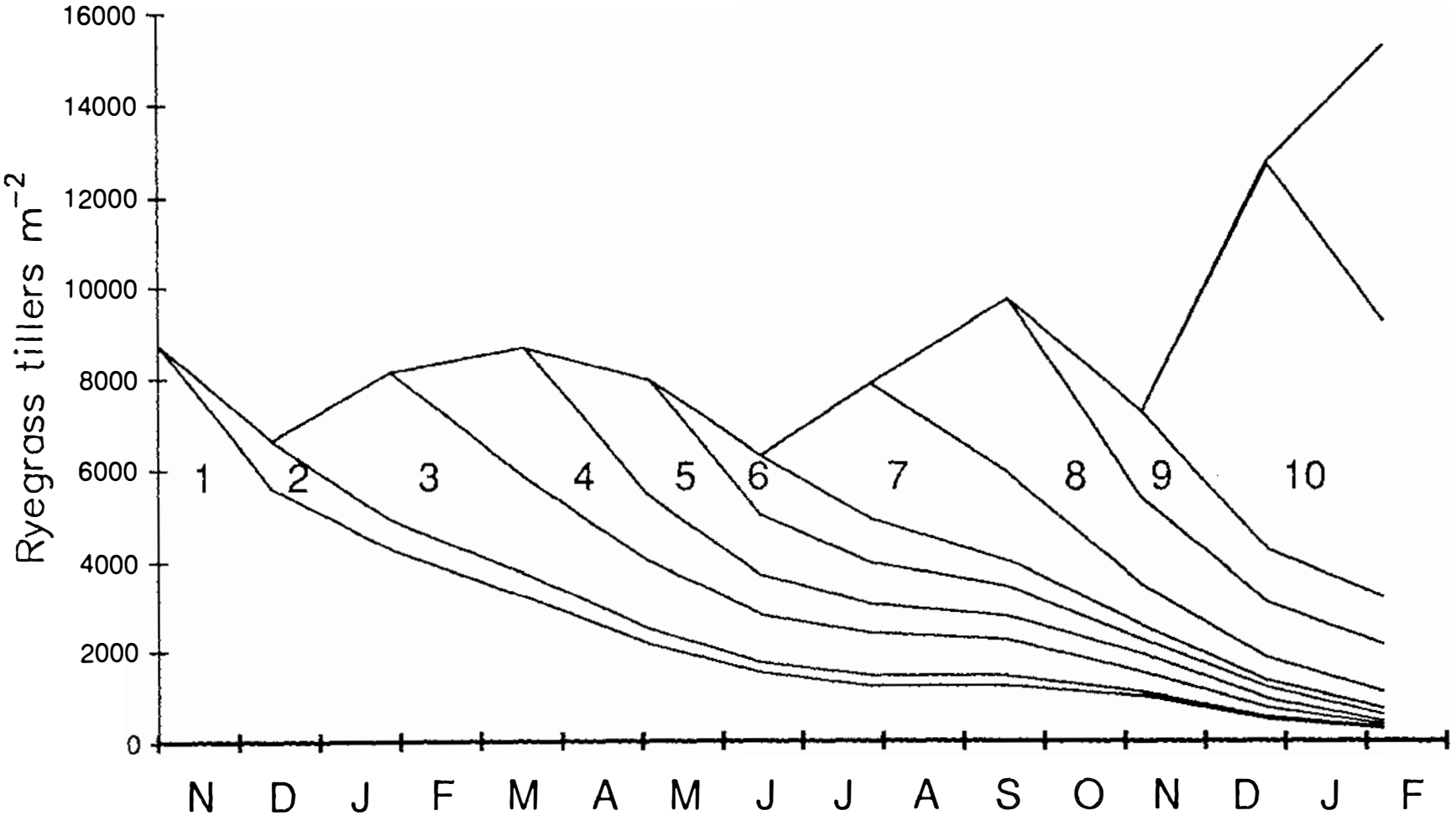


Figure 6.2: Tiller age-cohort survival for a 'Grasslands Ruanui' ryegrass sward (Experiment 3).



When the first set of measurements was made in November/December 1988, it was notable that for swards in Experiment 3 tiller appearance rates were extremely low (age cohort 2, Figure 6.2; Figure 6.1), whereas for swards measured over the previous three years in Experiments 1 and 2, tiller appearance had peaked in early summer (Section 5.3.4.3.2).

As measurements progressed other differences between swards monitored in Experiment 3, and those monitored in Experiments 1 & 2 became apparent. Apparent root growth rate (Figure 6.1) was lower than expected in the first measurements, did not fall to low levels in winter, and peaked in August. This resembles the seasonal pattern previously reported by Caradus & Evans (1977) with a late-winter peak, rather than the pattern reported in Chapter 4 (Figure 4.4). This period of high root growth in August coincided with a period of high tiller appearance and with a period when leaf extension rate was even lower than in mid-winter and leaf appearance was infrequent (Figure 6.1).

This raises the question as to why the seasonality of tiller appearance for Experiment 3 should be different from that observed in Experiment 2 (and probably also Experiment 1). The most obvious common denominator between Experiment 3 and the studies of Jacques (1956) and Caradus & Evans (1977) is that all three used the same ryegrass cultivar, 'Grasslands Ruanui'. These data therefore suggest that the tiller demography of 'Grasslands Ruanui' used in Experiment 3 might be different from that of the 'Ellett' cultivar, used in Experiments 1 & 2.

Further evidence in support of the view that tiller demography differed between cultivars is seen in the tiller age-cohort survival diagram (Figure 6.2). Comparison of this diagram with those of Jewiss (1966), Colvill & Marshall (1984), or Korte (1986), shows that one feature of this diagram is the almost complete lack of an annual replacement of tillers at flowering (December). This is not easily quantified, but qualitative evidence of the difference in behaviour is that surviving November tagged tillers in late March comprise some 35% of tillers in these swards (Figure 6.2), compared with 15% - 30% for Ellett swards in Experiment 2 (Figure 5.8, Section 5.3.4.3.2).

The visual impression gained when tagging tillers was that in Experiment 3 the generation turnover tended to occur in two phases. At the initial tagging in 1988 there were numbers of young tillers attached to flowering tillers (similar tillers appeared in 1989 as cohorts 7 & 8 in Figure 6.2). In the following

autumn, these daughter tillers of flowering tillers which had formed rather earlier than the main flush of tillers in Experiment 2, appeared to be active in producing cohorts 3 & 4 (Figure 6.2). In turn, tillers of age-cohorts 3 and 4 appeared particularly active in producing tillers of age-cohorts 7 and 8, and appeared to be an important source of flowering tillers in 1989. Tillers of cohort 1, unlike tillers tagged at about the same time of year in Experiment 2, did not appear to show a high propensity to flower. Many of them remained vegetative through the summer, then simply lost vigour and died of old age in autumn or winter (Plate 6.2).

As an illustration of this, percentage of tillers classified as reproductive in late November 1989 was 53% & 66% for age-cohorts 1 & 3, respectively. This difference between age-cohorts 1 and 3 was not statistically significant, but contrasts markedly with values of 93% & 45% for corresponding age-cohorts in Experiment 2 (Table 5.15, Section 5.3.4.6.2). Percentages of tillers flowering in Korte's (1986) study were much lower, and never exceeded 38%, although Korte's (1986) values apply to tillers produced in the establishment year, and are probably not typical of tiller dynamics in subsequent years (Section 5.3.4.6.1).

The fact that the different seasonal pattern of tiller appearance for Experiment 3 was associated with parallel changes in seasonal distribution of root growth supports the conclusion (Section 4.4.1) that high rates of root production in summer in Experiments 1 & 2 probably reflected new root production by newly-appeared tillers. In a British study also, tiller and root appearance peaks for an S23 perennial ryegrass sward occurred at the same time of year (April) (Garwood, 1969, 1967a,b). Allowing for climatic differences between Britain and New Zealand, these seasonal peaks observed by Garwood (1967a, 1969) correspond very well with data from Experiment 3.

While the above results suggest that Ellett ryegrass used in Experiments 1 and 2 may have different seasonal patterns of tiller demography from 'Grasslands Ruanui' ryegrass used in Experiment 3, it must be emphasized that because the results compare different swards in different years, the possibility that the results reflect climatic or other differences between the two paddocks and seasons cannot be ruled out. Different responses of the same genotype to different environmental surroundings (genotype x environment interactions) are the subject of a substantial body of literature, and a study of 12 ryegrass genotypes by Harris (1973) has shown that these effects can be substantial.

Plate 6.2: Non-flowering age-category-1 tillers (Figure 6.2) dissected from transplanted cores in May 1989. Extent of stolon formation suggests these tillers had been 6 - 12 months old when tagged in November 1988, and many tillers of this age lost vigour and died during winter.

However, if these differences in tiller demography do in fact represent genotypic variation, rather than genotype x environment interaction, then they are as substantial as the differences between two species, timothy and meadow fescue (Jewiss, 1986). This reinforces the conclusion drawn in Section 5.3.4.6, that a full demographic analysis of ryegrass behaviour would be valuable. Such an analysis would include data on propensity to produce daughter tillers and parentage of new tillers for tillers of different age classes.

The particular paddock used in Experiment 3 was chosen because it was later flowering than adjacent Ellett paddocks, and therefore more suitable in terms of timing of an initial measurement when commencement of the experiment was unavoidably delayed until November 1988. It was not considered at the time the experiment was set up, that different cultivars of ryegrass might have substantial differences in seasonal behaviour for parameters such as tiller appearance and root production, and there appears to be no literature suggesting such differences. Had differences between cultivars been expected, then the experiment could have been specifically designed to provide controlled comparisons between cultivars. This would obviously be a rewarding subject for further study. It has often been observed in evaluations of the Mangere ryegrass ecotype and its derivative cultivars 'Grasslands Nui' and 'Ellett' that performance relative to other cultivars is better in summer-autumn than at other times of the year (Cumberland & Honore, 1970; Harris *et al.*, 1977). It may well be that the high summer tillering observed in Experiment 2, and for Nui in the year following establishment (Korte, 1986) is a consistent feature of established swards of this ryegrass, and is the basis for the high herbage production in summer.

Another feature of the tiller age-cohort survival diagram is the very high tiller appearance in summer 1989/90 (age-cohort 10). This effect may be similar to the tillering effect seen on LH plots in Experiment 2, because plots received rather lax grazing in spring 1989, followed by a more severe grazing after measurements in Figure 6.1 had been completed, but there is no data on herbage mass to allow a more rigorous assessment of this point. However, even if the appearance of large numbers of tillers after flowering is parallel to that on LH plots in Experiment 2, the tiller dynamics still appear to be different in that the survival of pre-flowering tillers (age-cohorts 7, 8 & 9; Figure 6.2) is much higher than that of corresponding tillers for Experiment 2 (Figure 5.8).

6.3.2 Transplanted cores

6.3.2.1 Root mass and root length from transplanted cores

The quantity of root produced by transplanted cores was lowest in winter (May and July) and high at each of the other 4 harvests, but with some reduction of root growth in January (Table 6.1).

Table 6.1: Root mass (g Ash-Free DM), total length of new root (km), mean monthly temperature[1], and mean diameter[2] (mm) of nodal roots at point of origin for transplanted cores.

	Jan.	March	May	July	Aug.	Nov.	SE
Root mass	0.36	0.61	0.06	0.16	0.61	0.60	0.057
Root length	4.6	17.6	1.1	1.7	13.1	14.2	1.27
Temperature	19.1	16.8	11.8	8.7	7.2	16.2	-
Diameter at origin	0.35	0.56	0.50	0.64	0.70	0.66	0.011

1. Monthly mean 10 cm depth soil temperatures for DSIR, 0.7 km from experimental site.
2. Diameter differences also observed using image analysis, Cochrane *et al.* (1991).

For apparent root production in refilled cores there was a single peak in August, with moderate root growth at other times of the year (Figure 6.1b). By contrast, values for new root mass for transplanted cores were uniformly high in March, August, and November (Table 6.1), although with differences in mean diameter, and corresponding differences in root length (Table 6.1).

This raises questions as to what factors control root growth and why the seasonal pattern of root growth for refilled and transplanted cores should differ. The root production effects do not appear to correlate with temperature, as temperatures in August when root production was highest were lower than in May when root growth was lowest, although reduced root growth for transplanted cores in January might be a result of supra-optimal temperatures and root diameter effects do show a tendency for a negative correlation with temperature:

Mean diameter at origin (mm) = 0.77 - 0.015 x Temp. $r^2 = 0.36$ $P = 0.21$.

The hypothesis that high root growth in field swards in spring is primarily a response to loss of root apices in winter (Section 4.4.1), and is analogous to **a**

root pruning effect (Jacques & Edmond, 1952) is not supported by these results. If the primary stimulus for root growth had been loss of existing roots, then high root growth from transplanted cores might have been expected at all 6 Harvests, providing conditions of soil temperature and soil moisture were suitable for root growth. Instead, root growth was highest in late winter, even though temperatures at this time were lower than at any other measurement period. There also appeared to be a period of root dormancy in early winter, despite relatively mild temperatures. This low root growth in early winter and high root growth in August would seem to indicate some sort of seasonal signal, possibly hormonal, within the plant, although this is not to say that root growth from transplanted cores could not have been enhanced by a root pruning effect, in addition to the seasonal effect. The author attempted to quantify root pruning effects in transplanted cores and separate these from seasonal effects by staining roots with triazine dyes (Carman, 1982), but was unable to duplicate Carman's staining procedure.

The measurements on nodal root diameter at the point of attachment to the tiller axis indicate that there is seasonal variation in nodal root diameter. This is apparently a temperature effect and a similar effect has previously been observed by Garwood (1968). However there were also seasonal differences in the proportion of roots in fine and coarse diameter categories (Cochrane *et al.*, 1990), indicating that besides differences in diameter, there must also have been differences in the degree of branching. The significance of seasonal differences in root diameter is not known.

6.3.2.2 Root behaviour in relation to nodal position on the tiller axis

For each transplanted core, adult tillers were sampled randomly from among those dissected out, and tiller axes examined in detail to obtain information on the pattern of root development in relation to the segmental structure of the tiller axis (Section 2.4.1). One such tiller axis is shown in Plate 6.3.

Data collected were difficult to analyse because of wide variation from tiller to tiller, however there was a consistent pattern in that root initiation almost invariably occurred at the same node as leaf senescence, and root development was confined to the next 4 - 5 nodes on the axis, with roots at lower nodes in the process of senescence. To illustrate this, a summary of statistics for roots at successive positions on the tiller axis is given in Table 6.2

Plate 6.3: Tiller axis of tiller dissected from a transplanted core in August 1989. Note pairing of roots at each node, vertical alignment of root pairs and associated tiller buds, and death and disintegration of roots and phytomers lower on the axis.

Table 6.2: Root length and proportion of roots actively elongating, inactive due to disease, or severed during transplanting for roots at successive positions on tiller axes. Data are for transplanted cores harvested on 5 April (sample of 15 tillers) and 22 September (sample of 9 tillers).

	Position [1] 1	2	3	4	5	6	7	8	9	10	11	12
Harvested 5 April 1989.												
No. tillers[2]	15	15	15	15	14	14	12	11	9	8	8	8
No. active roots	15	10	5	10	5	8	4	6	3	2	3	2
No. diseased roots	-	5	10	5	8	5	4	3	2	2	1	1
No. severed roots[3]	-	-	-	-	1	1	4	2	4	4	4	5
Mean length active roots (mm)	4	29	126	156	168	272	160	258	233	365	167	255
Harvested 22 September 1989.												
No. tillers	9	9	9	9	9	8	8	8	8	8	8	8
No. active roots	9	7	7	9	8	5	3	1	1	0	0	0
No. diseased roots	-	2	2	0	1	0	0	0	0	0	0	0
No. severed roots	-	-	-	-	-	3	5	7	7	8	8	8
Mean length active roots (mm)	28	86	205	267	332	322	322	345	205	-	-	-

Average number of roots per tiller with lateral branches: 5 April = 1.93 $\pm$ 0.03; 22 Sept. = 2.89 $\pm$ 0.40.

1. Position = number of root counting basipetally from youngest root. Position 1 normally at senescent leaf, and usually 2 positions at each node.
2. Number of tillers remaining in sample - younger tillers in sample had less than 12 root positions.
3. Severed roots - cut by corer during transplanting.

In hindsight it would have been useful if details of tiller sites had also been recorded along with associated root sites. Also, it is very possible that severance of most existing roots at transplanting altered the pattern of root growth, so the data in Table 6.2 cannot be taken as typical of behaviour of roots in field swards.

Even so, it is evident that the morphological organisation and pattern of turnover is rather different from that commonly illustrated in texts (see e.g. Langer's (1979) Figure 3.3, or Jewiss' (1981) Figure 5.2). Rather than being produced at random sites around a basal crown, new roots are formed at a certain stage of maturation of the phytomer to which they are attached. After a relatively brief period it appears that roots die and decay, and the associated segment of the tiller axis also breaks down. In this study the last active root was on average only 6 - 9 positions (approximately 3 - 5 nodes) below the senescent leaf, and was seldom more than 12 positions distant (Table 6.2). This pattern is consistent with the continued production of roots in refilled cores throughout the year (Figure 4.4, Figure 6.1), although the seasonal pulse of root production in spring observed by previous workers (Section 4.4.1) is also confirmed.

The two roots per node were in the A and B positions (Klepper *et al.*, 1984) and were aligned obliquely away from the corresponding axillary bud and leaf scar so as to form 4 rafts of roots, 2 on each side of the axis (Plate 6.3). The fact that root initiation usually occurred at the same nodal position as the senescing leaf, suggests a possible utilisation of products of leaf senescence in root initiation, perhaps controlled through a product of leaf senescence triggering the root initiation.

Plate 6.3 shows some evidence of basipetal release of dormant tiller buds. This phenomenon was noted in a relatively small proportion of tillers, and only in the spring. Even so, this shows one mechanism whereby site filling ratios higher than Davies' (1974) theoretical maximum of 0.491 could arise without initiation of tillers from prophyll buds (Neuteboom & Lantinga, 1989) and may explain high site filling ratios sometimes observed in field studies (see e.g. Simon & Lemaire, 1987).

It was notable that only 2 - 3 nodal roots per axis on average bore lateral branches (Table 6.2), although this value might have been higher if older roots had not been severed at transplanting. Lateral branching and regrowth of roots

severed during the transplanting process was seen on approximately 10 roots of more than 1000 examined, so is a very rare event. Even where this appeared to have occurred, the new lateral was usually weak and short.

Overall, this pattern showed little change with season or in tillers of different age classes, apart from a smaller number of developed nodes on young tillers or between transplanting and harvest in winter (presumably due to lower winter temperatures, and hence fewer leaf appearance intervals). There also appeared to be an increased proportion of diseased roots in summer/autumn (Table 6.2) but this was not statistically tested.

6.3.2.3 The phytomer and root and tiller initiation.

An approximation of the 'normal' sequence of events at a phytomer can be constructed from the above data. The delay between leaf and root appearance (defined as n by Neuteboom & Lantinga, 1989) is approximately 3 phyllochrons, the number of root sites at each node is 2, initiation is in strict chronological sequence, and the number of phyllochrons for which a root persists is 3 - 6 (although the root-pruning effect of transplanting may have reduced the lives of severed roots and increased the number of potential root sites developed). The proportion of root sites which did develop roots was approximately 95%.

Site filling ratio (F_S) was estimated using the formula:

$$\frac{\text{Tiller appearance (Tillers m}^{-2}\text{ day}^{-1}) \times \text{leaf appearance interval (days)}}{\text{Tiller density (Tillers m}^{-2})}$$

Where data used are instantaneous values, this formula is equivalent to equation 9.6 of Davies, (1981). Values so obtained were 0.08, 0.10, 0.10, 0.18, 0.25 and 0.03, respectively for the six harvests represented in Figure 6.1.
These values would underestimate actual site filling, assuming that fixed-quadrat-tiller-density values used in the calculation were inflated (c.f. Section 5.3.4.4). Chapman *et al.* (1983) observed values for site filling in field swards ranging from 0.22 to 0.43. However, even if the higher values of Chapman *et*

al. (1983) are considered to be more correct, the proportion of tiller buds producing tillers appears to be rather lower than the proportion of root sites. This would indicate that there should always have been dormant tiller buds available for rapid release if environmental conditions determined this, whereas the root system is in a state of more continuous turnover, with few root sites not producing a root.

This is consistent with earlier evidence (Section 5.3.4.1) that swards have a capacity to vary tiller density, along a -3/2 power line, in order to optimise sward leaf area under different grazing managements or in response to other environmental stimuli. Responses involving an increase in tiller density could not occur quickly unless there was always a current surplus of sites available, or uninitiated axillary buds remaining on the tiller axis. This implies limitation to tillering in field swards would not likely arise through lack of available sites. By contrast, the high percentage of root sites forming a root indicates that there is little scope for grazing management to alter the number of new roots produced (although loss of leaf area at grazing might reduce root elongation indirectly through reduced carbohydrate status).

Also, these contrasting strategies of root and shoot systems confer unique properties on the grass plant. Formation of roots at the majority of available sites and slower turnover for root tissue than for leaf tissue, allows for continual opportunity to exploit differing soil depths with a response time reflecting seasonal changes in soil conditions. On the other hand, the more rapid turnover of leaf tissue and the fact that there are normally dormant tiller buds capable of rapid release, is probably a more appropriate strategy for coping with sudden removal of tissue, as at grazing. Even so, the facility to provide a rapid response does not preclude more gradual responses. Two examples of more gradual responses by the shoot system are the seasonal increase of tiller density which allows exploitation of increasing light levels in spring-summer (Section 5.3.4), and the tiller density response to changed grazing pressure in which case a shift along the -3/2 line to a higher density of smaller tillers appears to optimise light interception for more severe grazing, and vice versa for laxer grazing (Section 5.3.4.6.2).

One further possibility which arises from the analysis of transplanted core results, is that in future studies it might be possible to record data in such a way as to allow calculation of ratios of daughter tillers:phytomers and of roots:phytomers for a portion of the tiller axis, for example the first five nodes

below the senescent leaf. These ratios might be designated P_t and P_r, respectively, and would not be subject to the ambiguities of interpretation of the corresponding ratios F_s and F_r (Section 2.4.1). Values of P_t and P_r calculated in this way would also be intrinsically more logical. For example the maximum value of P_t would be unity, as compared to 0.69 for F_s (Neuteboom & Lantinga, 1989). Again, Hunt & Thomas (1985) commonly report values for F_{rl} of near 1.0, and it is easy to overlook the fact that this indicates approximately *two* roots per node, but depending on the delay in phyllochrons between leaf and root initiation.

6.4 Summary.

1. Seasonal pattern of change in seven above- and below-ground variables was different in Experiment 3 from that observed in Experiments 1 and 2. It is thought that these differences in seasonality of root and tiller dynamics may be a characteristic of the different ryegrass cultivar ('Grasslands Ruanui') used in this experiment.

2. Seasonal patterns of tiller age-cohort survival in Experiment 3 also appeared to differ from those in Experiment 2, but information on the identity of parent tillers producing new daughter tillers would be necessary to define these differences.

3. Data from transplanted cores showed that seasonal changes in root diameter could be only partly explained by differences in degree of branching; that root initiation in ryegrass is in strict chronological sequence with normally two roots per node produced at the same node as the senescing leaf; and that lateral branching of severed roots is extremely rare.

4. The tendency in field swards for only a proportion of available tiller sites to be used, while the proportion of root sites used is high, and the longer turnover time for roots than for leaves, provides a growth strategy whereby root and shoot systems can respond to changes in their respective environments.

CHAPTER 7: TOWARDS AN INTEGRATION OF ROOT AND SHOOT DYNAMICS.

7.1 Introduction and overview

In this chapter the extent to which the results obtained in Experiments 2 & 3 give information on the interaction of root and shoot systems, and on the potential for manipulation of swards for increased productivity, is examined.

The theoretical implications of the strict segmental morphology of graminaceous plants (Sections 2.4.1, 6.3.2.3) are briefly discussed, root-shoot partitioning in Experiments 2 and 3 is examined, and a multiple discriminant analysis (MDA) is used to derive a mathematical description of the data for the four treatments used in the latter part of Experiment 2. For Experiments 2 & 3, principal component analysis (PCA) is used to provide a mathematical description of the seasonal patterns of variation in, and relationship between above- and below-ground variables.

A possible strategy for improvement of pasture productivity is identified.

7.2 Aspects of the inter-relationship between root and shoot systems

7.2.1 The phytomer as a basis for integration of root and shoot dynamics

Most earlier studies of grass swards have attempted to define sward behaviour in terms of mass or tissue flow (e.g. Brougham, 1957; Parsons *et al.*, 1983ab; Bircham & Hodgson, 1983; Grant, *et al.*, 1988), and in many of these studies the tiller has been considered to be the basic independent unit of production. This leaves open the question of why a tiller behaves as it does, however, and a few studies have attempted to define behaviour of grass swards or grass plants in terms of the segmental morphology of the plant. Notable examples are the work of Etter (1951) and Silsbury (1970), although the approach is also implicit in the work of Hunt & Halligan (1981) and Hunt & Thomas (1985), and in the concept of site filling (Davies, 1974; Davies & Thomas, 1983).

This approach was pursued in Experiment 3, by detailed examination of axes of tillers dissected from transplanted cores (Section 6.3.2). This

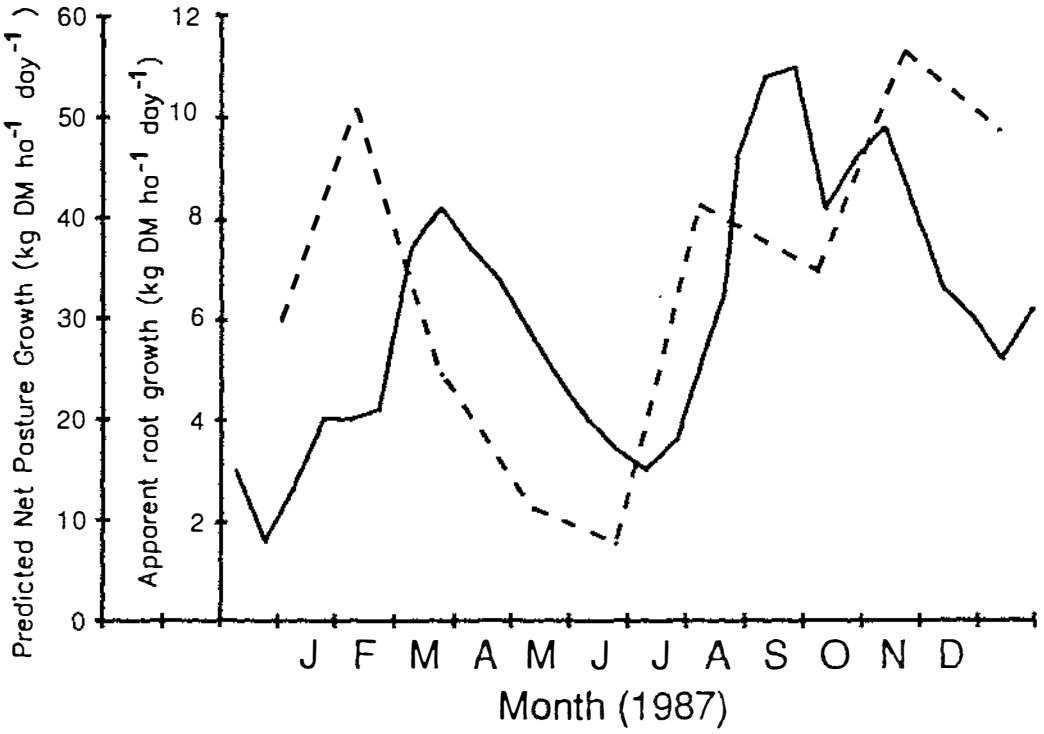
detailed examination of segmental morphology was helpful in understanding the ecology of ryegrass. For example, consideration of events at the phytomer level led to an understanding of the growth strategy of ryegrass, whereby the pattern of utilisation of growth sites (root and tiller initials) provides flexibility and allows adjustment to changing environmental conditions (Section 6.3.2.3).

Again, the tiller does not lie passively at the soil surface, but is an elongating axis moving upwards towards a soil surface intermittently raised (in relation to the growing point) by events such as earthworm activity or stock trampling. Where addition of phytomers to the axis does not keep pace with the rate of burial, underground internode elongation occurs (Section 5.3.2). This stolon formation would, at certain times, divert considerable substrate away from harvestable production. However, while it may be considered helpful to have detailed information of this type on the mechanisms of particular responses, it is difficult to see how this knowledge could be used to manipulate root/shoot relations in such a way as to lead to increased shoot production.

7.2.2 Ratio of root:shoot production as an estimate of root/shoot partitioning

Control of root/shoot allocation is one of the more important means by which plants adapt to environmental factors such as change in nutrient supply (Section 2.4.2). It would have been of interest to use data on apparent root production in refilled cores together with above-ground tissue turnover data to estimate root/shoot partitioning. However for Experiment 2, data on tissue turnover was collected for only part of the experiment. Therefore, in the absence of measured data spanning the full period of the experiment, the computer model GROW was used to predict pasture production (Grow predictions represent net production, growth - senescence) for a ryegrass sward growing under climate and soil conditions as in Experiment 2. The GROW model was designed to simulate pasture production for sites at various locations in New Zealand under 14 day or 28 day cutting regimes. For many sites, measured data match predictions to within 5 kg DM ha^{-1} day^{-1} (Butler *et al.*, 1990). The predicted net pasture growth rates, together with apparent root growth rates (averaged over LL & HH treatments, Figure 4.4) for Experiment 2 are plotted below (Figure 7.1).

Figure 7.1: Herbage accumulation obtained by computer prediction (————————), and apparent root growth rates (— — —) averaged for LL and HH plots for Experiment 2, January 1987 to January 1988.



In Figure 7.1, seasonal peaks of root and shoot production in spring and in autumn after summer drought approximately coincide, but with maximum root production in each case apparently preceding maximum shoot production by 4 to 6 weeks. Apparent root growth averaged approximately 20% of computer predicted net shoot production, though with some seasonal fluctuation. Since the pasture production values in Figure 7.1 do not include leaf formed but lost by senescence, this value of approximately 20% agrees well with observations of Parsons & Robson (1981b), who found that approximately 10% of ^{14}C applied to shoots was recovered from roots.

Ratios of root:shoot production appear to be lower in the early winter months of May and June than at other times of the year, an effect also consistent with observations of Parsons & Robson (1981b), who noted that percent allocation of ^{14}C to roots fell slightly in November/December. Low allocation to root growth in early winter does not appear to be attributable to stolon formation as the major increase in stolon density (Table 5.3) occurred between Harvests 7 & 8 (September). However, Lambert *et al.* (1986) have reported that ryegrass (shoot) growth rates at this time of year are high relative to browntop and seasonal variation in root/shoot allocation is one possible explanation for these inter-specific differences in seasonality of pasture growth reported by Lambert *et al.* (1986).

For Experiment 3, the data again allow only an approximate estimate of root shoot partitioning, because the contribution of species other than ryegrass to root growth in refilled cores was not known. Apparent root growth in refilled cores for all species (Figure 6.1b) was divided by ryegrass leaf tissue accumulation calculated from ryegrass leaf extension (Figure 6.1c) and ryegrass tiller density (Figure 6.1g). Ratios of root:leaf accumulation obtained in this way were, for the six harvests respectively, 0.07, 0.06, 0.08, 0.10, 0.36, and 0.08. The seasonally high ratio of root:shoot for July/August is noteworthy, and would seem to add further evidence in support of the view (Section 6.3.2) that the seasonal pattern of root appearance in Experiment 3 was different from that observed in Experiment 2, but similar to the pattern reported by Caradus & Evans (1977).

7.2.3 Multivariate analysis as a means of describing root/shoot relationships

Approximate coincidence of seasonal peaks in root and shoot growth (Figure 7.1) means that, when analysed over all seasons, root and shoot growth variables will tend to be correlated. This is precisely the type of correlation which is assessed in multivariate analysis, and which contributes to the canonical structures in a multiple discriminant analysis (MDA, Appendix 1.3), or to the principle components in a principle component analysis (PCA, Appendix 1.4). In addition, the proportion of the dispersion explained gives an assessment of the overall importance of an effect within the wider data set. Multivariate analysis could therefore be compared to a contour map, in that it is able to give a description, in mathematical terms, of the various features of the data set, and their size relative to other features in the data set.

In the following sections, results of three multivariate analyses are presented. MDA was used to analyse the relationships between five variables for Harvests 10 & 12 of Experiment 2; and PCA was used to analyse data for Harvests 2 to 10 of Experiment 2, and data for the 6 harvests of Experiment 3.

7.2.3.1 Multiple discriminant analysis

MDA has an advantage in the present context in that it can take account of the experimental design structure, but a disadvantage in that more than two

experimental treatments are required for a meaningful analysis (Appendix 1.3). Thus for Experiment 3, or for the first period of Experiment 2, when only LL and HH treatments were in place, MDA was not applicable.

One association which could be tested by MDA, however, was the low root production on LH plots (Section 4.3.3) at a time of high herbage production (Section 5.3.4), suggesting that developing tillers might be relying on roots of parent tillers as proposed by Garwood (1969). Variables entered into the analysis were: (i) intact core root mass length, 0 - 250 mm soil depth; (ii) apparent root growth, 0 - 250 mm soil depth; (iii) tiller density; (iv) tiller appearance; (v) herbage accumulation.

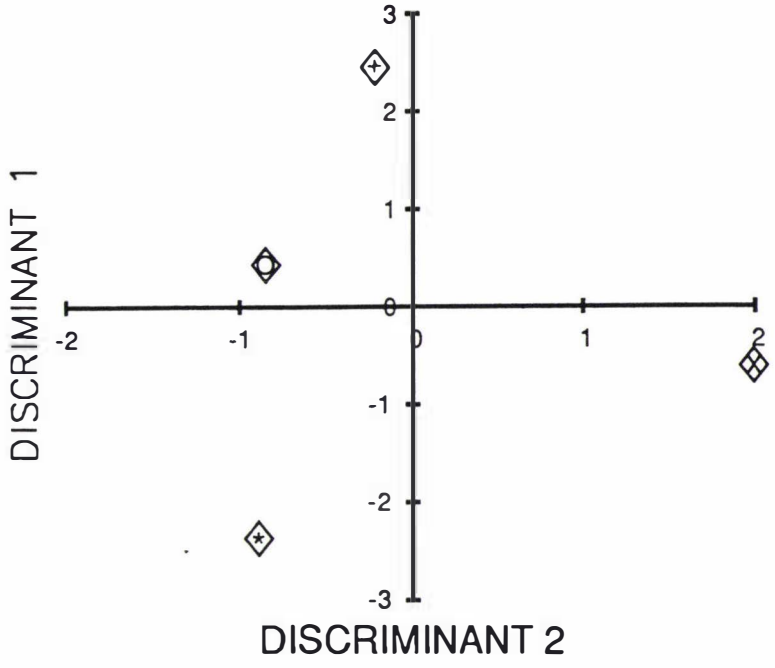
The first and second discriminant scores accounted for 63% and 29% of the dispersion respectively (Table 7.1). Evaluation of grazing treatment means for these two discriminant scores (Figure 7.2) showed that the first discriminant score contrasted LL and HH treatments with LH and HL being intermediate while the second picked out the LH treatment as being different form the other 3 treatments (Figure 7.2).

Table 7.1: Canonical structure and summary statistics, for multiple discriminant analysis of selected variables measured at Harvests 10 & 12, Experiment 2.

Variable	Discriminant 1	Discriminant 2
Intact core root mass	0.00	-0.99
Apparent root growth	0.21	-0.52
Tiller density	0.96	0.28
Tiller appearance	-0.09	0.99
Herbage accumulation	-0.71	0.61
Canonical r^2	0.92	0.83
P	0.002	0.014
Proportion dispersion (%)	63.5	28.5
Cumulative proportion	63.5	92.0

Canonical structure for the first discriminant function (DF) indicates scores for this DF were highly correlated with tiller density ($r = 0.99$; Table 7.1), and mean values of DF1 for the four grazing managements (Figure 7.2) reflect tiller population densities of Figure 5.3. Therefore, DF1 can be interpreted as indicating that, statistically, the effect of the grazing treatments on tiller density dwarfed all other effects in the data set.

Figure 7.2: Grazing management means for first and second discriminant scores described in Table 7.1. Symbols: (*) = LL, (+) = HH, (X) = LH, (O) = HL.



The canonical structure of DF2 indicates an association of high tiller appearance and herbage production with low root mass and root production (Table 7.1) and, for this characteristic, separates the LH grazing management from the other three treatments (Figure 7.2). Thus the statistical evidence confirms the association noted intuitively in Section 4.4.3 between high shoot growth and low root growth. However, this does not define cause and effect. The results of the MDA are consistent with a change in root/shoot allocation if new tillers from flowering tillers can utilise existing root systems for a time, as suggested by Garwood (1969). On the other hand, these results from MDA do not exclude alternative explanations for the coincidence of high shoot growth and low root growth on LH plots, such as an earlier peak in root growth on these plots (Section 4.4.3).

7.2.3.2 Principal component analysis

For both Experiments 2 & 3, it was also of interest to obtain a mathematical analysis of the seasonal pattern of inter-relationships among the variables measured. This was done using PCA, and a brief outline of PCA and its relationship to MDA is given in Appendix 1.4.

In the present context, PCA is of interest because it does not assume any experimental design structure, and can therefore provide a basis for

comparing the results from Experiment 2 and Experiment 3, despite the very different designs of those experiments. As an aid to interpretation of the PC's obtained from the analysis, but not to assess their statistical significance, analysis of variance of PC scores for particular PC's was performed.

Extensive discussion on PCA, including examples of analysis of time series data and discussion on the analysis of variance of PC scores, is given by Joliffe (1986). While it would be strictly more correct to adjust a PCA of time series data for possible correlation of successive observations over time, Joliffe (1986) notes that this is mathematically complex and the properties of the available mathematical solutions not well understood. In many cases authors have simply treated time series data as if they were independent observations (Joliffe, 1986), and results with common sense interpretations have been obtained. One example cited by Joliffe (1986) is an analysis of time trends in stock market prices for 30 companies' shares (Feeney & Hester, 1967). In this case, 66% of the variation in the data set was explained by a "size" PC, reflecting general increase in almost all stock prices over the period 1951 - 1963. The second PC accounted for 14% of the variation in the data set and indicated a contrast between "producer" and "consumer" stock prices.

The objective of the following section is therefore to provide an analysis of the pattern of variation in root and shoot data from Experiments 2 & 3 similar to that obtained for variation in stock market prices by Feeney & Hester (1967).

7.2.3.2.1 PCA for tiller and root appearance for January to December 1987 (Experiment 2)

A complete set of variables was not available for this analysis. Data on herbage production was not available, and data from intact core root sampling was omitted because the decrease in total root mass over time (Figure 4.2a) could have masked seasonal variation. Also root and tiller density data for Harvest 7 was omitted because there was not concurrent tiller appearance and tiller death rate data (Appendix 3). Thus 64 observations on 5 variables were used in the PCA, as follows: (i) root mass & (ii) root length in refilled cores, (iii) tiller density, (iv) tiller appearance and (v) tiller death rates for periods corresponding to Harvests 2 to 5 & 7 to 10.

Although 5 PC's were available from the analysis, only 3 are reported (Table 7.2) because the remaining two accounted for less than 8% of the variation in the data set (Table 7.2). Both the PC's which are not reported did contain statistically significant differences between harvests and/or grazing managements, but there was no obvious interpretation for these differences.

Table 7.2: Correlations between raw data and PC scores, and proportion of total variance explained by PCA of data from Experiment 2, January 1987 - January 1988.

	PC1	PC2	PC3
Refilled core root length (kg DM ha^{-1}).	0.73	-0.66	0.10
Apparent root growth (kg DM ha^{-1} day^{-1})	0.86	-0.46	0.14
Tiller density (Tillers m^{-2}).	0.64	0.55	0.40
Tiller appearance rate (Tillers m^{-2} day^{-1})	0.60	0.69	0.05
Tiller death rate (Tillers m^{-2} day^{-1})	0.65	0.17	-0.74
% variance explained	0.49	0.29	0.14
Cumulative %.	0.49	0.78	0.92

The first PC (Table 7.2) explains 49% of the variance and is highly correlated with all variables. Analysis of variance of the 64 PC scores indicated a grazing management effect, but showed that this PC score primarily contrasted values for Harvests 3, 9 & 10 with values for the other harvests. This can be interpreted as picking out the approximately coincident summer peaks for apparent root growth, tiller density, tiller appearance, and tiller death (Figures 4.4, 5.4, 5.7a. 5.7b); as the major feature in the data set. This PC is therefore analogous to the "size" PC in the stock market analysis mentioned above (Joliffe, 1986). The difference in grazing management means for this PC score probably reflects the much higher tiller densities on HH than on LL plots.

The second PC (Table 7.2) accounts for 29% of the variance and identifies a contrast between apparent root production and tiller density and tiller appearance variables. Again, analysis of variance of PC scores indicated this

PC contains information on both grazing management and seasonal effects in the data. However the most important of these was that PC scores were low for Harvests 8 & 9. This PC is therefore interpreted as describing a period of high root growth, associated with low tiller density and low tiller appearance, especially for LL plots (Figures 4.4, 5.4, 5.7a). It is probably also significant that the events described by this PC coincide with peak reproductive development (Table 5.10).

The third PC is strongly correlated with variation in tiller death rate and there is a weak association between tiller death and tiller density (Table 7.2). This is not easy to interpret, but seems to indicate that apart from an increase in tiller death associated with high values for all variables in summer (PC1), there is seasonal variation in tiller mortality which is largely independent of other measured variables.

It is interesting to compare and contrast the information obtained from MDA (Table 7.1) with that from PCA (Table 7.2). In MDA where the analysis is constrained mathematically so that discrimination between grazing managements maximised, grazing management effects on tiller density are seen as the major feature in the data set. In PCA where no such constraint is placed on the analysis, results of analysis of variance on PC scores suggest that grazing management effects on tiller density do influence PC scores, but the grazing management effects are dwarfed by seasonal events.

7.2.3.2.2 Principal Component Analysis for Experiment 3

In this case data used in the analysis were the 18 observations (6 harvests x 3 replicates) for the 7 variables presented in Figure 6.1. Three of the 7 available PC's are reported (Table 7.3).

The first PC again described the seasonal changes in sward activity as accounting for a substantial proportion of the variation in the data set (Table 7.3). Scores for this PC were highest for the August harvest, lowest for the November harvest, and intermediate for other harvests (Figure 7.3).

Figure 7.3: Mean scores for PC1 (Experiment 3) at six harvests.

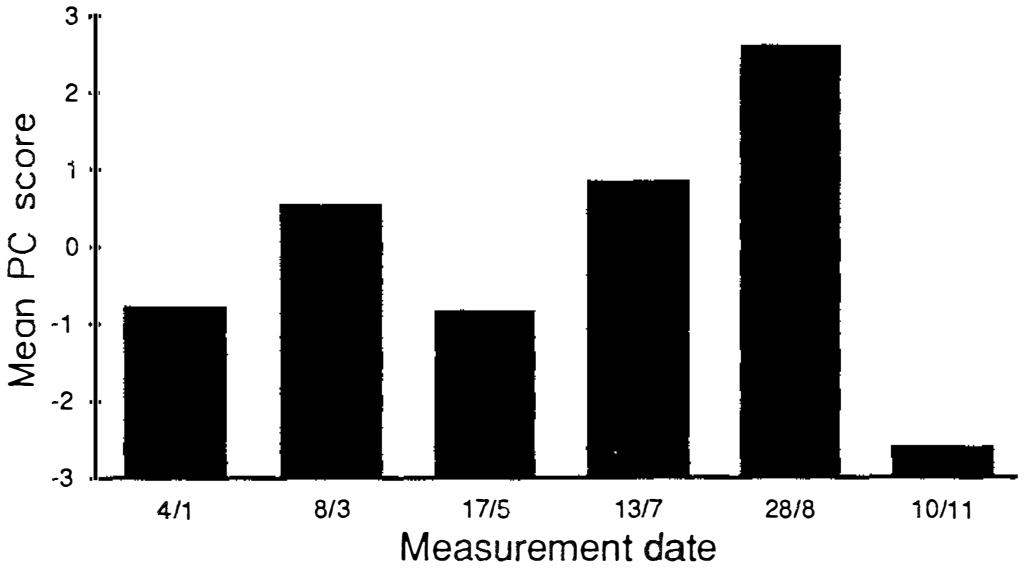


Table 7.3: Correlations between raw data and principal component scores, and proportion of variance explained for PCA analysis of data for above- and below-ground variables (Experiment 3).

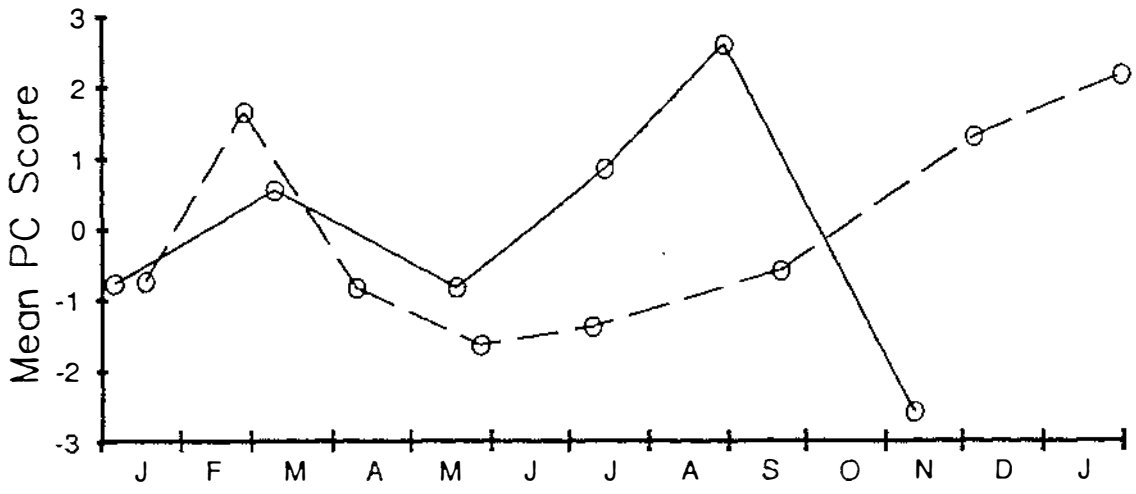
	PC1	PC2	PC3
Intact core root mass (kg DM ha^{-1}).	-0.66	0.53	0.25
Apparent root growth (kg DM ha^{-1} day^{-1}).	0.40	0.64	0.57
Leaf extension (mm day^{-1}).	-0.90	0.17	0.07
Leaf appearance (Leaves $tiller^{-1}$ day^{-1}).	-0.74	-0.37	-0.02
Tiller appearance (Tillers m^{-2} day^{-1}).	0.83	0.00	-0.23
Tiller death (Tillers m^{-2} day^{-1})	-0.66	0.23	-0.41
Tiller density (Tillers m^{-2})	0.17	0.71	-0.60
% variance explained	44	20	14
Cumulative %	44	64	78

Essentially PC1 identifies a period of high tiller and root appearance associated with low tiller death, low leaf appearance, low leaf extension and low root mass occurring in late winter-early spring (Figure 6.1). This implies a degree of internal co-ordination within the plant, and differential allocation of assimilate to different organs at different times. Such differential allocation has recently been reported for tall fescue. In tall fescue, drymatter deposition in regions of cell division at leaf elongation zones occurs during the day and continues in darkness, whereas in adjacent cell expansion zones drymatter deposition occurs only in light when presumably current photosynthate is available (Schnyder & Nelson, 1988). It is believed that this diurnal difference in allocation to different regions of the expanding leaf occurs because the different regions of the leaf metabolise fructans of different chain length (C. J. Nelson, pers. comm.). It is possible that similar mechanisms could account for the seasonal differences in growth of 'Grasslands Ruanui' ryegrass described in PC1, if the average chain length of storage compounds varies at different times of the year.

The second PC (Table 7.3) was most strongly associated with tiller density and root variables and values for the PC scores were highest for replicate 3 at August & November harvests. Values for these variables were lower for replicate 3 than for the other 2 replicates for these harvests, and so this PC is assumed to reflect sward damage which occurred on replicate 3 during grazing in wet conditions in June. PC 3 is assumed to reflect either minor seasonal variation or random variation among the data and is not interpreted. Given the small number of observations (18) in the analysis, it is not surprising that PC's without any obvious interpretation should arise in this way.

The August timing of the peak for seasonal mean scores for PC1 in Experiment 3 (Figure 7.3) differs from the corresponding peak for PC1 in Experiment 2 (Figure 7.4). This different seasonal pattern does not appear to be attributable to the smaller number of variables used for the PCA for Experiment 2, because a PCA for Experiment 3 using only tiller density, tiller appearance & death, and apparent root growth (4 variables), still produced a PC 1 with an August peak and with contrasts between variables. This appears to provide further confirmation of differences in sward behaviour between Experiments 2 & 3; which were thought to be most likely due to genetic differences between cultivars (Section 6.3.1).

Figure 7.4: Seasonal changes in mean score for PC1 for Experiments 2 ('Ellett' ryegrass, — — —) and 3 ('Grasslands Ruanui' ryegrass, ————).



7.2.4 Functional equilibrium

The concept of a functional equilibrium between root and shoot systems of plants has been presented in a number of forms by different authors, and the literature on this topic has been reviewed by Wilson, (1988, Section 2.4.2). A recent major study on this topic is that of de Willigen & van Noordwijk (1987) (Section 2.4.2). Those authors used a different approach from that adopted in the present study, in that they first constructed a detailed mathematical model to define nutrient uptake, shoot growth, and the inter-relationship between the two, then proceeded to collect field data for various parameters specified by their model. The complexity of their model is illustrated by the fact that some 15 parameters are required to define root adsorption

By contrast the experiments reported above have been designed to monitor events in the field such as tissue flows and changes in tiller population, in the expectation that the information obtained would allow deduction about questions of function, and about opportunities for manipulation. This coarse approach can never supply the detail provided by the approach of de Willigen & van Noordwijk (1987), but can ensure that the effort of detailed investigation is channeled to answering actual questions of sward behaviour, and can ensure that questions addressed in field studies are important in field swards, as

distinct from the laboratory environment. Thus the following section does not seek to provide a comprehensive synthesis of root and shoot behaviour. Instead, more detailed work of other researchers is drawn on to identify specific questions where a more detailed study could provide information to meet the original objective (Section 1.2) of identifying possible strategies whereby grazing management manipulation of swards might increase their productivity. In this context, several points can be made from the above results.

Firstly, previous research on this topic such as that by Deinum (1985), or de Willigen & van Noordwijk (1987), has tended to explore the operation of functional equilibrium for steady state conditions, whereas it seems likely (Figure 7.1) that seasonal change in partitioning between roots and shoots occurs, and forms a part of a plant's growth strategy. Such changes could be due to a number of factors, and for ryegrass greater root longevity (Garwood, 1967b) and consequent lower root replacement (Figure 4.4) probably play a part. It is doubtful if seasonal change in root/shoot partitioning could be manipulated through grazing management, but as noted previously, such effects may well explain inter-specific variation in seasonal growth patterns between ryegrass and browntop identified by Lambert *et al.*, (1986).

Secondly, it is possible to identify events, for example stolon formation following winter burial (Section 5.3.2), which represent a cost to the plant. However, there is no obvious avenue for manipulation which would reduce the need for stolon formation, and allow the substrate needed for stolon formation to be diverted to above ground growth.

Thirdly, some manipulations which can be achieved do no more than adjust compensating aspects of sward structure to new equilibria reflecting the changed environment. Thus change in grazing height resulted in changes in tiller size/density relations (Figure 5.6a,b, Table 7.1), but these changes appear to largely reflect equilibria defined by the -3/2 power relationship (Section 5.3.4.1) and do not necessarily lead to increased or decreased sward productivity. Similarly differential grazing height also resulted in the development of swards with different herbage masses for pseudostem and dead components, but similar quantities of leaf (Table 5.6).

Fourthly, some of the observations are consistent with (though not proof of) evolved responses to particular environments. For example, the fact that peaks

of root growth preceded shoot growth (Figure 7.1) and occurred independently of stimuli such as temperature (Section 6.3.2), could be interpreted as an 'evolved anticipation' of seasonal periods when conditions favour growth. Presumably a plant having the capacity to supply water and nutrients already in place when conditions become favourable for shoot growth would have a competitive advantage over a plant which did not. On the other hand, the behaviour of swards of 'Grasslands Ruanui' ryegrass (Table 7.3) is more consistent with an evolved strategy for coping with a period of winter dormancy followed by a period of rapid spring growth. Such a growth strategy could be a disadvantage in regions with mild winters, and from the incomplete evidence available (Section 6.3.1, Tables 7.2, 7.3) there are some indications that 'Ellett' ryegrass may have a different growth strategy.

Finally, there was one event observed in Experiment 2 which did appear to be associated with increased sward productivity. This occurred on treatment LH in summer 1987/1988 where lax grazing in spring and a consequently greater degree of reproductive development was followed by hard grazing. Daughter tiller formation from stubs of flowering tillers was involved (Section 5.3.3.2) and resulted in the LH plots having tiller size/density values significantly greater than those of HH and HL plots by late summer (Figure 5.6b). At least two possible explanations for this response arise from the data.

One mechanism could have been a transfer of yield from non-harvestable below-ground organs to shoot production (Discriminant function 2, Table 7.1). It has previously been noted (Garwood, 1969), that such effects appear to occur, and could be explained by roots of decapitated flowering tillers supplying water and nutrients to new daughter tillers formed from stubs of those flowering tillers. Such an effect would also parallel the case of higher productivity in crops with smaller root systems cited by de Willigen & van Noordwijk (1987).

Another possible explanation could be re-allocation to daughter tillers of substrate from decapitated reproductive tillers on these plots. In Experiment 1 (Sections 3.4.3.1, 3.4.3.2), increased root growth was observed in conjunction with increased reproductive growth on RUHE plots and there were also possible increases in tillering on these plots. In Experiment 2 increased reproductive growth occurred on LL and LH plots (Table 5.10), and increased daughter tiller formation was a feature of the subsequent sward dynamics of LH plots (Section 5.3.3.2). Also the new tillers were more productive than new

tillers on other treatments (Table 5.14). In Experiment 3, tiller appearance was much greater in the second summer than in the first summer and this effect might have been due to a laxer grazing regime in the second spring (Section 6.3.1).

This second explanation would be consistent with evidence of high assimilation rates for reproductive swards (Parsons & Robson, 1982); and also with reports that daughter tiller formation from stubs of flowering tillers was reduced where stubs were cut short (Davies, 1977, 1988; Davies *et al.*, 1981).

7.3 A hypothesis for further investigation

From the above it was concluded that manipulation of root growth *per se.* would not provide a strategy for increased herbage production, and that functional equilibrium responses are only useful if they result in a transfer of yield from non-harvestable components to harvestable components.

Alternatively, a hypothesis was developed that it might be possible to capitalise on the high gross photosynthesis of reproductive swards in spring, and devise a grazing strategy which would result in pre-flowering assimilate being expressed more as post flowering daughter tillers than as seedheads. Accordingly, a more detailed investigation of factors increasing or reducing daughter tiller formation from flowering tillers was undertaken. This investigation is reported in Chapter 8.

7.4 Summary

1. Computer simulation of net pasture growth rates suggests that in Experiment 2, seasonal peaks of herbage production in spring and autumn were preceded by increased root production; and also that ryegrass may maintain high shoot growth in winter (relative to other species) by reduced allocation to roots.

2. Multivariate analysis was used to derive mathematical descriptions of the salient features of the data. PCA for data from Experiment 2 indicated that the main feature of this data was a general increase in summer for all variables measured, whereas MDA indicated that greatest effect of grazing management treatments was the effect on tiller density of swards.

3. PCA was also used to describe data from Experiment 3, and seasonal patterns of variation for this Experiment (using 'Grasslands Ruanui' ryegrass) appeared to be different from those for Experiment 2 (using 'Ellett' ryegrass). These differences in seasonal pattern might reflect either year to year variation or fundamental differences in tiller dynamics between 'Ellett' and 'Grasslands Ruanui' cultivars of ryegrass, but more probably the latter.

4. Attempts to manipulate root/shoot balance to the advantage of shoot production are likely to be defeated by internal compensations within the plant, but it may be possible, through grazing management similar to that used on LH plots in Experiment 2, to stimulate tillering in spring, with consequent increase in pasture production. Specifically it appears that high assimilation rates of reproductive tillers may provide substrate for daughter tiller formation. Further investigation of this possibility is reported in Chapter 8.

CHAPTER 8: EFFECT OF MANIPULATION OF REPRODUCTIVE GROWTH ON THE POTENTIAL FOR TILLER INITIATION IN THE LATE-SPRING SWARD.

8.1 Introduction and overview

The field studies reported in Chapters 3 to 7 indicated that allowing some reproductive development to occur in late spring enhanced both root formation and tiller production, as compared to swards maintained in a vegetative state through greater grazing pressure. From these observations the hypothesis was developed that transfer of assimilate from flowering to daughter tillers is important in stimulating the development of late-spring tillers in ryegrass swards. (Section 7.3, Matthew *et al.*, 1989). These tillers can be important for summer productivity (Section 5.3.3.1), and probably also for sward persistence, because tiller death and replacement is high at this time (Figures 5.7ab). This chapter reports studies designed to give information on the possible scope for using grazing management manipulation to enhance tiller appearance rates of the late-spring sward.

Three experiments were designed. In the first of these (Experiment 4, November 1988 - February 1989) individual flowering tillers in the 'Grasslands Ruanui' ryegrass sward used previously for Experiment 3 were tagged, and the effect of various cutting regimes on the number and weight of daughter tillers formed by each tagged flowering tiller determined. The final two experiments involved measurements similar to those performed in Experiment 4, but on plugs of tillers transplanted from the field to a glasshouse. Transplanting was carried out in September 1989, and measurements made in November - December, 1989. Experiment 5 determined the effect of 4 cutting regimes on numbers and weight of daughter tillers formed by flowering tillers of 'Grasslands Ruanui' ryegrass, and Experiment 6 used $^{14}CO_2$ to determine the extent of translocation from parent to daughter tillers in flowering tillers of 'Ellett' ryegrass under different cutting regimes. Number and weight of daughter tillers formed per flowering tiller were also determined in this experiment.

8.2 Numbers and weight of daughter tillers formed in early-summer after decapitation of flowering tillers in a 'Grasslands Ruanui' ryegrass sward on different dates or at differing heights (Experiment 4)

8.2.1 Experimental

In order to make a preliminary evaluation of the hypothesis that reproductive development of a tiller promotes daughter tiller formation, a field experiment (Experiment 4) was set up on an area adjacent to and within the same paddock as that used for Experiment 3. Site details were therefore the same as for Experiment 3.

For Experiment 4, three plots were divided into 4 mowing strips (defoliation treatments), laid out in a completely randomised design. Within each mowing strip 20 individual tillers in the process of stem elongation (a total of 240 tillers) were pre-tagged for later analysis of daughter tiller formation. Treatments applied to the mowing strips and to the tagged tillers within them were:

(1) Mown to 50 mm height on 16 November 1988, and individual tagged tillers cut at ground level also on 16 November (pre-head emergence).

(2) Mown to 75 mm height on 16 November 1988, and individual tagged tillers cut at ground level on 3 December 1988 (head emergence-anthesis).

(3) Mown to 75 mm height on 16 November 1988, and individual tagged tillers cut at 50 mm height on 3 December 1988 (head emergence-anthesis).

(4) Not mown between 16 November 1988 and 17 January 1989. All tagged tillers left uncut and allowed to set seed.

For treatments 1, 2, and 4 the three cutting heights were designed to result in a gradation in the extent to which reproductive development occurred, so as to examine differences in daughter tiller formation from flowering tillers for swards undergoing different degrees of reproductive development. Treatment 3 was included to evaluate the effects on daughter tiller formation of an increase in stubble height, when decapitation occurred at about head emergence. The cutting height comparison (Treatments 2 & 3) was included because Davies (1977, 1988) had noted that reduced stubble height reduced daughter tiller formation from cut stubs of reproductive tillers.

For ease of relocation at the end of the experiment, tagged tillers were placed on fixed transects. The transects were at right angles to the mowing strips with 2 tagged tillers per mowing strip in each transect (8 tillers per transect). Treatments 1-3 were mown at regular intervals to maintain sward heights close to those initially established on 16 November 1988, and all tagged tillers were harvested on 17 - 19 January 1989. Plate 8.1 shows one replicate on 18 January 1989. Harvested tillers were removed to the laboratory where measurements made included the number of live and dead daughter tillers per tagged tiller and the total number of roots initiated on all daughter tillers of a tagged tiller.

Plate 8.1: Layout of mowing strips and fixed transects for replicate 3, Experiment 4. Treatment 1 at left, Treatment 4 at right, Treatments 2 & 3 centre.

For statistical analysis, each of the 12 mowing strips was treated as a plot, and means for the 20 tillers within each strip analysed for treatment and replicate effects (11 d.f.).

8.2.2 Results

Statistical significance of the results was marginal, in that even where significant differences occurred, these generally involved only the highest and lowest values for the measurement in question. However, some trends can be identified.

The number of daughter tillers formed per flowering tiller was higher for treatments 3 & 4 than for treatments 1 & 2, though the higher daughter tiller formation was partly offset by higher tiller death (Table 8.1). The total number of daughter tiller roots and the mean number of roots per tiller are given as a measure of the degree of tiller development and show that tillers formed were less developed for the uncut treatment 4 (Table 8.1). Treatment 4 also had the greatest death and lowest mean weight for daughter tillers (Table 8.1), but these effects were not statistically significant at the 5% probability level.

Table 8.1: Numbers of daughter tillers, roots, and average daughter tiller weight for flowering tillers subjected to four defoliation treatments from 16 November 1988 and harvested January 1989.

	Treatment[1]					
	1	2	3	4	SEM	P[2]
Tiller number:						
Live	1.8	1.7	2.3	1.9	0.25	NS
Dead	0.6	0.9	1.2	1.3	0.17	*
Total	2.4	2.6	3.5	3.2	0.33	*
Root number	6.8	6.6	8.2	5.6	0.6	*
Roots/tiller	2.9	2.5	2.6	1.8	0.35	*
Live tiller weight (mg)	37	39	37	30	10.9	NS

1. For details of treatments see text.
2. For Table 8.1 and following Tables, + = P < 0.10, * = P < 0.05 ** = P < 0.01, *** = P < 0.001)

8.2.3 Discussion

Co-efficients of variation were not unduly high (approximately 10% for daughter tiller number), so the fact that only treatments with highest and lowest means differed significantly indicates that effects on numbers of daughter tillers formed were fairly small.

These results are consistent with the hypothesis that photosynthate from reproductive tillers may promote daughter tiller formation and there does

appear to be an increase in daughter tiller formation with the increased stubble height for treatment 3, although the degree of enhancement of tillering was small. The reasons for this small response are not known, but the swards became clover dominant after mowing (Plate 8.1), and the resultant shading might have suppressed tillering. The results do show that reproductive growth must be removed to facilitate development of the new daughter tillers produced by flowering tillers, and do not support the popular view (as reflected by Hughes (1983), for example) that removal of flowering tillers at ground level before head emergence promotes daughter tiller formation.

8.3 Effects on daughter tiller formation of height and date of cutting of flowering tillers in plugs transplanted to a glasshouse from a 'Grasslands Ruanui' ryegrass sward (Experiment 5)

8.3.1 Background

To gain further information on the effect of different spring defoliation regimes on daughter tiller formation by over-wintering reproductive tillers, two further experiments were set up in early spring (September) 1989. Both of these experiments involved use of cores collected in exactly the same way as transplanted cores (Section 6.2.2), but potted in plastic planter bags and grown on in a glasshouse for close observation. One of these (Experiment 5) used cores collected from the 'Grasslands Ruanui' ryegrass plots used in Experiments 3 & 4; while the other, reported in Section 8.4, used cores from an 'Ellett' ryegrass sward (Experiment 6).

8.3.2 Experimental

Cores were collected in September 1989, and stored under refrigeration (7 °C) for up to 1 week until potted. At potting each of 32 cores was packed firmly with sand into an 8 litre plastic planting bag and 20 g "Osmocote" slow release fertiliser (N:P:K = 14:16:12) placed beneath the core.

Cores were then allowed to grow on undefoliated until early November when a 2 x 2 factorial regime of 2 cutting heights and 2 cutting dates was applied to 28 pots, 7 replicates (4 pots being rejected because of uneven growth).

The cutting heights were 20 mm or 100 mm (LO or HI, respectively) and the cutting dates (1 or 17 November, early (E) or late (L), respectively). After the initial cutting, pots were trimmed fortnightly to the original cutting height. Plate 8.2a (page 167) shows trimmed pots in late November.

Shortly after potting in September, all live ryegrass tillers were tagged with split plastic rings. In early November pots were re-examined, and new tillers formed since the first tagging identified with a tag of a second colour. In addition, three randomly selected flowering tillers per pot were marked with tag of a third color for later evaluation of numbers and weight of daughter tillers formed per parent tiller.

The 3 marked flowering tillers per pot were harvested on 21 December. At harvest, number and weight of tillers formed by marked tillers during November/December (i.e. after the second tagging) was determined for the following categories of daughter tiller:

(1) Primary tillers formed during the interval between the September and November taggings. (Existing daughter tillers, EDT)

(2) Secondary tillers formed from EDT during November/December. (Existing daughter secondary tillers, EDST).

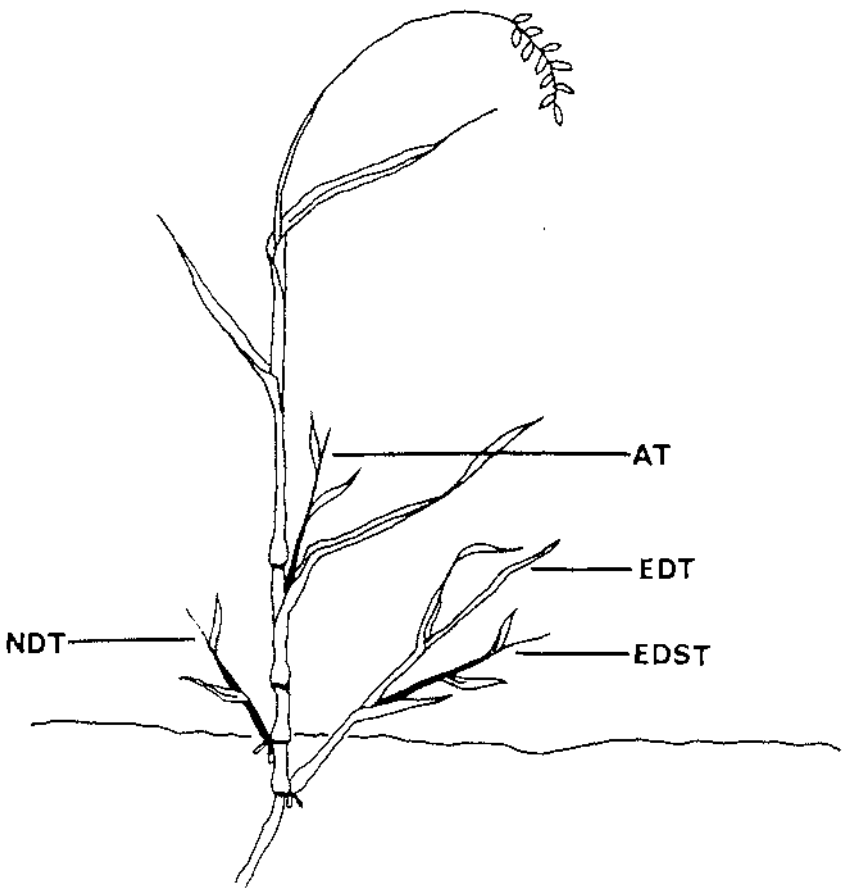
(3) Primary tillers and their secondary tillers formed on the main axis at ground level or below ground during November/December. (New daughter tillers, NDT). These NDT were almost all primary tillers, and secondary tillers, if present, were usually small.

(4) Primary aerial tillers and their secondary tillers formed from the main axis during November/December. (Aerial tillers, AT.)

These 4 categories are illustrated diagrammatically in Figure 8.1 (page 166).

In Experiment 5, tiller number and tiller size data tended to be positively skewed, and so was log transformed before performing analysis of variance. Standard errors are therefore presented as the least significant ratio between two treatment means (Steel & Torrie, 1981), but means presented are those for the untransformed data. Data analysed were the means for the three marked tillers in each pot.

Figure 8.1: Schematic diagram of position on parent reproductive tiller axis of tiller categories reported in Table 8.2.



8.3.3 Results

Numbers of daughter tillers produced by the tagged flowering tillers were fewest for pots cut to 20 mm height on 1 November (LO/E cutting treatments), and in pots with either increased cutting height (HI) or later cutting (L) the tagged flowering tillers produced increased numbers of daughter tillers (Table 8.2, Plate 8.2b). For the two most extreme treatments, (ExLO and LxHI) there was a more than four-fold difference in numbers of daughter tillers formed between early November and mid December.

Plate 8.2a: Experiment 5 - view of pots after imposing LO or HI and E or L cutting treatments, November 1989.

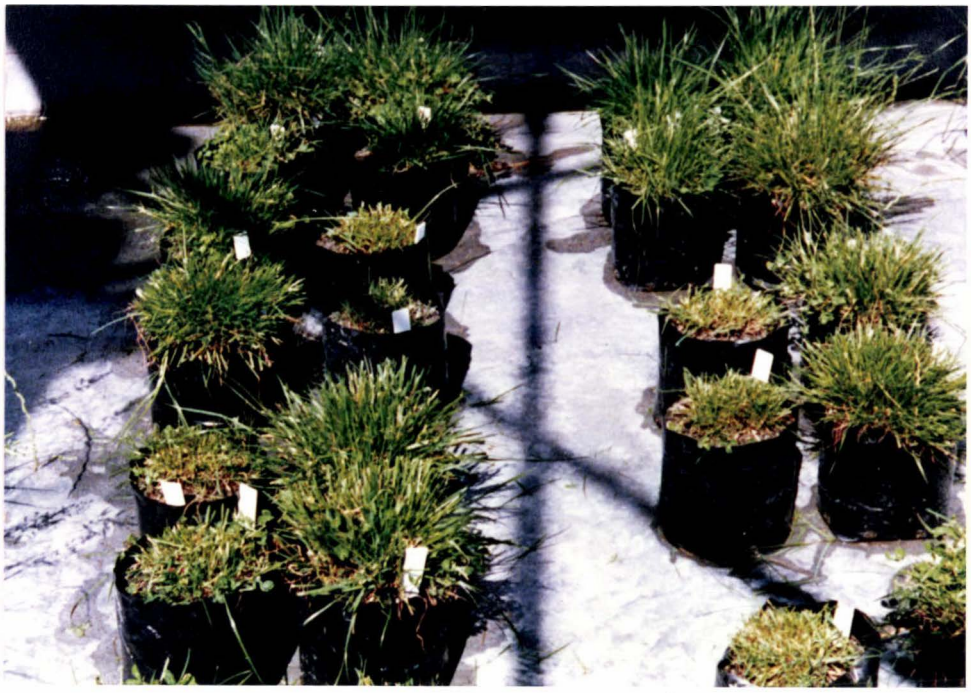


Plate 8.2b: Experiment 5 - tagged flowering tillers dissected from pots at harvest on 21 December, and showing extent of daughter tiller formation for 4 cutting treatments, first cut on 1 or 17 November (E or L, respectively) to 20 mm or 100 mm height (L or H, respectively).

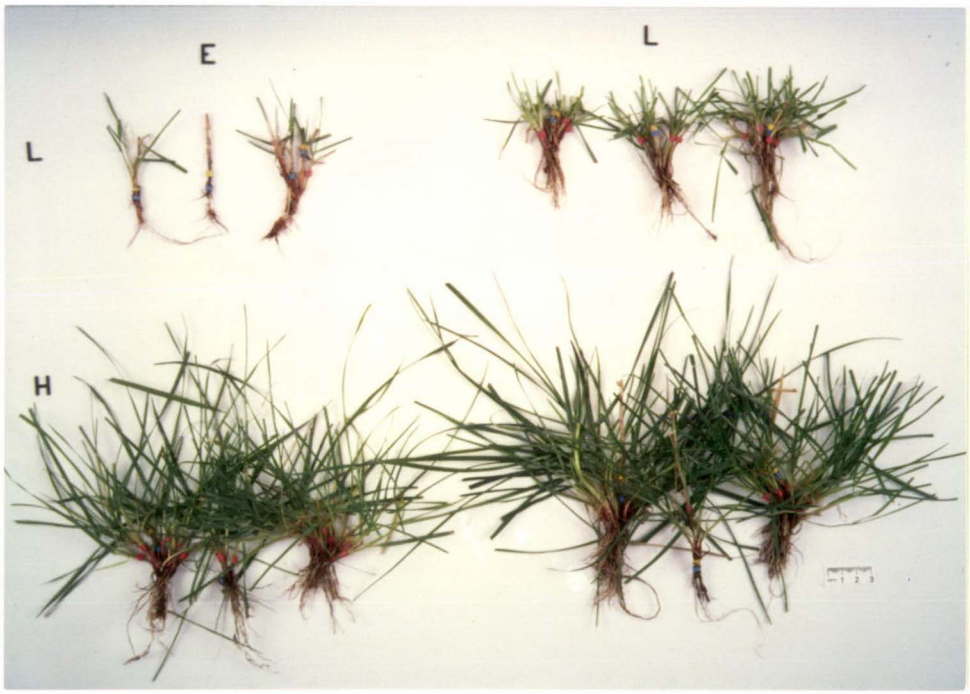


Table 8.2: Numbers and weights (mg DM tiller^{-1})of daughter tillers formed per parent tiller from reproductive tillers of 'Grasslands Ruanui' ryegrass under differing spring defoliation regimes.

Category of daughter[1]		Cut 1 November (E) LO	Cut 1 November (E) HI	Cut 17 November (L) LO	Cut 17 November (L) HI	LSR 5%
2 EDST	Number	1.5	6.7	6.3	7.1	1.81
	Total Wt.[2]	10.4	51.8	108	125	2.33
3 NDT	Number	0.76	1.9	2.2	2.1	1.40
	Total Wt.	9.0	22	62	58	1.50
4 AT	Number	0.33	1.0	0.56	1.2	1.50
	Total Wt.	0.67	8.1	5.8	24	2.26

1. Category 1 data (EDT) did not show treatment effects and so are not presented.
2. Mean individual tiller wt. = total wt (mg) for category divided by number.

8.3.4 Discussion

Tiller weight data (Table 8.2) need to be interpreted with caution, because the fortnightly trimming of pots would have removed material from developing tillers as well as their parents. Numbers of tillers formed would not have been affected in this way by trimming, except for the possible loss of a small number of AT bud sites in the LO cutting height, however.

These results thus confirm the findings from Experiment 4, that defoliation regimes which allow opportunity for reproductive development of over-wintering tillers are likely to increase daughter tiller formation by those tillers. This response involves both an increase in the number of tillers formed and an increase in their weight. It seems likely that transfer of substrate from parent to daughter tillers could account for the enhancement of tillering observed with later or laxer defoliation of reproductive tillers, as suggested by Davies (1988). It would appear also, that control of lateral bud development on reproductive tillers of perennial ryegrass is different from that in *L. multiflorum* where there is evidence of both an apical dominance effect, and an inhibitory effect exerted by the upper leaves (Clifford & Langer, 1975; Clifford, 1977).

8.4 Translocation of ^{14}C from flowering tillers to daughter tillers

8.4.1 Introduction

This experiment (Experiment 6) aimed to use $^{14}CO_2$ to obtain information about the quantity of photosynthate translocated from parent (flowering) to daughter tillers and to test whether different cutting regimes would result in differing recoveries from daughter tillers of radiocarbon fed to reproductive tillers. Ellett ryegrass was chosen because this had been the cultivar used in Experiments 1 - 3. Information on translocation between developing seedheads and their daughter tillers would test the hypothesis (Sections 5.3.4.6.3, 7.3, 8.1) that enhanced daughter tiller formation under certain management conditions was due to increased transfer of assimilate, and also provide a basis for development of objective criteria for grazing management decisions in order to maximise late-spring herbage production, if the hypothesis were confirmed.

8.4.2 Experimental

Potted plants were prepared in September 1989 in a similar way to those for Experiment 5 (Section 8.3.2). Two main differences were that the sward from which the cores were collected had been sown in 'Ellett' perennial ryegrass (no other species included at sowing) approximately 15 months previously, and that cutting treatments were applied to single tillers, with surrounding tillers remaining uncut (Plate 8.3a). As before, all live ryegrass tillers were tagged in September, shortly after transplanting, and all new tillers in late October. Initially, 64 pots were prepared.

In early November 24 pots (6 replicates of 4 treatments, see below) were selected for uniformity of growth, and two randomly selected flowering tillers per pot were each fed 740 kBq[1] (20 μCi) $^{14}C0_2$ tracer, using a method adapted from that of Pasumarty (1987). The technique is shown in Plate 8.3b. Briefly, the portions of flowering tillers to be fed radiocarbon (in this case the emerging seedhead, the flag leaf, and the two penultimate leaves) were enclosed in a plastic bag, sealed around the stem with plastic sealing compound, ^{14}C-labelled Na_2CO_3 injected into a pocket in the bag by means of a hypodermic syringe, and $^{14}CO_2$ liberated by further injection of excess 20% v/v lactic acid.

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1. Note: 1 kBq = 1000 radioactive disintegrations per second.

Plate 8.3a: Experiment 6 - view of pots shortly after feeding radiocarbon tracer and applying cutting treatments to selected individual flowering tillers (November 1989).

Plate 8.3b: Experiment 6 - injection of radiocarbon tracer into plastic envelope surrounding inflorescence, flag leaf, and 2 penultimate leaves of selected flowering tillers.

Needle holes were sealed with petroleum jelly after the hypodermic syringe had been withdrawn. Release of $^{14}CO_2$ was not immediate and bags were left in place until Geiger counter readings showed that radioactivity had transferred from the solution in the pocket of the bag to the tiller being labelled, usually 2 to 3 hours. The tracer was applied in three applications of 185, 185 and 370 kBq over a 4 day period. It was hoped that multiple applications of radioactive tracer might reduce variability of uptake commonly noted in studies using $^{14}CO_2$ tracer.

Radiocarbon was not 'chased' with $^{12}CO_2$, a practice often used to overcome the difficulty that C_3 plants such as ryegrass are unable to remove CO_2 below concentrations of approximately 50 ppm, and therefore not all radiocarbon fed to plants is taken up (Parsons, 1981). However, given the small volume of air enclosed in the bags themselves, and that the labelling period of several hours would probably have allowed some diffusion of $^{12}CO_2$ into bags during labelling via the clip seal at the bottom of the bag, it is unlikely that not chasing radiocarbon with non-radioactive CO_2 resulted in any significant loss of applied tracer.

The design was a 2 x 2 factorial and treatments applied were:

(1) Cutting treatments - Seedheads of radioactively-labelled tillers either left undecapitated or removed at the flag leaf node. In contrast to Experiment 5 where all tillers in a pot were cut, in this experiment tillers other than the two labelled tillers per pot, were left uncut.

(2) Shade treatments - Pots either left in full sunlight or placed inside an inverted bag made from plastic shade-cloth. Tests with a light meter showed that light levels inside these shade-bags were about 50% of light levels a few cm above the bags.

On the day following the last application of $^{14}CO_2$ (7 November), shade treatments as above were applied and cutting treatments were imposed on the individual labelled tillers for 24 pots (6 replicates). In addition to the above treatments, 2 tillers per pot not labelled with radioactive tracer were removed as near to ground level as practicable. This was to provide information on the level of daughter tiller formation which would occur if there was effectively no possibility of further translocation from flowering tillers to daughter tillers after radioactive labelling had taken place.

The 2 marked tillers per pot and any tillers basally attached to them were harvested 3½ weeks later on 30 November, and dissected into the four

categories as in Experiment 5 (Section 8.3.2). Labelled tillers themselves were also dissected to give a further 4 dissection categories - parent tiller live leaves (PT-LLF), dead leaves (PT-DLF), basal stem (PT-STM), and (if still present) parent tiller seedhead (SHD). A 9th category was parent tiller seedhead removed 24 hours after completion of labelling. The 2 tillers per pot removed at ground level were also harvested in this way, but for these tillers only daughter tiller categories EDT, EDST and NDT could be determined because sites for AT tillers and the parent tiller itself had been removed when cutting treatments were imposed.

Samples dissected as above were dried 24 hours at 65 °C, weighed and stored in a deep freeze. Samples were later ground, oxidised and scintillation counts made to quantify ^{14}C distribution within the plants four weeks after labelling. The method used followed closely that described by Jeffay & Alvarez (1961), and the scintillation cocktail used was ethanolamine:methyl cellosolve:toluene in the ratio 1:7:10 with 6.0 g l^{-1} PPO. The sample oxidiser used was a Harvey OX600 which typically gives recoveries of radioactivity from test samples of approximately 95% (D. H. Greer, pers. comm.) and the scintillation counter was a Beckman LS3801.

For each category of daughter tiller a subsample of approximately 100 mg was oxidised and counted in this way to determine specific activity (Bq mg^{-1}), and total activity calculated by simple proportion. Where less than 100 mg tissue was available, the entire sample was oxidised. For some categories such as AT, samples were frequently as small as 10 mg, so checks were carried out to confirm linearity and repeatability of results obtained by the above procedure (Appendix 7.1). Duplicate analysis of samples was ruled out because of cost.

At the time samples were processed, no radioactive standard was available to determine quenching coefficients for the samples in this experiment so counts per minute were converted to disintegrations per minute (DPM) using a counting programme for the Beckman LS3801 supplied by another user of the machine. Subsequently 3 Amersham CFR.101 calibration discs with a known activity of 5000 DPM were obtained and were oxidised and counted using the same procedure. This provided a check, both of recovery of radioactivity and on the accuracy of results from counting programme which had been used for liquid scintillation counting (Appendix 7.2).

Data for weight and number of daughter tillers formed per labelled tiller and data on ^{14}C recovery were averaged for the two labelled tillers in each pot and analysed for decapitation and shade effects as a 2 x 2 factorial design. Data were not markedly skewed, and log transformation was not used. For tillers cut to ground level, means and standard errors were derived by one-way analysis of variance. Because tillers cut to ground level were from the same pots as labelled tillers, a 3 x 2 factorial analysis of 3 cutting heights x 2 light levels would have artificially increased degrees of freedom in the analysis, and would have been invalid. Assumptions of independence between observations might also have been violated.

Tillers from a further four spare pots were labelled as above and pressed for autoradiography at five and twelve days after labelling with radiocarbon. This provided qualitative information on radiocarbon distribution within labelled plants, but after autoradiography these additional tillers were dissected so as to isolate particular leaf segments identified in the autoradiographs as having high concentrations of radiocarbon. Specific activities (Bq mg^{-1}) for these leaf segments were then quantified by oxidation and scintillation counting as above.

8.4.3 Results

8.4.3.1 Tiller number and tiller size

The weight and number of NDT formed from flowering tillers was greatest when tillers were decapitated at the flag leaf node. Both leaving seedheads intact and removing them at ground level reduced daughter tiller formation (Table 8.3). Removing seedheads at ground level appeared to affect daughter tiller formation more severely than leaving seedheads intact (Table 8.3). Placing pots in 50% shade did not cause statistically significant reductions in weight or numbers of daughter tillers formed, although values for the 50% shade treatment were consistently lower than those for the full light treatment (Table 8.3). For EDST similar trends were noted, but were not statistically significant (Table 1). No treatment differences in weight of EDTs were observed.

Table 8.3: Effects of cutting height and light or shade treatment on total weight and number of new daughter tillers (NDT) and existing daughter secondary tillers (EDST) formed from flowering tillers.

	Cutting height					
	Ground level		Flag leaf node		Seedhead uncut	
Tiller category	NDT	EDST	NDT	EDST	NDT	EDST
Total weight (mg)						
Full sunlight	38.3	32.4	130	62.2	49.0	45.5
50% shade	25.5	20.0	114	54.5	47.8	33.3
SEM	23.0	27.9	38.3	42.5	38.3	42.5
Number of tillers						
Full sunlight	1.83	3.25	5.00	6.50	2.40	4.40
50% shade	1.65	1.83	3.20	4.60	2.20	1.70
SEM	0.68	2.22	0.54	2.77	0.54	2.77

Note: average individual tiller weight can be computed as weight of new tillers/number of new tillers.

8.4.3.2 Recovery and distribution of radiocarbon

Seedheads decapitated at the flag leaf node immediately after the labelling period were found to contain approximately 5% of the applied radiocarbon. Undecapitated seedheads contained about 1/2 this amount when harvested approximately 3 weeks later, with a further 10% of applied radiocarbon still present in the lower stem and leaves (Table 8.4). Translocation to daughter tillers (EDT, EDST, NDT, and AT) accounted for only 3.5% of tracer recovered (Table 8.4).

Treatment effects on the distribution of radiocarbon within labelled tiller hierarchies were found (Table 8.5). Removal of the seedhead at the flag leaf node increased the recovery (kBq) of tracer from new daughter tillers but decreased the recovery from parent tiller leaves (Table 8.5). The shade treatment yielded higher recovery values than the light treatment for all plant parts, though the differences were not always significant (Table 8.5).

There were parallel differences in specific activity (Bq mg^{-1}) of plant parts from the various dissection categories, although paradoxically increased total radioactivity (3.99 kBq cf. 2.21 kBq) of NDT + AT daughter tillers formed by decapitated seedheads was associated with lower specific activity (24.5 Bq mg^{-1} cf. 36.2 Bq mg^{-1}) for these same tillers. Specific activity of NDT + AT was increased when plants were placed in 50% shade ($P < 0.001$). Shading also resulted in higher specific activity values for most dissection categories, although these differences were again not always significant (Table 8.5).

Table 8.4: Total activity per tiller (kBq), specific activity (Bq mg^{-1}) and % recovery (% tracer applied and % tracer recovered) for 9 dissection categories. Data are means for 48 labelled tillers from 24 pots. Quantity of tracer applied was 740 kBq $tiller^{-1}$.

Dissection category	Recovery (kBq)	Specific activity (Bq mg^{-1})	% of tracer applied	% of tracer recovered
1. + 2. EDT + EDST	1.73	2.91	0.23	1.25
3. NDT	1.91	11.8	0.26	1.39
4. AT	1.13	16.7	0.15	0.82
5. PT-LLF	29.9	117	4.04	21.7
6. PT-DLF	1.53	36.3	0.21	1.11
7. PT-STM	48.7	106	6.58	13.08
8. SHD (uncut)	35.7	214	2.41	35.32
9. SHD (cut day 1)	69.8	743	4.72	25.32
Total[1]	137.6	NA	18.6	100

1. Note: Failure to sum because categories 8 & 9 each apply to 12 pots, only. These values averaged to calculate totals.

Table 8.5: Effects of seedhead removal and light or shade treatments on total activity (kBq) and specific activity (Bq mg^{-1}) for selected dissection categories of labelled tillers.

	Uncut	Cut	Signif.	Light	Shaded	Signif.
Total activity recovered from 740 kBq/tiller applied as $^{14}CO_2$						
EDT + EDST	1.95	1.50	NS	1.56	1.99	NS
NDT + AT	2.21	3.99	**	2.77	3.32	NS
PT-LLF	38.7	21.1	***	25.3	34.5	**
PT-TOTAL	104	56.6	***	71.1	89.2	*
Specific activity (Bq mg^{-1})						
EDT + EDST	9.01	5.58	+	5.73	8.86	NS
NDT + AT	36.2	24.5	**	23.1	37.6	***
PT-LLF	332	175	*	203	304	NS
PT-TOTAL	283	170	+	190	263	NS

TOTAL = LLF + DLF + STM.

8.4.3.3 Autoradiography

Autoradiography of 1 labelled tiller 5 days after labelling showed obvious translocation to daughter tillers (Plate 8.4). It was noted that radiocarbon was not evenly distributed in daughter tillers, with some leaves showing very little uptake (A2, A3; Plate 8.4) and others showing localised bands of higher specific activity (B1, B2, B3; Plate 8.4). Subsequent oxidation and quantitative ^{14}C determination of leaf segments from these high-activity bands revealed specific activity values ranging from 63 to 193 Bq/mg (Table 8.6). These values were on average 3.6 times greater than those found in adjacent leaf segments (C1, C2, C3; Plate 8.4; Table 8.6), and were similar to values observed later for parent tiller leaves which were actually fed radiocarbon (PT-LLF, Table 8.4).

Table 8.6: Specific activity values (Bq mg^{-1}) for leaf segments dissected from 3 individual tillers after autoradiography to identify regions of higher uptake (see Plate 8.4).

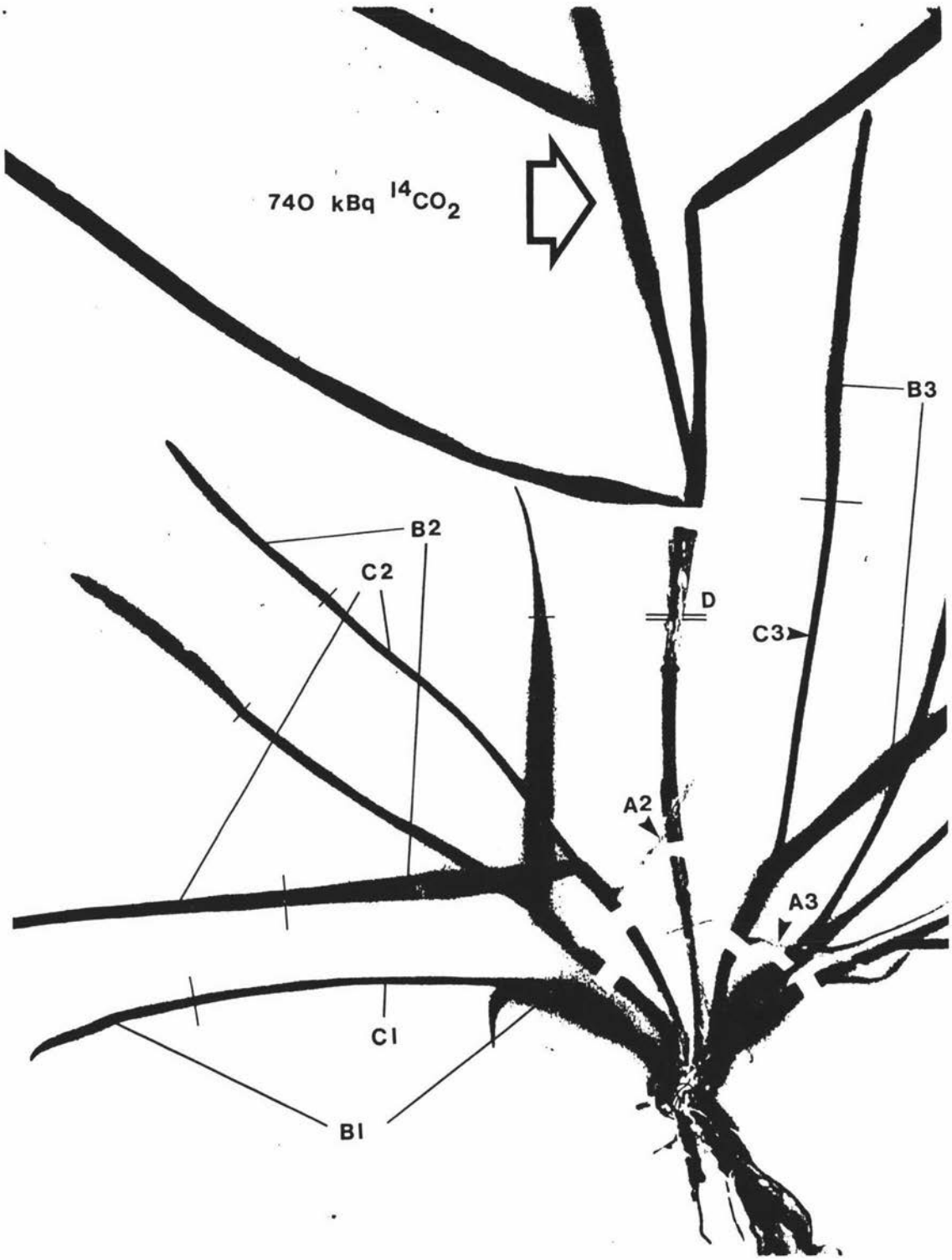
	Tiller number			
	1	2	3	Mean (SE)
High uptake segments (B, Plate 8.4)	193	63	115	124 (53)
Adjacent segments (C, Plate 8.4)	51	22	29	34 (12)

Autoradiography of 1 labelled tiller after 12 days also showed uneven distribution of ^{14}C in daughter tillers, with a clear impression that successive nodal daughter tiller clusters down the axis had each had lower specific activity than the tiller or cluster above. This was confirmed by oxidation and scintillation counting (Table 8.7), although total activity of the youngest tiller was low due to its small size (Table 8.7).

Table 8.7: Specific activity (Bq mg^{-1}) and total activity (kBq/tiller) for tillers at successive nodal positions of one flowering tiller, counting basipetally and beginning with node of attachment of youngest daughter tiller.

	Nodal position			
	1	2	3	4
Specific activity (Bq mg^{-1})	25.4	14.6	5.85	2.16
Total activity (kBq $tiller^{-1}$)	0.629	1.26	1.19	0.729

Plate 8.4: Autoradiograph of labelled tiller harvested 5 days after radiocarbon application. (A) daughter tiller leaf showing minimal uptake, (B) high specific activity bands located contiguously at the base of the second leaf and tip of emerging leaves. (The position of this band coincides with the expected location of cell expansion during the 4 days of radiocarbon application). (C) Lower specific activity bands indicating leaf formed after radiocarbon application. (D) Point at which plastic envelope was sealed around parent tiller during tracer application. 1,2,3 - three tillers for which specific activities of leaf segments were determined (Table 8.6).



8.4.4 Discussion

8.4.4.1 Experimental strategy and radiocarbon recovery

The different cutting treatments were expected to result in differing quantities of assimilate being translocated from parent to daughter tillers. In undecapitated tillers the developing seedhead would likely act as a competing sink, and limit translocation to daughter tillers, while removal of the parent tiller at ground level would clearly preclude further translocation from parent tiller to daughter tillers.

The shade treatment was expected to reduce carbohydrate status of parent tillers and so reduce availability of assimilate for export to daughters. In the shade treatment, effects of reduced carbohydrate status, and effects of light flux and quality (Deregibus *et al.*, 1983) would be confounded, but this artificial stress would provide a measure by which to asses the magnitude of differences in tiller initiation resulting from different cutting heights of the reproductive tillers.

Previous experiments using radiocarbon to measure translocation have usually been concerned with measurements over much shorter time periods, often 24 hours or less, although Colvill & Marshall (1984) did measure ^{14}C distribution in ryegrass tillers 40 days after labelling. The reason for a 28 day period between labelling and quantification of radiocarbon distribution in this experiment was to allow time for remobilisation from cut stems as suggested by Davies (1977). A relatively large amount of radioactivity (740 kBq per tiller) was used to compensate for anticipated respiration losses over the 3½ weeks between application of radiocarbon and harvest of labelled tillers.

Radiocarbon was not applied to tillers to be removed at ground level because the results of Colvill & Marshall (1984) suggest redistribution of carbon from storage in the reproductive stubble is the major source of substrate exported from reproductive to daughter tillers. It was therefore presumed that removal of the tiller at ground level would remove the potential source of radiocarbon and that recovery of tracer in such a treatment would be very low. However, if radiocarbon had been applied to these tillers some information on how rapidly assimilate from parent tillers is passed to daughters would have been obtained. In this case there would have been no opportunity for re-mobilisation and distribution of stored

assimilate from previous photosynthesis and any radiocarbon recovered could have been assumed to be allocated from current photosynthesis at the time of labelling.

It was expected from the results of earlier studies (Clemence & Hebblethwaite, 1984; Hampton *et al.*, 1987) that the seedhead would retain the bulk of the assimilate and the current experiment was not designed to differentiate between assimilate fixed in leaves and exported from there to the seedhead, and assimilate fixed in the seedhead itself. Nor was there any attempt to measure movement of carbon into or out of temporally different sinks in the developing seedhead, such as stem elongation and spikelet formation. Rather, it was intended to capture total radiocarbon reaching daughter tillers by translocation from all sources over a period of time and to look for differences in the amount of radiocarbon recovered from daughter tillers as a result of differences in cutting treatments applied to parent reproductive tillers.

The overall recovery of 18.6% applied radiocarbon (Table 8.4) reflects respiration losses, translocation to organs not harvested (e.g. roots), and possible leakage of some radiocarbon from the clip seals of the plastic enclosures, before assimilation. No attempt was made to quantify the magnitude of these different losses. The reason for a higher recovery of radiocarbon from shaded plants than from plants in full sunlight (Table 8.5) is uncertain but might be due to reduced photorespiration losses of current photosynthate.

One issue not addressed by this experiment is the possibility of reciprocal translocation from daughter tillers to parents (Clifford *et al.*, 1973). Hampton *et al.* (1987) found that less than 10% of carbon fixed in daughter tillers was exported, however, so reciprocal translocation is unlikely to have seriously affected conclusions reached below.

8.4.4.2 Translocation to daughter tillers

The hypothesis that translocation from flowering tillers is important to the initial establishment of daughter tillers from the flowering tillers is supported by the above results. Where the opportunity for translocation from the parent tiller to daughters was limited by removal of the parent tiller, or by the

presence of a competing sink (ie. the seedhead), both the total weight and the number of daughter tillers formed were reduced (Table 8.3). Furthermore, shading of plants, which presumably also would have reduced the assimilate supply, also appears to have reduced daughter tiller formation, although less strongly than removal of the parent tiller.

In addition the fact that more tracer was recovered from daughter tillers where the seedhead of the parent tiller had been removed than from daughter tillers where the seedhead of the parent remained intact confirms that reduction in the absolute amount of substrate translocated from parent tillers to daughters was involved in the reduction in daughter tiller formation where seedheads remained intact. It is true that the amount of label transferred to daughter tillers was small in percentage terms (Table 8.4), however, the specific activity of some daughter tiller tissues was high (Table 8.6). Localised zones of daughter tillers were found to have specific activity values up to 193 Bq mg^{-1} 5 days after labelling (Table 8.6).

By comparison the mean specific activity value for leaves actually fed radiocarbon and harvested approximately 3 weeks later was 117 Bq mg^{-1} and judging by relatively low long term respiration losses measured by Danckwerts & Gordon (1987) would not have been appreciably higher than 117 Bq mg^{-1} when the autoradiographed tillers were harvested 5 days after labelling. This would indicate that the high specific activity regions of daughter tillers were themselves strong sinks, and that due to their small size, even the small percentage allocation of substrate of 3.5% (Table 8.4) from the large parent tiller was relatively important to their carbon economy.

The lower specific activity values for NDT of cut parent tillers is not necessarily inconsistent with the hypothesis because absolute recovery of radiocarbon in NDT was higher in daughter tillers of cut than in daughter tillers of uncut parent tillers and because localised regions of the daughter tillers did have high specific activity values (Table 8.6). These observations are consistent with the interpretation that emerging daughter tillers which received an increased carbon supply from their parent tiller utilised this carbon for cell elongation, so attaining photosynthetic self sufficiency more quickly. After this, increased growth rates on these more rapidly establishing tillers could result in a greater dilution of the radiocarbon initially received from the parent tiller. This would explain the lower specific activities for

whole daughter tillers, despite higher absolute quantities of radioactivity in daughter tillers of decapitated flowering tillers.

8.4.4.3 Location of sinks

The observation that several daughter tillers on the plant autoradiographed after 5 days (Plate 8.4) showed bands of high specific activity located at contiguous elongation regions at the base of the 2nd leaf and the tip of the emerging leaf is consistent with preferential uptake of translocated ^{14}C tracer by cells in the leaf expansion zone of NDT. Leaf elongation rate was not measured during the experiment, but as the high specific activity band on the emerging leaf was approximately 100 mm from the tiller base on each tiller, a leaf extension rate of approximately 20 mm per day would have been required for the high specific activity bands to have been located at the cell elongation region over the four day period of tracer application. As leaf extension rates of 20 mm per day are easily attained (Davies *et al.* (1989) reported values ranging from 13.7 to 39.9 mm/day.) it would seem highly likely that a major deposition region for tracer imported by daughter tillers from parent flowering tillers was the cell elongation zone of expanding leaves, and that photosynthate from parent tillers reached this destination within hours of fixation. For *Lolium multiflorum* Clifford *et al.* (1973) found evidence for such translocation directly between vegetative tillers without involving roots, and Bell (1976) has described in detail vascular pathways which would allow such transport of current assimilate by diffusion from parent tiller leaves to daughter tillers. It is probably safe to assume that similar transport occurs in perennial ryegrass, but further research to determine the physiological reasons for sink strength would be worthwhile. As noted in Chapter 7, it is suggested that for tall fescue, tissues of different organs metabolise fructans of different chain lengths, and that this can result in differential growth of particular organs (C. J. Nelson, pers. comm.). It may be that a similar mechanism operates for ryegrass.

Two previous studies (Clemence & Hebblethwaite, 1984; Colvill & Marshall, 1984) have noted that smaller daughter tillers tended to have higher specific activity than larger daughter tillers, and although these authors did not consider nodal positions of tillers, it is likely that their smaller tillers were also younger. Thus the finding (Table 8.7) of lower specific activity values for tillers originating from successively lower nodes on the parent tiller axis

helps explain the earlier results. A possible mechanism is that photosynthate moving down the stem towards the root system is able to be withdrawn by daughter tillers at each node with successively smaller amounts passing on towards tillers at the next node. The fact that the youngest daughter tiller had the highest specific activity, while the next youngest tiller had the highest total activity, suggests that the small size of the younger tiller (the tillers weighed 25 mg and 86 mg respectively) limited the quantity of radiocarbon taken up by the younger tiller.

An incidental observation also of interest is that only some 6 roots show any appreciable radiocarbon uptake (Plate 8.4), whereas the tiller autoradiographed had some 17 apparently live roots attached. A future study to provide information on the uptake of assimilate by roots at differing points of attachment on the tiller axis could well aid understanding of the cycle of root formation and death and on root-tiller interactions (Section 6.3.2; Matthew *et al.*, 1991).

8.4.4.4 Evidence for remobilisation

Davies (1977, 1988) has suggested that remobilisation from dying tissue of cut tillers may provide substrate to promote daughter tiller formation. The extent to which remobilisation of assimilate from dying tissues of parent tillers could occur is demonstrated by the different specific activity values of 117 and 36.3 Bq mg^{-1} for live and dead leaves respectively (Table 8.4). While specific leaf weight measurements (mg mm^{-1}) would also be needed to quantify carbon fluxes fully, the dead leaves at the end of November would have been live when labelled, so the above values can perhaps be taken to estimate the proportion of radiocarbon recovered from live leaves one month after fixation which could be available for remobilisation on the death of those leaves.

There was also evidence of remobilisation from seedheads. Comparison of radiocarbon recovery for seedheads removed immediately after labelling (69.8 kBq) and for seedheads removed a month later (35.7 kBq, Table 8.4) gives some indication of the amount of label lost from seedheads between labelling and harvest. The fact that recovery from parts of the plant other than the seedhead was greater where seedheads remained intact (Table 8.5) suggests that some of this loss occurred through remobilisation and

translocation out of the seedhead rather than as respiration, although whether tracer leaving the seedhead was fixed there or first imported from the leaves is unknown. Hampton *et al.* (1987) did detect export of carbon fixed in stems immediately below the ear to other parts of the plant, including basal daughter tillers, however.

It is evident from earlier studies that the carbon economy of flowering perennial ryegrass tillers is complex and not yet well understood (Hampton *et al.*, 1987). In view of the likely remobilisation and re-export of carbon from various organs of parent tillers at different times it would be of interest to obtain more detailed information on the location of sinks at different stages in seedhead formation, and on the extent to which deposition in particular sinks is reversible at later times. Another area requiring investigation is the extent of remobilisation of carbon from senescing tissue, and whether carbon retained by mature leaves is more available on leaf death than carbon deposited in expanding cells at leaf elongation and stem elongation.

8.4.4.5 Comparison between tillering responses in Experiment 5 ('Grasslands Ruanui' ryegrass) and In Experiment 6 ('Ellett' ryegrass)

Given that Experiment 3 had indicated possible genetic differences in tiller dynamics between Ruanui and Ellett ryegrass, comparison was also made of results from Experiments 5 & 6.

Ratios of tiller weights and tiller numbers for categories NDT and EDST were derived from data in Tables 8.2 and 8.3, and are presented in Table 8.8. While tillering responses from both Experiments 5 & 6 were consistent with the hypothesis that substrate from parent flowering tillers is important for daughter tiller formation (Sections 8.3.4, 8.4.4.2), the data in Table 8.8 suggest that different categories of tiller were more strongly involved in the responses in respective experiments. In Experiment 5 responses were more strongly expressed by the formation of EDST and in Experiment 6 by the formation of NDT. While these differences cannot be interpreted with certainty because of the lack of appropriate experimental controls, the above differences would be expected if, at tiller initiation, tiller buds in 'Ellett' ryegrass (Experiment 6) were able to develop their vascular connection to their parent tiller (Bell, 1976) more quickly than tiller buds in 'Grasslands Ruanui' ryegrass (Experiment 5).

Table 8.8: Ratios of NDT:EDST for tiller weight and tiller number in Experiments 5 & 6.

	Experiment 5				Experiment 6		
Treatment	LOxE	LOxL	HIxE	HIxL	Ground level.	Flag leaf node.	Uncut.
Tiller weight	0.87	0.62	0.53	0.66	1.67	2.09	0.87
Tiller Number	0.51	0.28	0.35	0.30	0.95	0.75	0.97

In the case of a vascular connection occurring more quickly, relatively less assimilate would be available for tillers developing lower on the parent tiller axis. This view that tiller dynamics of a reproductive sward can be understood in terms of export of assimilate from flowering tillers, with progressive withdrawal of assimilate at successive nodes down the axis, supports that obtained from consideration of distribution of radiocarbon in tillers at successive nodal positions (Table 8.7 & Section 8.4.4.3). However, because the results in Table 8.7 are based on a single tiller and those in Table 8.8 are for two separate Experiments with no common treatments, further research is needed for confirmation of these findings.

8.5 Implications for farm practice

The results from Experiments 4, 5, & 6 reported in this chapter all support the hypothesis (Sections 7.3, 8.1; Matthew *et al.,* 1989) that assimilate exported from flowering tillers is an important factor in determining tiller initiation in late-spring swards. Given that tillers formed from flowering tillers were a large proportion of the total tiller population by mid December (Section 5.3.4.6.1) and that these tillers had a greater propensity to flower and produce new tillers in the following year (Section 5.3.4.6.2), these results do suggest that deliberate encouragement of reproductive growth in ryegrass swards might be a way to improve summer-autumn pasture productivity, providing that seedheads were not allowed to develop to the stage where daughter tillers were suppressed, as in Treatment 4 of Experiment 4 (Section 8.2.3). This contrasts with popular views held by many in the industry and expressed by, for example, Hughes (1983) who has recommended a "bottoming" or grazing as close to the ground as possible in October to remove seedheads and promote vegetative growth.

The question of the application of these results to farm practice is further discussed in Chapter 9.

8.6 Summary

1. In a field sward, there was evidence that individual flowering tillers formed more daughter tillers when cut to 50 mm height near anthesis. Earlier, later, or lower cutting appeared to have reduced numbers of daughter tillers formed between mid November and mid January.

2. Translocation of substrate from parent flowering to young daughter tillers was verified by radioactive tracer. Translocation to daughter tillers was approximately 3% of tracer recovered, and was greater where the parent tiller seedhead had been removed than where the parent tiller seedhead remained intact.

3. Radiocarbon translocated to daughter tillers was largely localised in leaf regions which had probably been zones of cell elongation at the time of tracer application. Data suggested that radiocarbon from parent tillers reached cell elongation zones of daughter tillers within hours of assimilation and was deposited there in concentrations almost equal to those remaining in labelled leaves of parent tillers.

4. These findings confirm that a period of reproductive growth in spring swards could be expected to result in increased tiller formation, and presumably also root formation, as observed in Experiments 1 & 2. This would be contrary to popular belief, and suggests that current recommendations for late spring-early summer pasture grazing management in New Zealand should be revised.

CHAPTER 9: OVERVIEW AND CONCLUSIONS.

9.1 Synthesis of results

9.1.1 Root growth of ryegrass swards

The underlying aim at the commencement of this study (Objective iii, Section 1.2) was to identify possible ways to increase production from field swards of perennial ryegrass, through manipulation by grazing management. Initially it was felt that the most likely way to achieve this objective would be through greater understanding of the dynamics of the root system, and of the inter-relationship between root and shoot systems. Information on tissue turnover of tillers and leaves has greatly increased understanding of sward dynamics (Section 2.2.1), yet parallel data has not been available for roots, especially not for field conditions, because of difficulties in accessing roots for non-destructive measurement. Further, because collecting data on any one facet of sward dynamics (tiller dynamics, tissue turnover or root behaviour) tends to be highly labour-intensive, most studies have been confined to one of these areas, and there have been few previous attempts to integrate root and shoot data from field swards.

The initial experiment (Experiment 1) and subsequent calibration studies (Appendix 2.6) established that a variation of the mesh bag, or refilled core technique recently adapted from forestry research by Steen (1983, 1984) for study of root growth in grass swards in the field was able to provide data on seasonal production of new roots in terms of mass flow, and was also sensitive enough to detect differences in root growth arising from grazing management manipulation. Experiment 1 also established that root turnover in ryegrass swards in early summer was much higher than expected on the basis of previous studies (Jacques 1956, Caradus & Evans, 1977), and was enhanced where seedheads were allowed to develop to head emergence or anthesis, as compared to where seedheads were removed earlier or allowed to mature.

The second field study (Experiment 2) was then set up to provide data on seasonal patterns of root formation and on differences in root production under contrasting lax and hard grazing managements. Results from Experiment 2 confirm the high turnover in root mass in summer (Section

4.4.1) as observed in Experiment 1, and provide a picture of root behaviour which suggests continual replacement of roots throughout the year with dry matter accumulation below ground typically being about 10 to 20% of herbage accumulation above ground (Sections 4.3.1.2) but with seasonal increases in root production after summer drought or after winter preceding corresponding increases in above ground production (Section 7.2.2).

The fact that apparent root production was as little as 10 to 20% of net herbage production while root mass was approximately similar to or even greater than shoot mass indicates a difference in turnover time for root and shoot tissue. The estimate of a 650 day root turnover time (Section 4.4.1) would have been inflated because refilled core data underestimated actual root production (Appendix 2.6) and because intact core samples would have included dying roots, but even if actual root turnover were only third this value, the turnover time is still much longer than for leaf tissue. Leaf turnover time can be estimated by multiplying the reciprocal of values for leaf appearance rate (leaves tiller $^{-1}$ week^{-1}) by the number of leaves per tiller. Based on data of Davies (1977), leaf turnover time ranges from 25 days in May, to 90 days in January, in Britain. For data in Figure 6.1, the corresponding range of turnover times is 34 days in December to 63 days in August.

This indicates one way in which root and shoot systems are adapted to their respective environments (Section 7.2.2). The faster turnover time for leaves would allow for rapid recovery after grazing. Root systems are not normally subjected to sudden losses of tissue, but a slower rate of turnover allows for the zone of exploitation within the soil profile to move, depending on seasonal changes in soil moisture level at different depths in the soil profile (Section 4.4.1). For example, in Experiment 2 root mass at depth increased during dry conditions in summer, but the reverse occurred in winter as the water table rose and soil conditions became anaerobic at depth (Sections 4.3.1.1, 4.4.1). Another difference between shoot and root system behaviour which also allows a relatively fast response by shoots after loss of tissue by grazing is that a high proportion of root primordia, but a lower proportion of tiller primordia normally develop (Section 6.4.3). This means that when tiller size is reduced by increased grazing pressure, it is possible for numbers of new tillers to develop very rapidly, resulting in a new equilibrium size/density combination (Sections 5.3.4.1, 5.3.4.6.2).

Increased grazing pressure (more frequent and more severe defoliation) reduced root production (Section 4.4.2), as expected from earlier research (Evans, 1971a, 1976). However, the magnitude of the grazing management effect on established swards was much smaller than expected from the earlier work on seedling plants. Root dry matter accumulation on the LL treatment averaged over time was only 15% greater than on the HH treatment (Table 4.4). This relatively small reduction in root growth for the HH treatment in Experiment 2 (and also for the VEGH treatment in Experiment 1), can be attributed partly to the fact that the grazing regime for HH plots imposed rather less severe defoliation than, for example, the experimental cutting treatments applied by Evans (1971a). Another factor contributing to the smaller than expected differences in root growth between LL and HH plots would be the morphological adaptation to cutting height, which was evidenced by similar before-grazing leaf mass (Table 5.6). Similar morphological adaptaion of swards has previously been reported by Jackson (1976), who found that swards cut repeatedly at 60, 90 or 120 mm height all developed a similar leaf mass of approximately 1500 kg DM ha^{-1}.

It has also been suggested that reduction of root growth at depth under increased grazing pressure may reduce ability of swards to take up water and nutrients (Evans, 1976). In this study there was some evidence of a transient difference between rooting patterns of LL plots and of HH plots, during summer moisture stress (Section 4.4.4), but no evidence that increased grazing pressure, as in the HH grazing management, reduced root growth at depth disproportionately.

Since the dominant feature of root system behaviour was the seasonal pattern of change, which reflects influences outside a farmer's control, and responses of the root system to grazing management for established swards were much smaller than expected, it was concluded that manipulation of root growth *per se.* would not offer an effective way to achieve pasture production efficiencies.

9.1.2 Above-ground growth of ryegrass swards

9.1.2.1 Tiller dynamics

When multivariate analysis maximised discrimination between treatments, tiller population density responses to the contrasting LL and HH grazing

managements in Experiment 2 were found to be the outstanding statistical feature of the results from that experiment (Table 5.9). Such responses are well known from numerous previous studies and can be explained in terms of the so-called -3/2 power rule (Sections 2.2.3, 5.3.4.1), which for grass swards appears to operate so as to optimise leaf area under differing grazing regimes (Section 5.3.4.6.3). However, in this study tiller size/density plots (on a log/log scale) frequently had a slope more negative than -3/2 (Figure 5.6). This steep slope could be partly due to there being a higher percentage of leaf in herbage on HH plots than in herbage on LL plots, but probably also reflects some limiting factor preventing full expression of the environmental potential for tillering on the harder-grazed plots to the lower right of the diagrams. One such limiting factor may well have been reduced substrate supply on HH plots, and to the extent that this was so the increased negative slope of the tiller size/density line (Figure 5.6) is an above-ground parallel to the effect of the HH grazing management in reducing apparent root growth rates.

One of the reasons for choosing the contrasting LL and HH grazing treatments was to create deliberately, swards with different tiller population densities, and hence different mean tiller size, to test the possibility that tiller populations with different size/density relationships might have inherently different rates of root production (Section 4.2.1). However, there was no evidence of any such effect, and in hindsight it might have been realised that tiller size/density responses are shifts of equilibria in response to external factors such as change in grazing pressure or light levels. Such responses do not necessarily shift tissue deposition from un-harvestable organs such as roots to harvestable organs such as leaves (Section 7.2.4), and even where manipulation of one sward characteristic is possible, compensatory changes often occur in other sward characteristics. For example differences in leaf mass and leaf area index between LL and HH plots had largely disappeared within 6 - 8 months of the start of the experiment, as a result of increases in the proportion of stem and dead components in the sward (Table 5.6).

Another facet of tiller dynamics is the way in which patterns of tiller natality, tiller mortality, and propensity to produce daughter tillers for tillers of different ages define the tiller demography of perennation in grass swards. This aspect of tiller dynamics appears to be independent of the -3/2 power effect, and would likely be under genetic rather than environmental control (Section

5.3.4.6), although it is clearly possible through grazing management to influence the proportions of tillers flowering (Table 5.10). This presumably in turn affects the tiller age structure of the post-flowering sward (Figure 5.8).

The present study was not designed to answer questions of tiller demography, because the significance of such effects was not appreciated at the outset. It was possible to recover some preliminary information of a demographic nature and this information showed that older flowering tillers are important for perennation because they produce disproportionately large numbers of summer daughter tillers (Tables 5.14, 5.15). An examination of earlier literature shows that this point has been largely overlooked by most authors writing on tiller dynamics, but has been previously noted by Silsbury (1964), Langer (1979), Jewiss (1981), & Colvill & Marshall, (1984) who were not able to present supporting data, because even laborious fixed quadrat observations do not provide such data. The provision of this type of data will require destructive harvesting of fixed quadrats, and this will present formidable logistical problems.

However, if available, information on tiller demography would be extremely useful, because it can be inferred from tiller age-cohort survival diagrams (Figures 5.8, 6.2) and from more detailed information on the origin of new tillers (Table 8.8), that 'Ellett' and 'Grasslands Ruanui' cultivars of ryegrass have different demographic patterns of perennation (Section 6.3.1), and may therefore respond to different managements appropriate to their respective demography. For 'Ellett' ryegrass there was enhanced daughter tiller formation on the LH treatment (Section 5.3.3.2) in Experiment 2, which was associated with high productivity on these plots, both on a per unit area basis (Section 5.3.3.2) and on a per tiller basis (Table 5.12).

This tillering response on LH plots in Experiment 2 appeared to involve a number of factors, including an availability of substrate for tiller formation because of high photosynthesic rates in parent flowering tillers (Table 3.6), environmental potential for high tiller formation due to the -3/2 equilibrium moving to the right in summer (Figure 5.6b), an increased percentage of flowering tillers under laxer grazing management (Table 5.10), and demographic pattern which resulted in naturally high levels of daughter tiller formation from flowering tillers (Tables 5.14, 5.15).

From these four factors apparently involved in the tillering event on LH plots, the first was chosen as a subject for detailed follow up study. Experiments 4, 5, & 6 confirm that export of assimilate from reproductive tillers is a factor in determining tiller initiation and development from flowering tillers. Moreover, the 2 - 5 fold increases in tiller numbers formed from tagged tillers in Experiments 5 & 6 where opportunity for daughter tillers to receive such assimilate was optimised, indicate that this effect is large enough to explain, at the sward level, two observations associated with increased reproductive growth in Experiments 1 & 2. These effects were the increase in root formation under the RUHE and RUAN treatments and the associated increase in tiller density (Experiment 1, Section 3.4.3), and the high herbage accumulation associated with high levels of daughter tiller formation on LH plots (Section 5.3.3.2). The high tiller appearance in the second season of Experiment 3 (Figure 6.2) might also be such an effect. This information on the way in which timing or height of decapitation of reproductive tillers affects daughter tiller formation from the flowering tillers should therefore be useful in formulating grazing management strategies to optimise herbage production in summer.

9.1.2.2 Herbage accumulation

In Experiment 1 only limited herbage accumulation data was collected. However, the data do show a substantial difference in herbage accumulation, and in the balance between grass leaf, grass stem and clover production, depending on the timing of removal of reproductive growth. During a 30 day period commencing mid November, herbage accumulation was more than doubled on plots where seedheads remained intact, as compared to mown plots (Matthew, *et al.*, 1986; Table 3.6).

Herbage accumulation and tissue turnover measurements for Experiment 2 did not commence until part way through the experiment due to the heavy workload of root and tiller measurements, and when they did commence formed part of a study reported in more detail elsewhere (Xia, 1991). The principal finding to emerge from these measurements was that highest net herbage accumulation occurred in plots switched from the lax grazing regime in spring to the hard grazing regime in early summer (LH plots). As noted above, the data indicate that this high herbage accumulation on the LH plots was associated with high rates of daughter tiller production.

Exploitation of this response does appear to offer a means for meeting the objective (Section 1.2) of varying grazing management in order to obtain pasture production advantages on a farm scale. The seasonal timing of this effect is such that implementation by farmers of such grazing management plans should be feasible. This is because farmers tend to face a surplus of pasture growth over animal requirements in spring, and therefore have some degree of flexibility in grazing management during reproductive growth. (By contrast, winter grazing management is constrained by the need to ration feed at a time when animal demand exceeds pasture growth.) The implications for farming practice in New Zealand are briefly discussed below (Section 9.3).

9.1.3 Root-shoot relations

Apart from limited multivariate analysis of root and shoot data (Section 7.2.2) and a simple description of the seasonal patterns of change in root and shoot parameters using PCA analysis (Section 7.2.3), more detailed analysis of root/shoot relations was largely precluded by the rather coarse time frame of some of the experimental measurements in Experiment 2. For example, the complete harvest sequence of grazing, herbage mass sampling, extracting refilled cores and setting up new cores, intact core sampling, and tiller tagging occupied several man-weeks. It was therefore impossible to synchronise fully all the measurements, and even if that had been possible, reductions of root growth following grazing probably lasted only a few days (Section 4.4.2), and hence would not have been evident in refilled core data collected on a six-weekly basis. Experiment 3 represented an attempt to synchronise the various root and shoot measurements more closely. However, in hindsight there was an experimental design error in locating the experiment on a sward with substantial content of species other than ryegrass. This meant that measured root growth could not be unambiguously attributed to the ryegrass component of the sward on which the tiller and tissue turnover measurements were made.

Because of lack of time for collection of full herbage accumulation data, a computer model was used to provide estimates of herbage accumulation to compare with apparent root growth data from Experiment 2 (Figure 7.1). This comparison is approximate in the sense that the herbage accumulation values estimate net production, whereas the root growth rates approximate gross production estimates. However despite this, the comparison suggests

values for root/shoot partitioning similar to those obtained by direct measurement of ^{14}C distribution in labelled plants (Parsons & Robson, 1981b), but rather different from those obtained by Deinum (1985) who based his calculations on change over time of total root mass.

Storage of dry matter in the root system and subsequent utilization is a feature of the seasonal behaviour of some plants, for example *Lotus pedunculatus* (Sheath, 1981). One early study has claimed evidence for such behaviour in ryegrass (Roberts & Hunt, 1936) but there was no indication from root mass data in the present study of this type of effect (Table 4.1). Similarly, seasonal variation in carbohydrate levels in ryegrass roots do not indicate storage and later re-utilisation either (Thom *et al.*, 1989).

However, there was evidence that ryegrass may be able to maintain relatively high herbage accumulation rates in early winter through reduction in allocation to roots at this time (Figure 7.1). There was also evidence that in late winter substantial quantities of true stem or stolon are formed underground. Where tiller burial during winter dictates stolon formation there must certainly be a corresponding loss in potential herbage production (Section 5.3.2).

Also, the prevalence of stolons among root samples led to the idea of examining new root and tiller production in terms of the segmental morphology of the grass plant. The transplanted core technique developed for this purpose was time consuming and so only limited numbers of samples were processed (Section 6.3.2). However, the data do show that it is possible to define the number of potential sites for root production and the life expectancy of an individual root as a function of the turnover of phytomers on the tiller axis, as indicated by leaf appearance interval. In future, integrated models of root/shoot dynamics might well be based on this principle.

9.2 Further work.

In the above discussion, and also in the preceding chapters, a number of questions have been raised which could not be followed up during the study, but which would merit further investigation.

In Experiment 2, there was evidence that soil moisture withdrawal profiles in summer differed between HH- and LL-grazed plots, and that root formation was greatest at depths where moisture was most abundant (Section 4.4.4). If this was confirmed it might be possible to some extent to manipulate rooting depth to anticipate seasonal changes in soil moisture status. Again in Experiment 2, some observations of tiller population change over time (such as the apparent oscillation around an equilibrium population density after disturbance of swards at harvesting) or in response to grazing, raise questions on how signals for release of axillary buds on tiller axes are generated and on precisely how the -3/2 power rule applies to tiller populations. More detailed study of tiller size/density equilibria in grass swards could be rewarding.

In Experiment 3, data suggested the operation of some mechanism for co-ordination between organs, and for determining priority growth of particular organs at particular times (Section 7.2.3.2). More information on this aspect of sward behaviour may help in identifying "time windows" when particular grazing managements might be beneficial or detrimental. There were also a number of results which did not match those from Experiment 2. PCA analysis (Section 7.2.3) suggested a different seasonal pattern of root and tiller formation for the two experiments, and the tiller age-cohort survival diagram for Experiment 3 (Figure 6.2) indicated a greater survival of pre-flowering tillers than in Experiment 2 (Figure 5.8). Plugs of tillers taken from the respective swards appeared to have different sites of new tiller formation in a common glasshouse environment (Section Table 8.8). These effects are assumed to be at least partly due to genetic differences between the two cultivars used ('Ellett' in Experiment 2, 'Grasslands Ruanui' in Experiment 3). Further investigation to confirm that different ryegrass cultivars do in fact exhibit different demographic patterns, to quantify these patterns (Section 9.1.2.1, above), and to model the effect of variation in demographic pattern on tiller population stability over time would be valuable.

Finally, the observation that there are seasonal changes in the mean diameter of ryegrass roots, and that such changes involve both differences in the degree of branching, and differences in diameter at the origin on the tiller axis (Section 6.3.2) invites the questions of why these seasonal changes occur, and of whether or not the diameter differences are associated with any seasonal differences in root uptake characteristics. This aspect of ryegrass root dynamics would also be worth further study.

9.3 Implications for farm practice in New Zealand

This study has identified a strategy for manipulating swards so as to optimise herbage production. In Experiment 2 the LH treatment produced some 0.5 t DM ha^{-1} more herbage than other treatments during December - April (Section 5.3.3.2). Subsequently, a paddock-scale experiment designed to investigate this response further has been conducted at Massey University No. 4 Dairy Unit. In this Experiment, a gradation in the degree of reproductive development in spring swards was achieved by mowing to 40 mm height in late October (early control), mid November (late control), or early December (very late control). Herbage accumulation for the period 9 October to 18 December was 4400, 5000, and 5700 kg DM ha^{-1} for early, late and very late control treatments, respectively. Herbage accumulation from 18 December to 11 April was 3300, 4900, and 4500 kg DM ha^{-1} for the same treatments (P.N.P. Matthews, pers. comm.). The increased herbage accumulation on late and very late control plots, compared to early control plots, was accompanied by statistically significant increases in tiller population density (C. Matthew, unpublished data). These results therefore appear to confirm that tillering responses investigated at the individual tiller level in Experiments 5 & 6 (Chapter 8) can lead to pasture production responses in field swards.

However, within the constraints of a farm system, it is another thing to exploit such an increase in pasture production, so as to increase animal production. The following discussion is specific to the New Zealand situation. In a different context different constraints might apply. For example, in New Zealand, grazing management hinges around strategies to overcome the deficit between pasture growth and animal demand in the winter months of June and July; whereas in Britain the key element in seasonal grazing management is the summer conservation strategy in order to provide winter feed for animals housed indoors.

In New Zealand, current industry practice favours removal of seedheads as early as possible and as low to the ground as possible. For example, Hughes (1983) recommends mowing pastures to 25 mm height in October, and claims that such management does not reduce pasture growth. Such views probably arise from a concern to avoid the decline in herbage quality (Browse *et al.*, 1981) associated with reproductive growth and from a belief

that developing ryegrass seedheads exert apical dominance and prevent daughter tiller formation. Also, it is considered beneficial to reduce pasture growth in order to reduce accumulation of ungrazed pasture at a time when pasture growth exceeds animal demand (Sheath *et al.* 1987). Because of these factors a recommendation to farmers that seedhead development in November be encouraged is likely to meet some resistance.

There is in fact little scientific evidence for suppression of tillering in perennial ryegrass due to apical dominance, as much of the research which has been carried out has been for Italian ryegrasses (see e.g. Clifford, 1977). In Experiments 4, 5, & 6 it was found that tillering from flowering culms continues freely through the stem elongation phase, and this is supported by information from seed production studies, where the daughter tiller development at this time is considered detrimental to seed yield (Hampton *et al.*, 1987). However, although suppression of tillering does not appear to be a limitation in reproductive swards, there are situations where increased herbage accumulation and reduced herbage quality associated with reproductive growth limit animal intake, if not pasture production. Indeed, on more extensive hill-country sheep/beef properties, pasture growth rates often swing from less than 10 kg DM ha^{-1} day^{-1} in winter to more than 50 kg DM ha^{-1} day^{-1} in late spring, a ratio of winter:spring growth of around 1:5 to 1:10. For example, pasture growth rate averaged over 10 years for hill country near Palmerston North was 8 kg DM ha^{-1} day^{-1} for July and 47 kg DM ha^{-1} day^{-1} for November (J. Mc Crone, unpublished data). Stocking rate tends to be limited by the low winter growth, so that the need to prevent accumulation of ungrazed herbage in late spring is an overriding consideration. On a property of this type, Clark *et al.* (1982) demonstrated that rotational grazing management which reduced herbage intake by animals in late spring (compared with continuous grazing management) and increased accumulation of ungrazed herbage, resulted in lower animal live weight through summer/autumn, presumably due to declining digestibility of the ungrazed herbage.

By contrast, on lowland dairy farm properties the ratio between winter and spring peak pasture growth is much narrower, the ten year average growth rates for lowland pasture near Palmerston North (mean of 5 sites) being 16 and 48 kg DM ha^{-1} day^{-1} for July and November, respectively (J Mc Crone, unpublished data). With the ratio of winter:spring pasture growth rate typically about 1:3 or 1:4, utilisation of additional herbage produced by

encouraging reproductive growth may be feasible. It is therefore possible that extra herbage grown through encouragement of reproductive growth could be converted to milk production on dairy farms, providing that management was such as to remove seedheads rapidly in late November or early December, before any marked decline in herbage quality, and before death of newly formed daughter tillers due to shading. Management which increased tiller replacement in summer would likely also have implications for longer term pasture persistence, because survivors of tillers formed at this time are very active in producing daughter tillers when they themselves flower in the following season (Table 5.14).

Thus it should be feasible, in New Zealand dairy farm systems, to develop a grazing management strategy which will potentially increase spring/summer pasture production, relative to current industry practice. The increased production involves enhanced daughter tiller appearance from stubs of flowering tillers, and appears to be based on increased supply of assimilate for daughter tiller initiation and leaf elongation when seedheads are not removed before head emergence, but allowed to continue development until about anthesis. It is suggested that the next stage in this research should be to investigate how best to implement this "late control" grazing management strategy within the context of a farming system. It needs to be confirmed that the higher summer pasture production can be obtained in a systems context (as distinct from controlled experiments), and also converted to animal product.

APPENDIX 1: NOTES ON STATISTICAL ANALYSES.

A1.1 Measurements repeated over time

In Experiments 1, 2 and 3 a number of measurements were repeated over time on the same plots. In such cases the data from successive observations are not strictly independent.

Many authors (e.g. Barry, 1976; Matthew *et al.*, 1991) have analysed such data as if it came from a split-plot experiment with time as the split-plot effect. Another approach (Steel & Torrie, 1981) is to treat time as a split-block effect, and the analysis is similar to that for the split-plot analysis, except that the sum of squares for the replicate x time interaction is used as an error term to test time effects, while the treatment x time interaction is tested against the treatment x time x replicate sum of squares. However, Steel & Torrie (1981), and a number of other authors, including Cole & Grizzle (1966) and Rowell & Walters (1976), note that both these analyses may be invalid if there is heterogeneity or correlation of error variance across sampling times, and that in such cases multivariate analysis is required.

On the other hand, multivariate analysis raises problems also. For example, in Experiment 2 results are for 8 plots (2 replicates of two treatments) over 9 harvest dates (Section 4.3), and in this case there are more times (multivariate variables) than there are plots (degrees of freedom), so that multivariate analysis is impossible. A similar problem applies to results for Experiment 3 (Section 6.3), where 6 observations were made on 3 plots. Also, while multivariate analyses correct for heterogeneity or unequal correlation of error variance across sampling times (Cole & Grizzle, 1966; LaTour & Miniard, 1983), there are some types of error which can not be corrected by any analysis, univariate or multivariate. For example, in this study there was evidence that there was a tendency for the 21 mm corer used in the later part of Experiment 2 to push ryegrass stolons aside, rather than cut them, when soil was moist (even though the quantity of root collected by two different diameter corers agreed well, (Table A2.1), and so the lower stolon lengths for Harvest 12 (Section 5.3.2) may be an artifact of the change in measurement technique, rather than a seasonal effect, as implied by the analysis of treatment effects over time.

For these reasons, the analyses of repeated measurements in this thesis were carried out using the univariate split-plot in time approach, but with attention to the detection of conditions which might invalidate the conclusions drawn. In this respect the split-plot in time analysis does not present as many difficulties as some advocates of multivariate procedures have led their readers to believe.

Analyses were normally carried out using the "repeated measures" option of the SAS general linear models procedure. This SAS procedure in fact calculates univariate sums of squares as for a split-plot in time model, but also gives results of certain multivariate tests of hypotheses where there are sufficient error degrees of freedom. There are three types of effect tested in the split-plot in time analysis; (i) tests of treatment effects averaged over times, (ii) tests of differences between times, and (iii) tests for treatment x time interactions.

Taking these 3 cases in turn, tests of treatment effects averaged over times using error (a) of the split-plot in time analysis **are** valid (Cole & Grizzle, 1966; Rowell and Walters, 1976) and such tests are used to determine the statistical significance of grazing management effects in Experiments 1 & 2 (Sections 3.3, 4.3, & 5.3).

The second and third cases, testing of significance of differences between times or of treatment x time interactions, require closest attention here. In these cases it is necessary to ascertain that the statistical significance has not been inflated by heterogeneity of error variance across times. This can happen if a large "within harvest" standard error at a particular time is artficially reduced by averaging over other harvests with smaller "within harvest" standard errors. Similarly, the error (b) mean square in a split plot analysis is a function of the correlation across time (Gill, 1986) and there can be optimistic estimates of significance where the correlation over times of data from the same plots is on average quite high, but for two particular harvests being compared is rather lower.

It follows from the above that unequal correlation across times is not an issue when there are only two times in the analysis, and in this special case the use of the conventional split-plot in time model is valid (LaTour & Miniard, 1983). This applies to the split-plot analyses for sand filled cores harvested on Days 56 & 80 in Experiment 1 (Section 3.3.1.3).

In Experiments 2 & 3, where there are respectively 9 and 6 times in the analysis, tests of differences between times and of treatment x time interactions were handled in two steps. Firstly, an initial analysis was performed for the entire data set, (and tests of grazing main-effects carried out where applicable). Secondly, where time effects or interaction effects of interest were identified, the statistical significance of these was confirmed by re-analysing data but including in the analysis just two harvest dates of interest. This procedure is logically equivalent to, but computationally simpler than the method recommended by Rowell & Walters (1976), of extracting orthogonal comparisons of two means of interest from the analysis of variance of the entire data set.

Finally, in order to provide a mathematical description of seasonal variation for measured variables, principal component analysis was used and is described further below (Section A1.4).

A1.2 Measurements repeated over successive depths of the same soil cores

Analysis of samples from different depths of soil cores on the same plots (or the analysis of data from sand filled and silt filled cores as if the core type had been a split-plot effect) raises problems closely parallel to those of split-plot in time analyses. One approach has been to use a multivariate analysis based on coefficients of polynomial regressions of variation with depth of treatment effects (Roberts & Raison, 1983). However, these authors had divided soil cores into 10 segments, and with only 2 depths considered in Section 3.3.1.3, the split-plot analysis is not invalid. Alternatively, where 3 depth categories are analysed in the present study, it has been more convenient to compute statistics which can be validly analysed, for example the ratio root mass for 0 - 70 mm depth:root mass for 250 - 600 mm depth (Section 4.3.1.1).

A1.3 Multiple discriminant analysis

Multiple discriminant analysis (MDA) is a form of multivariate analysis, and as such, allows several variables to be analysed simultaneously for common treatment effects. MDA was used in this study to gain an overview of the effects of the 4 grazing managements on components of herbage

accumulation in the respective swards (Section 5.3.3); and again in Chapter 7 to examine aspects of the relationship between measured root (Sections 4.3.1, 4.3.2, and 4.4) and shoot (Section 5.4.1) parameters.

A number of introductory texts on multivariate analysis are now available, among these Cooley & Lohnes (1971), Chatfield & Collins (1980), and Manly (1986). Manly (1986) considers that where a number of variables are to be analysed in concert multivariate analysis is more appropriate than univariate analysis because it not only takes proper account of the correlations between the data, but also reduces the risk of type I statistical error where the treatment differences for one variable are declared significant by pure chance.

Analyses were performed using the MANOVA option of the SAS general linear models procedure. This procedure uses the correlations between variables in a data set to compute a number of 'composite' variables or multiple discriminant functions (called "canonical variables" in the SAS programme output). Often one or two of these discriminant functions account for much of the variation in a larger data set. The discriminant functions have the form:

$$DS_{in} = a_1 X_{1n} + a_2 X_{2n} + ... a_p X_{pn}$$

Where:

X_n denotes the standardised value of the n th observation from a data set of N observations, for each of the 1 .. p variables in the analysis. (The use of standardised data removes the effects of scale when analysing a number of different variables together, and prevents a numerically large variable (e.g. tiller density) from swamping a numerically smaller one (e.g. herbage accumulation rate).

a_1 .. a_p are constants, called canonical co-efficients by SAS. For each discriminant function, the analysis derives a unique constant for each of the P variables included in the analysis. The number of discriminant functions (i) determined in the analysis is i = T - 1, where T is the number of experimental treatments.

DS_{in} is the "score" obtained by evaluating the i th discriminant function for the n th group of P observations.

The correlations between the n discriminant scores in the i th set and the original variables is often termed the canonical structure and can be used to interpret the canonical variate. One property of these correlation co-efficients is that when squared and summed accross the i discriminant functions, they sum to unity for each of the P variables in the analysis (Chatfield & Collins, 1980). This means that if the above analysis is applied where there are only two treatments, only one discriminant function is determined and all the correlation co-efficients are positive or negative 1. More than two experimental treatments are therefore required for a meaningful MDA.

Under the above procedure the matrix algebra equations which provide the canonical co-efficients are constrained so that scores for the first discriminant function possess the maximum possible difference between experimental treatments, and the N scores for each discriminant function have a mean of zero and unit variance. Also, each of the i successive sets of N discriminant scores is uncorrelated with, and accounts for a smaller proportion of the multivariate dispersion than the previous set. Multivariate analysis therefore reduces a set of inter-correlated observations to a number of unrelated features or dimensions. For each of these dimensions, a measure of the relative size or importance within the overall data set is given by the percentage of the multivariate dispersion explained.

As mentioned above, this statistical technique was used to examine patterns of herbage accumulation (Section 5.3.3.2), and the relationship between above- and below-ground variables (Section 7.2.3.1). To display results, tables of correlations (canonical structure) between original variables and the first two discriminant scores are presented, and treatment means for the first two canonical variates are evaluated and plotted graphically on opposing axes, a procedure described by Chatfield and Collins (1980).

A1.4 Principal component analysis (PCA)

Superficially, PCA is similar to MDA, and the mathematical basis of PCA is discussed by many authors, including Cooley & Lohnes (1971), Manly (1986) and Jolliffe (1986). Like MDA, PCA also generates new variables (principal component scores) which are a linear function of the original variables, and which have a mean of zero. Each set of N principal component scores is referred to as a principal component (PC). As in MDA, successive PC's are

uncorrelated with each other, and so express different features or dimensions within the data. The number of PC's determined is the same as the number of variables, and this is different from the T - 1 discriminant functions in a MDA (See section A1.3, above).

The essential difference between MDA and PCA is that PCA does not take account of any experimental design structure. Instead, PCA maximises the proportion of the overall variance in the entire data set of P variables which is expressed by each successive PC.

For example, where P standardised variables are entered into a data set the total variance of all the variables is by definition 1 x P, or P. On performing PCA, the variance of the first PC is the maximum possible for a linear combination of the original variables, and is usually much greater than 1. Successive PC's have successively smaller variances, the last one or more PC's often having a variance near zero. PC's which have a low variance can be discarded where the objective of the analysis is to reduce the number of variables in the data set without major loss of information, and the first few PC's which explain most of the variation can then be interpreted in terms of correlations or contrasts between the P variables of the original data set.

The fact that PCA does not specifically analyse for effects of experimental treatments needs to be taken into account when interpreting PC's. For example, in the present study, if grazing management and season effects had both contributed to variation in particular variables for particular observations in the data set, then these grazing management and season effects would be confounded in a single PC. One method of interpretation of PC's in such cases is to perform analysis of variance on the PC scores for particular PC's (Jolliffe, 1986). Comparison of the treatment means and F-statistic values for the various experimental design effects then gives information on the extent to which variation in the scores for a particular PC is due to a particular experimental design effect such as grazing management.

The particular interest in PCA in the present study is that data from Experiments 2 & 3 can be reduced to a similar format for comparison of the seasonal pattern of behaviour of the two swards studied in the respective experiments (Figure 7.4), despite the radically different design structures of those two experiments (Section 7.2.3.2). It is emphasised, however, that while the PCA's presented in Chapter 7 do indicate differences in behaviour of

swards of two different ryegrass cultivars in two different years at two adjacent sites; it is not possible to separate statistically the effects of cultivar, site, year, or of interactions between those factors. For this a further experiment, appropriately designed, would be required.

Another issue is the use in a PCA of data collected from repeated harvests of the same plots. This is discussed in Section 7.2.3.2.

APPENDIX 2: REFINEMENT OF ROOT SAMPLING TECHNIQUES.

Although the preliminary experiment succeeded in identifying an effective root sampling strategy, a number of problems of root measurement identified in Sections 3.3 and 3.4 were addressed as the experimental programme continued. One concern was to reduce the sampling time of approximately 2 hours per core (Section 3.4.1), and the rather high coefficients of variation of 30% - 50% in Experiment 1. A second concern was to determine the effect of refilled cores on root growth within the core, and to check for other potential errors such as possible loss of root dry weight through respiration, when samples could not be washed immediately or leaching of solids from roots during storage in alcohol prior to root length determination.

A2.1 Root sampling time

Because time taken for root washing was roughly proportional to the amount of soil to be removed it was realised that smaller volumes of soil would wash faster. Therefore a second corer of reduced diameter (61 mm) was constructed for Experiment 2. On the other hand to reduce co-efficients of variation, four cores per plot were collected and bulked. As a result of this sampling strategy data from Experiment 2 did not require log transformation.

In addition, sampling depths were changed to 250-600 mm for the lower depth. This was because 250 mm was the approximate depth of the A horizon and therefore a more logical delineation between sampling depths; and little root was found below 600 mm yet significant time savings were made by sampling to the shallower depth.

For the later harvests in Experiment 2 and in Experiment 3 core diameter was further reduced to 21 mm, and the number of cores increased to two independent samples of ten cores. Root mass densities (t. DM ha^{-1}) obtained from 21 mm cores compared well with those obtained from 61 mm cores (Table A2.1), but there was some indication that amounts of ryegrass stolon (Section 5.3.2) detected were reduced when a 21 mm core was used. This could have been due to stolons being pushed aside by the 21 mm corer more than by the 61 mm corer, and such effects are also more likely to have occurred when soil moisture levels were high, than when soil was drier and firmer.

For Experiments 2 and 3, root length determination was speeded up by using a 4 cm grid. This reduced counts per subsample to approximately 500. (Conversion factor to convert intersects to cm root length = 3.1428). The combined effect of changing to 21 mm cores and a 4 cm counting grid was to reduce total time for sampling and sample processing to less than 1 hour per plot, and coefficients of variation for root mass to close to 10%.

In Experiment 3 the 250-650 mm soil depth was not sampled to further save sampling time.

Table A2.1: Comparison of results for (A) root mass (kg ha^{-1} AFDW) and (B) root length (km m^{-2}) for two soil depths sampled using 21 mm or 61 mm diameter corers.

Sampling depth	Core diameter	Grazing management[1] LL	HH	LH	HL	SEM
A. AFDW (kg ha^{-1})						
0 - 70 mm	21 mm	2230	2200	1800	1910	246
	61 mm	2610	2200	1840	2170	321
70 - 250 mm	21 mm	730	650	700	750	119
	61 mm	710	540	620	720	87
B. Root length (km m^{-2})						
0 - 70 mm	21 mm	35.4	36.5	32.1	33.5	5.2
	61 mm	37.9	37.7	36.1	36.3	5.5
70 - 250 mm	21 mm	13.2	16.5	18.7	19.8	2.3
	61 mm	16.8	15.2	18.0	15.7	2.4

1. For explanation of treatment codes see Section 4.2.3

A2.2 Root extraction from cores

For Experiment 2 a "hydropneumatic elutriation system" or root washing machine (Smucker *et al.* 1982) was used for recovering root samples from soil cores. This avoided the double counting of picked and washed samples from the same soil core (Section 3.2.5) although in some circumstances useful information on different types of roots could be derived from the proportion of root in each category when two categories of root were determined separately (Mwebaze, 1986).

The hydropneumatic elutriation system had some design faults. In particular, sieves designed to retain roots tended to block with the large quantities of roots from pasture samples. Therefore a machine described by Smucker *et al.* (1982) was redesigned at Massey University and used for samples from Experiment 3.

The major design modifications made were:

i) Open sieves of large surface area to reduce blockage.

ii) Root washing manifolds aligned along a wall instead of around a central pillar to save floor space, and allow for easier washing.

iii) On/off control of individual jets to vary the washing action for different soil textures.

It was found that the diameter and operating pressure of the jets was critical to the performance of the machine. If the pressure was too low or the diameter too small samples took too long to wash. If the jets were too large, or the circular current too fast, small fragments of soil were carried out of the manifold and collected with the root sample, necessitating re-washing. and The completed machine is shown in Plate A2.1.

A2.3 Determination of sample root length

Determination of root length using the grid intercept method was found to be critically dependent on the rules applied when counting root-grid intercepts, and initially, counts of the same sample made by different operators frequently varied by 30% or more. For example, Figure A2.1a shows a case where a decision is required whether to count 0 or 1, and Figure A2.1b, shows a situation where a decision must be made between a value of 0 or 2. These ambiguities largely arise from the fact that both the grid-line and the root have a finite thickness. Thus, the rule adopted for processing samples was that in ambiguous cases the number of intercepts was determined by whether or not an imaginary centre line of the root crossed an imaginary centre line of the grid. To ensure consistency, different individuals assisting with root length determination were asked to re-count samples already counted by the author until values agreed to within 5%. It is worth noting that while automatic counting devices would produce consistent results, they

would still be subject to biases arising from the fact that roots being counted have finite thickness, and such effects would need to be corrected by appropriate calibration.

A2.4 Effects of storage under refrigeration after sampling and of storage in alcohol on subsequent root AFDW determination

An experiment to determine the effect of storage under refrigeration and of storage in 95% ethanol on root mass recovered was conducted at the conclusion of Experiment 3. Thirty samples of ten 21 mm diameter cores for the 70 mm to 250 mm soil depth were harvested on 17 January 1990 (Day 0). 6 samples were washed on Days 0,7,14,21 and 28 (Times 1 - 5). In each case 3 of the six samples were dried and weighed immediately, and the other 3 stored 7 days in 95% ethanol, then dried and weighed. After storage of roots, ethanol was recovered and evaporated to dryness to determine dissolved solids. Data for the 30 samples was analysed as a factorial design. The effects of storage time and storage in alcohol on recovered root ash-free dry weight were both non-significant at P = 0.05 (Table A2.2). Co-efficient of variation for root AFDW was 12.4% and recovery of dissolved solids for 15 samples stored in alcohol averaged 11 mg (<3% sample ash-free dry weight).

Table A2.2: Effect of storage under refrigeration and storage in alcohol on recovery of root (sample ash-free dry weight, mg).

Storage method	Storage time (days) 0	7	14	21	28	Mean
Stored in alcohol 7 days	0.22	0.36	0.27	0.23	0.29	0.273
Dried immediately after washing.	0.26	0.29	0.26	0.31	0.29	0.283

Co-efficient of variation (2 samples of 10 cores): 12.4%
Significance: Times NS, Storage method NS, P = 0.05.

These data show that respiration losses prior to washing, and leaching of alcohol soluble dry matter during storage in alcohol should not have had any major effect on root mass results. Errors from these sources would have been smaller than sampling error associated with within plot variation.

Plate A2.1: Modified root washing machine (Section A2.2)

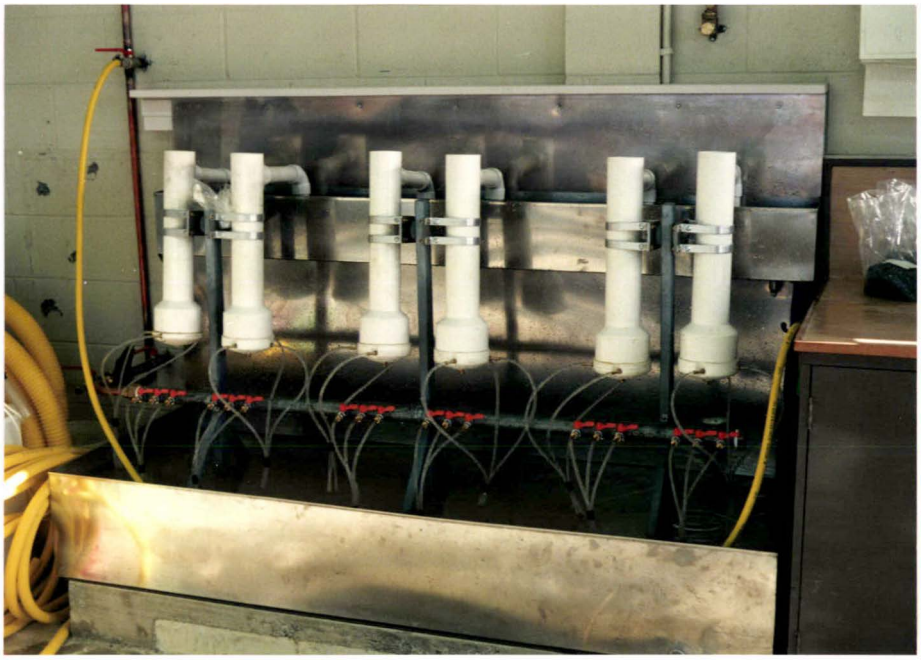
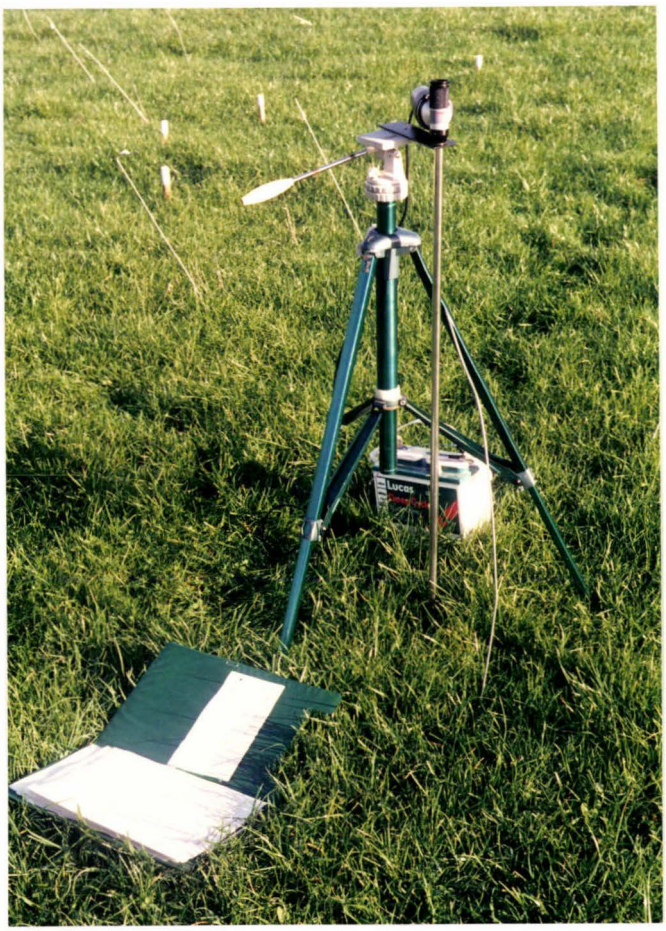


Plate A2.2: Method of mounting fibre-optic endoscope for counting of roots in minirhizotron tubes.



A2.5 Root length determination using image analysis

Recently the author has participated in the development of an image analysis system for root length determination. This system is based on the thinning of a binary image of a root sample down to a skeleton of one pixel width. The number of pixels in the skeleton image estimates the total root length of the sample, and information on distance of pixels from the edge of the image recorded during the thinning process is used to subdivide the total root length into diameter categories (Cochrane 1989, Cochrane *et al.*, 1990).

The image analysis system has the advantages, compared with hand counting, of consistency between samples and of not being as psychologically demanding for the operator. However, in order to provide the magnification required to image fine grass roots for diameter analysis, as opposed to simple root length determination. the field of view of the camera had to be reduced to approximately 50 mm x 50 mm allowing a maximum sample size of some 600 mm of root. Therefore, in common with other automated root counting machines available commercially, care is needed in planning an experiment, to stay within the capacities of the machine. The system was not available when samples from Experiments 2 and 3 were processed, but was used for diameter determinations on preserved samples from transplant cores in Experiment 3 (Section 6.3.4.1)

A2.6 Evaluation of refilled core data in relation to root growth in surrounding soil

Minirhizotron tubes were used to compare the number of root apices appearing in refilled cores with that in undisturbed soil. The apparatus used was a fibre-optic endoscope mounted on a camera tripod as illustrated in Plate A2.2.

On 10 May 1988 3 sand-filled cores were installed as described in Section 3.2.4, but with a 21 mm internal diameter perspex tube at the centre of the core. A second perspex tube was installed in undisturbed soil 500 mm distant from each refilled core. A 20 mm hole was drilled with a hand auger, and the perspex tube gently tapped into this hole with a mallet. Tubes were vertical, sealed top and bottom with rubber bungs, and prior to installation were scribed with a vertical reference line and with 3 mm wide counting

windows spaced every 20 mm to 200 mm depth, then every 40 mm to 450 mm depth. No roots were seen in one of the tubes checked shortly after installation, so all roots seen later at the counting windows were considered to be new roots. Total numbers of roots visible in the counting windows were recorded on 10 June, 27 June and 21 July (Table A2.3)

This data suggests that refilled cores underestimate root appearance in the 0-70 mm soil depth, and overestimate root appearance in the 70-250 mm soil depth, however neither of these results was significant at $P=0.05$. In order to obtain further data comparing refilled core root appearance with that in undisturbed soil, the 3 perspex tubes were installed as above for each of the six measurement periods in Experiment 3 (Section 6.2.1).

Data was analysed as a factorial design with a nested factor. Sampling method (refilled core or undisturbed soil) and time (measurement periods 1-6) were analysed as main factors and soil depth was nested within sampling method. Block effects were also removed. Values for the 250-450 mm soil depth were omitted from the analysis of variance, and are not presented because there was a large proportion of zero counts, which would have invalidated statistical assumptions.

In this experiment root counts averaged over 6 sampling times were significantly lower in refilled cores than in undisturbed soil ($P<0.001$) for both 0-70 mm and 70-250 mm soil depths (Table A2.4).

In addition to the significantly lower counts in refilled cores there was a significant sampling method x time interaction. This interaction arises because of seasonal differences in the RC:US ratio (Table A2.4). The greatest difference between refilled core and undisturbed soil counts was at Time 4 when large numbers of fine lateral branches from new nodal roots were visible at the soil surface in undisturbed soil, but not in refilled cores.

From these data it is concluded that refilled cores did not substantially overestimate root production as some observers predicted would be the case (Section 3.4.1). To the contrary there is good evidence that estimates of root production obtained by the refilled core method in this study may actually underestimate root production and turnover.

Table A2.3: Comparison between refilled cores (RC) and undisturbed soil (US) for numbers of roots seen in minirhizotron tubes for three soil depths.

	Soil Depth 0-70 mm			70-250 mm			50-450 mm		
Date	10/6	27/6	21/7	10/6	27/6	21/7	10/6	27/6	21/7
RC	9	42	47	8	37	60	0	0	2
US	26	64	90	5	15	35	0	0	1
RC:US	0.35	0.66	0.52	1.60	2.46	1.71	-	-	-

Table A2.4: Comparison between refilled cores (RC) and undisturbed soil (US) for root numbers seen in minirhizotron tubes during Experiment 3.

Core type	Soil depth (mm)	Time 1	2	3	4	5	6	Mean
RC	0-70	10.7	37.7	14.3	8.7	13.3	9.0	15.6
	70-250	13.7	3.3	2.0	5.0	13.3	16.7	9.0
	Total	24.4	41.0	16.3	13.7	26.6	25.7	24.6
US	0-70	23.0	40.7	11.3	83.0	49.3	24.3	38.6
	70-250	26.0	21.7	3.0	17.0	39.3	26.7	22.4
	Total	46.0	62.4	14.3	100.0	88.6	51.0	61.0
RC:US		0.53	0.65	1.14	0.14	0.30	0.50	0.40

Significance: RC vs. US ***, Time **, Depth **, TimexRC/US *.
SEM: Times 1-6 = 6.3, overall mean RC or US = 3.6.

A2.7 Derivation of mean diameter formula

Since volume of a cylinder = $\frac{1}{4}\Pi D^2 L$

Where:

L = length (cm)
D = mean diameter (cm)

Then, assuming: (i) mass (g) = volume (cc)
(ii) root dry weight = 8% root fresh weight.

$$g.m^{-2}/0.08 = \tfrac{1}{4}\Pi D^2.km.m^{-2}.10^5 \quad \text{(convert km.m}^{-2}\text{ to cm)}$$

$$g/0.08 = \tfrac{1}{4}\Pi D^2.km.10^5$$

$$D^2_{(cm)} = 4g/0.08\Pi.km.10^5$$

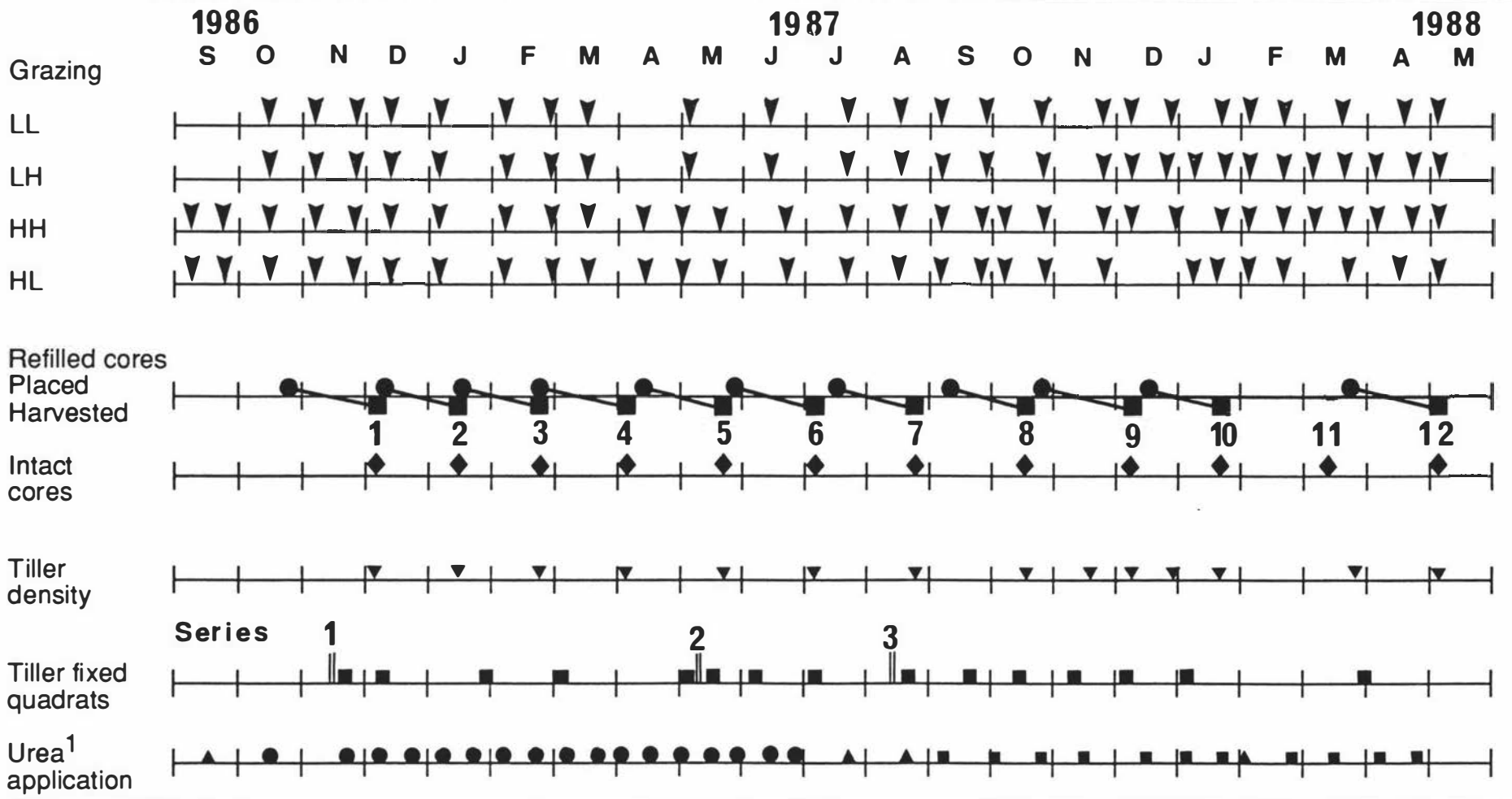
$$D^2_{(cm)} = 4/\Pi * 0.08 * 10^5 * g/km$$

$$D_{(cm)} = \sqrt{4/\Pi} * 0.08 * 10^5 * \sqrt{g/km}$$

$$D_{(mm)} = 10 * \sqrt{4/\Pi} * 0.08 * 10^5 * \sqrt{g/km}$$

$$D_{(mm)} = 0.1262\ \sqrt{g/km}$$ (Section 3.2.6)

Appendix 3: Schedule of grazing dates, measurements and urea application for Experiment 2.



1. Urea: (●) 200 g per plot, (■) 300 g per plot, (▲) 400 g per plot.

Appendix 4: Summary of results of split-plot in time analysis of variance for root data from Experiment 2, see Chapter 4.

Text Location	Core type	Soil Depth	Data	Grazing (LL v. HH) F	Sig.	Harvests (2 - 10)[1] F	Sig.	Grazing x time[1] F	Sig.	SEM Grazing	SEM Time[1]
Table 4.1	Int	Upper	AFDW	6.3	+	13.7	***	0.3	NS	247	168
Table 4.1	Int	Middle	AFDW	20.6	*	9.2	***	1.2	NS	40	38
Table 4.1	Int	Lower	AFDW	0.0	NS	4.0	**	0.9	NS	73	30
Table 4.2	Int	Upper	Length	7.9	+	8.3	***	0.8	NS	4.8	3.4
Table 4.2	Int	Middle	Length	26.4	*	8.2	***	0.3	NS	0.5	1.3
Table 4.2	Int	Lower	Length	0.0	NS	2.9	*	0.4	NS	1.9	0.8
Table 4.3	Int	Upper	M. diam	0.0	NS	21.2	***	1.4	NS	0.013	0.007
Table 4.3	Int	Middle	M. diam	1.6	NS	26.8	***	1.5	NS	0.010	0.006
Table 4.3	Int	Lower	M. diam	2.8	NS	1.1	NS	1.8	NS	0.009	0.012
Table 4.4	Ref	Upper	AFDW	0.0	NS	13.0	***	0.8	NS	0.4	0.6
Table 4.4	Ref	Middle	AFDW	0.2	NS	8.4	***	0.6	NS	0.3	0.4
Table 4.4	Ref	Lower	AFDW	3.8	NS	7.5	***	1.5	NS	0.3	0.3
-	Ref	Upper	Length	0.5	NS	20.1	***	1.2	NS	1.2	0.8
-	Ref	Middle	Length	0.6	NS	9.4	***	0.5	NS	0.9	0.9
-	Ref	Lower	Length	0.3	NS	12.4	***	0.5	NS	0.2	0.4
Fig. 4.2a	Int	Up+Mid	AFDW	10.7	*	19.8	***	0.5	NS	278	109
Fig. 4.2b	Int	Up+Mid	Length	13.9	*	13.8	***	0.8	NS	4.6	2.8
Fig. 4.4	Ref	Up+Mid	AFDW	0.1	NS	13.1	***	0.5	NS	0.5	0.3
-	Ref	Up+Mid	Length	4.0	NS	26.3	***	0.7	NS	1.5	1.2
Fig. 4.5	Ref	Up+Mid	M. diam	0.7	NS	28.9	***	0.9	NS	0.010	0.015

Int = Intact, Ref = refilled, Upper = 0 - 70 mm soil depth, Middle = 70 - 250 mm soil depth, Lower = 250 - 600 mm soil depth.
1. Used as a preliminary estimate of significance only, significance of effects confirmed by further testing, See Appendix 1.1 & Section 4.2.3.

Appendix 4 - continued.

Text Location	Core type	Soil Depth	Data	Grazing (LL v. HH) F	Sig.	Harvests (2 - 10)[1] F	Sig.	Grazing x time[1] F	Sig.	SEM Grazing	SEM Time[1]
Table 4.5	Ref	Upper	M. diam	0.4	NS	19.9	***	1.3	NS	0.017	0.010
Table 4.5	Ref	Middle	M. diam	0.5	NS	18.7	***	1.0	NS	0.010	0.010
Table 4.5	Ref	Lower	M. diam	0.0	NS	11.6	***	1.9	NS	0.019	0.013
Table 4.7	I:R	Middle	Turnover	2.4	NS	14.3	***	3.5	**	246	169
Tiller density measurements (Chapter 5).											
Figure 5.4	Tillers m^{-2}			108	***	29.3	***	2.5	*	594	300
Figure 5.7a	Tillers m^{-2} day^{-1}			3.4	+	67.4	***	2.7	*	14.7	9.3
Figure 5.7b	Tillers m^{-2} day^{-1}			0.1	NS	13.1	***	2.8	*	5.6	0.6

Int = Intact, Ref = refilled, Upper = 0 - 70 mm soil depth, Middle = 70 - 250 mm soil depth, Lower = 250 - 600 mm soil depth.
1. Used as a preliminary estimate of significance only, significance of effects confirmed by further testing, See Appendix 1.1 & Section 4.2.3.

APPENDIX 5: SEASONAL CHANGE IN TILLER DENSITY OF *POA* Sp.

In conjunction with seasonal change in tiller population density for ryegrass (Section 5.3.4.1), tiller densities for *Poa* sp. showed seasonal increase in summer ($P<0.001$), with values averaged over LL and HH plots declining from 2760 m-2 at Harvest 1 to 190 m-2 at Harvest 5, then increasing again to 1840 m-2 at harvests 9a and 10 (Table A6.1). Ratio of Poa:ryegrass tiller density was 0.53 and 0.39 at Harvest 1, fell to 0.06 and 0.01 at Harvest 5, then incresed to a peak of 0.34 and 0.22 at Harvest 10 for LL and HH plots, respectively (Table 5.6). Averaged over time, the grazing management differences in ratio of *Poa*:ryegrass tiller density were statistically significant.

These data indicate that for *Poa*, differences in tiller density between LL and HH plots were proportionately less than for ryegrass; and that the summer peak in tiller density was both more pronounced and earlier in the season than the summer peak for ryegrass tiller density. Analysis of log-transformed data showed the seasonal variation in *Poa*:ryegrass ratio and the reduction in this ratio on HH plots to be highly significant ($P<0.001$ in both cases), and also revealed a significant grazing x time interaction. These differences in behaviour of *Poa* relative to ryegrass would be consistent with *Poa* having a shallow rooting system and being adversely affected by HH grazing management which lowered soil moisture levels near the soil surface.

Table A5.1: Tiller density (tillers m^{-2}) for *Poa* sp. and ratio *Poa* sp.:ryegrass tiller density for period December 1986 to January 1988.

Grazing	Harvest[1] 1	2	3	4	5	6	7	8	9	9a	10	10a	Mean	SEM[2]
	Tiller density													
LL	2798	1173	525	846	276	590	703	1061	1142	1485	1609	1719	1160	} 304
HH	2715	593	208	276	98	317	873	990	372	1847	1840	1390	960	
Mean	2756	883	367	561	187	453	788	1025	757	1666	1725	1554	1060	215
	Ratio Poa:Ryegrass tiller density													
LL	0.53	0.28	0.07	0.15	0.06	0.15	0.13	0.24	0.34	0.34	0.21	0.27	0.23	} 0.04
HH	0.39	0.11	0.02	0.04	0.01	0.05	0.13	0.15	0.06	0.22	0.17	0.12	0.12	
Mean	0.46	0.20	0.05	0.09	0.03	0.10	0.13	0.19	0.20	0.28	0.19	0.19	0.18	0.03

1. For dates of harvests, see section 4.2.5
2. Standard error derived from analysis of untransformed data and appropriate for comparing grazing managements averaged over time. Log transformation of data greatly increased significance levels.

Appendix 6: Fourier equations fitted by least squares regression and used for interpolation of tiller data from fixed quadrats, Experiment 3, see Section 6.2.1.

1. Tiller appearance rate:

Rep. 1: TAR = 55.6	+ 0.4 S(D)	- 14.8 C(D)	+ 16.9 S(2*D)	- 6.9 C(2*D)	- 2.9 S(3*D)	+ 8.9 C(3*D)	r^2 = 76%
Rep. 2: TAR = 64.3	+ 3.0 S(D)	- 4.5 C(D)	+ 34.6 S(2*D)	+ 12.8 C(2*D)	+ 16.1 S(3*D)	+ 16.1 C(3*D)	r^2 = 79%
Rep. 3: TAR = 41.9	+ 7.2 S(D)	+ 3.1 C(D)	+ 21.3 S(2*D)	- 4.9 C(2*D)	+ 2.6 S(3*D)	+ 11.3 C(3*D)	r^2 = 81%

2. Tiller death rate:

Rep. 1: TDR = 51.1	- 7.4 S(D)	+ 10.8 C(D)	- 12.4 S(2*D)	- 11.4 C(2*D)	+ 3.4 S(3*D)	- 0.2 C(3*D)	r^2 = 94%
Rep. 2: TDR = 66.9	- 1.6 S(D)	+ 9.9 C(D)	- 23.4 S(2*D)	- 32.7 C(2*D)	- 9.1 S(3*D)	+ 3.1 C(3*D)	r^2 = 93%
Rep. 3: TDR = 55.3	+ 20.1 S(D)	- 12.9 C(D)	- 28.5 S(2*D)	+ 15.2 C(2*D)	+ 10.6 S(3*D)	- 7.8 C(3*D)	r^2 = 99%

3. Ryegrass tiller population density:

Rep. 1: TPD = 8444	- 1942 S(D)	- 888 C(D)	+ 49 S(2*D)	- 1091 C(2*D)	+ 161 S(3*D)	+ 183 C(3*D)	r^2 = 92%
Rep. 2: TPD = 9183	- 757 S(D)	+ 21 C(D)	+ 1287 S(2*D)	- 1702 C(2*D)	+ 170 S(3*D)	- 583 C(3*D)	r^2 = 93%
Rep. 3: TPD = 6554	+ 1794 S(D)	+ 1431 C(D)	- 706 S(2*D)	- 1275 C(2*D)	+ 338 S(3*D)	+ 123 C(3*D)	r^2 = 76%

S = Sin, C = Cosine, D = Day of year * 360/365.

Appendix 7: Calibration tests for ^{14}C sample oxidation and liquid scintillation counting.

The method used for detection of radiocarbon tracer and determination of specific activities for the various dissection categories (Section 8.4) followed closely that of Jeffay & Alvarez (1961). However, there were a number of uncertainties about the method, and therefore some informal checks were carried out to give information on accuracy and repeatability of results.

A 7.1 Linearity for differing sample sizes and repeatability of method

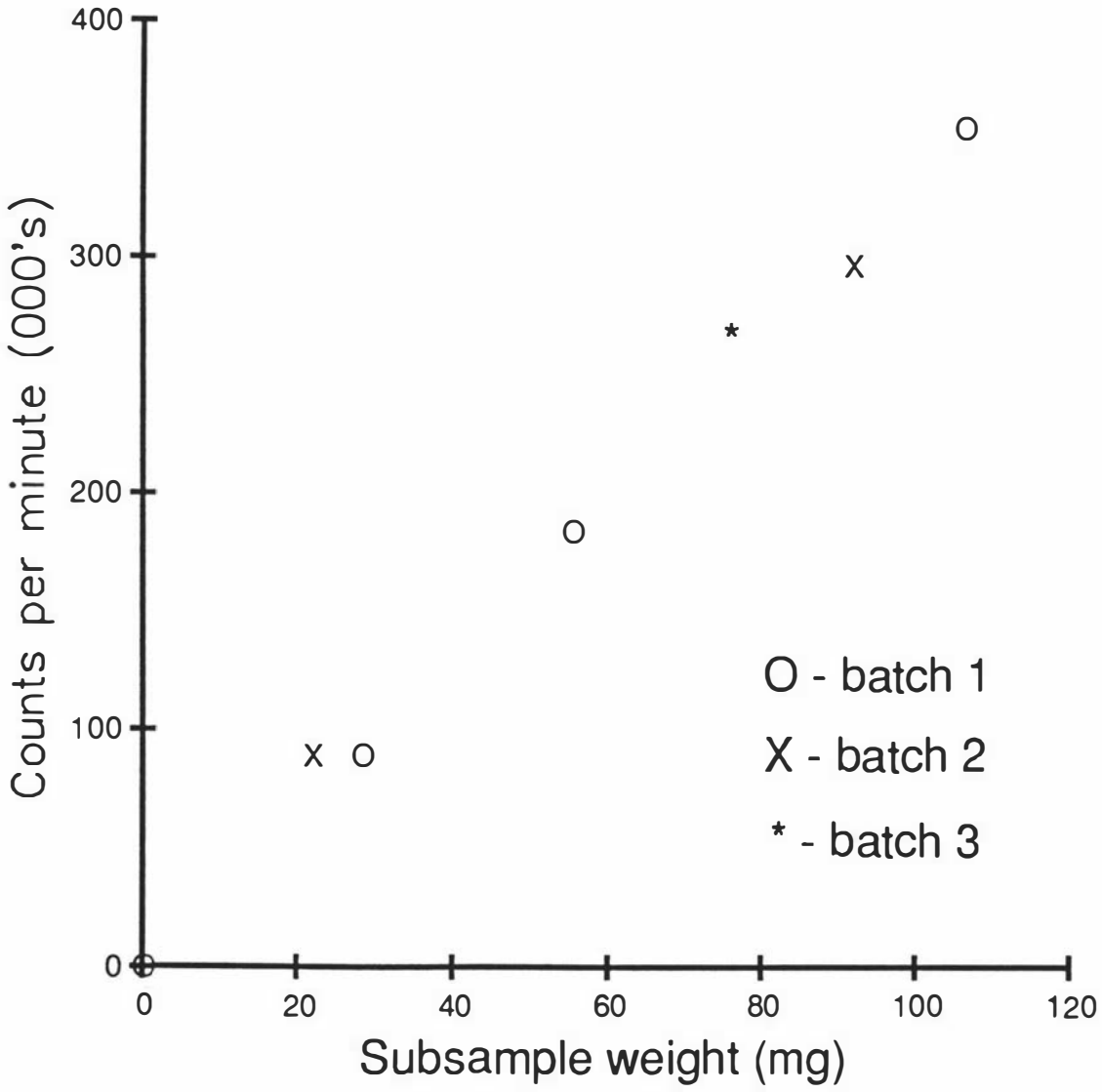
The weight of tissue for the various dissection categories in Tables 8.4 & 8.5 ranged from a few mg to several hundred mg. Samples larger than 100 mg were subsampled and approximately 100 mg plant tissue processed, but for samples smaller than 100 mg it was of interest to ascertain that the recovery and counting of radiocarbon was not affected by quantity of plant tissue in the sample. For example, it was not known at what sample size the ethanolamine carbon-dioxide-scavenger in the scintillation cocktail would begin to become saturated. If this had happened, then with the larger samples some radiocarbon might have passed the trap.

To check for possible errors of this type, radioactively labelled tillers from a spare pot not used in the experiment were dried and processed as described in Section 8.4.2, and this material used as a standard sample. Three subsamples of 107 mg, 55 mg, & 28 mg, together with a blank sample, were oxidised and scintillation counts obtained. These 4 samples were processed in order of descending sample size and the blank sample processed last, so that any tendency for radiocarbon to be carried over from one sample to the next in the sample oxidiser would also be detected. Samples were processed in several batches, and with each new batch of samples processed, a further subsample from the standard sample was included to check for consistency of results between different batches of samples.

In all 7 standard samples were analysed, as shown in Figure A7.1. The r^2 for regression of radioactive counts per minute on sample size, for samples ranging in size from 0 to 108 mg, was 99.5%. Also, the intercept of the

regression line did not differ significantly from zero, and a quadratic term when introduced was not statistically significant. On the basis of these test samples it was concluded that there was linearity over the range of sample sizes measured, no evidence of carry-over of radiocarbon from sample to sample, and no evidence of variation in results between batches of samples. Since errors at any stage of sample processing would have carried through and would have been reflected in the scintillation counts (Figure A7.1) the above validation applies to sample oxidation, carbon dioxide trapping, and scintillation counting procedures.

Figure A7.1: Relationship between sample size (mg) and scintillation count (DPM) for 7 subsamples from a standard sample.



A7.2 Determination of ^{14}C single-label DPM using the Beckman LS3801

A7.2.1 Background

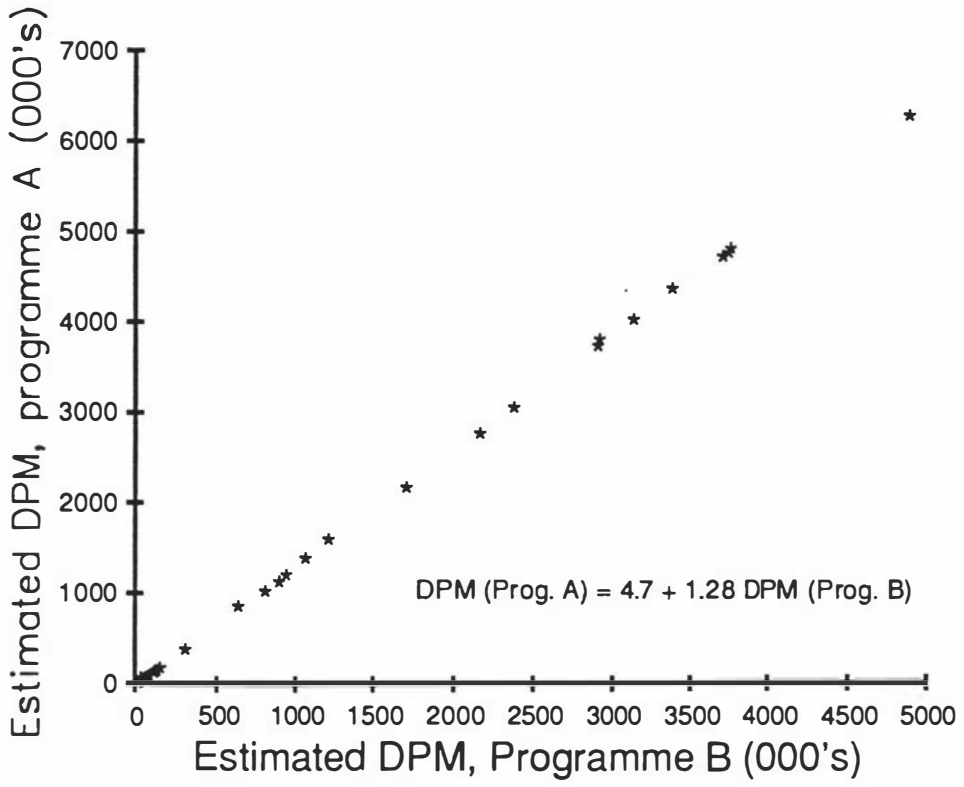
Liquid scintillation counting of radioactive samples requires a quench correction. That is the readings of counts per minute (CPM) generated by the machine must be corrected for the proportion of radioactive disintegrations which do not generate a flash of light, and are therefore not counted, so as to give an estimation of the actual activity of the sample in disintegrations per minute (DPM).

The quench correction is programmed into the machine by the user. Quench correction programmes require the entry of so-called channel settings and 4 co-efficients. At least two different programmes have been in current use at Massey University (Table A7.1). It appears that these programmes have been "handed-down" for some years. Samples were initially counted using programme A, but when thirty samples were counted using programme A, then recounted using programme B, it was found that the two programmes gave different results when run for the same batch of samples (Figure A7.2). It was felt necessary to test the two programmes in question against standard samples of known activity in order to clarify the above uncertainty.

Table A7.1: Channel 1 window and cubic co-efficients for programmes A & B for determination of ^{14}C DPM, using the Beckman LS3801.

	Window for Channel 1	Cubic co-efficients A	B	C	D
Programme A	0 - 670	2.847674	0.00914280	-0.0000054	-0.0000000308
Programme B	0 - 1000	4.598000	-0.0008770	0.00000571	-0.0000000222

Figure A7.2: Comparison of values for DPM obtained when programmes A & B were used to count the same batch of 36 samples.



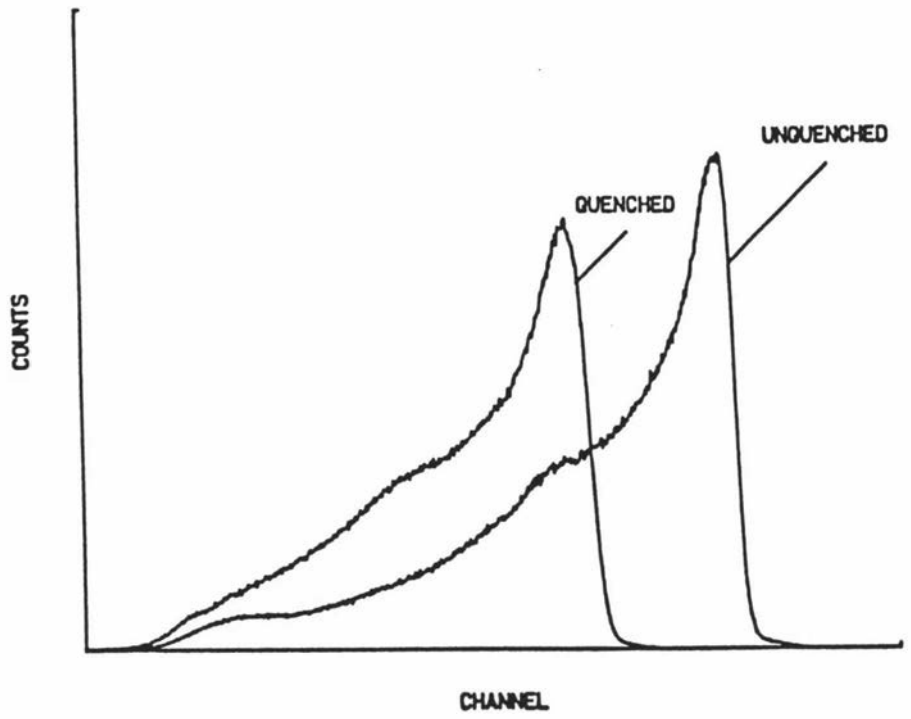
A7.2.2 Mode of operation of the Beckman scintillation counter

The Beckman LS3801 records both the number and intensity of the scintillation events monitored. The individual particles counted are categorised according to their energy level, and 1000 energy levels (channels) are recognised. The total number of counts can then be represented as a histogram, showing sub-totals for each energy level. When quenching occurs, both the number of particles counted and the energy level of individual particles is reduced. The histogram of counts by energy level for a quenched sample therefore has a peak which is both numerically lower and shifted to a lower energy level than for a hypothetical unquenched sample. (Figure A7.3).

The first step in counting a sample is that the machine exposes the sample to ^{137}Cs, a gamma-emitter, which elicits a shower of electrons from the sample (Compton electrons). These Compton electrons interact with the scintillation cocktail in almost the same way as beta-particles released on radioactive decay (beta-particles are also electrons). The machine is

programmed to measure the extent to which the histogram of energy levels of the Compton electrons is shifted to lower energy levels than expected. This shift (measured in number of channels) is a measure of the extent of quenching in the sample and is printed out by the machine as the sample "H-number".

Figure A7.3: Histogram of number of scintillation events classified by energy level, for an actual sample and a hypothetical unquenched sample (Source: Beckman LS3801 Instruction manual).



The next step is the determination of the number of scintillation events at each of the 1000 energy levels or channels. Three totals of counts are printed, and these are for 3 windows, or ranges of energy levels specified by the user. For example the instruction manual recommends that ^{3}H be determined on the basis of counts in channels 0 - 400, ^{14}C on the basis of counts in channels 0 - 670, and ^{32}S on the basis of counts in channels 0 - 1000, and these are the default settings on the machine. Just for confusion, these counting windows specified by the user are also called channels, when referred to in the instruction manual.

Finally, the machine takes the count from the first window (named channel 1 in the printout) and converts this total from CPM to DPM. The totals from the other windows (channels 2 & 3) are not used in the calculations to determine single label DPM.

The conversion from CPM to DPM is a two stage arithmetic process as follows:

1. The four co-efficients (A, B, C, D) from the user programme are used to convert the H-number to a counting efficiency. The equation used in this calculation is:

$$I_n \text{ efficiency}(\%) = A + Bx + Cx^2 + Dx^3$$

Where A..D are the four co-efficients
and x is the sample H-number.

2. The total count (CPM) from window 1 (channel 1) is divided by the counting efficiency (expressed as a decimal fraction) in order to estimate the actual DPM.

The co-efficients A..D are obtained in a totally separate calibration procedure by counting a series of standard samples, say 10 samples, all with the same known value of DPM, but with a range of different quench values. This range of quench values can be achieved by substituting successively larger volumes of scintillation fluid from the standard samples with a known quenching agent, such as alcohol (ml quantities) for water based scintillants or chloroform (µl quantities) for toluene based scintillants. The machine records the H-numbers for the successive samples and derives the observed counting efficiency from the ratio of observed counts in the defined window (channel 1):known value of DPM (assumed constant). The equation describing the relationship between the H-number and observed counting efficiency is then derived by the spline method, and the 4 co-efficients for the cubic curve stored.

A7.2.3 Possible errors

From the above it can be seen that before a previous cubic curve to convert H-number to counting efficiency can be safely re-programmed into the machine for a new batch of samples, it should be ascertained (i) that the channel window setting for channel 1 when counting the samples is the same as that used in generating the original curve and is appropriate for the isotope now being counted (because different isotopes emit beta-particles of different energies) and (ii) that the range of quench values (or H-numbers) in the samples being counted are within the range of quench values of the standard samples used to generate the cubic curve.

A7.2.4 Calibration exercise

Three Amersham CFR.101 calibration discs with known ^{14}C activity of 5000 DPM were oxidised and counted, as above. The Harvey OX600 oxidiser typically gives carbon recoveries in excess of 95% (D. H. Greer, pers. comm.) so that expected counts for the three samples so obtained were in the range 4750 - 5000 DPM. The samples were then counted on a Beckman LS3801 scintillation counter using programme A then recounted using programme B (Table A7.1). Finally, samples were counted a third time, using Programme A, but with channel 1 window setting redefined as channels 1 - 400 instead of 1- 670. Results are given in Table A7.2.

Table A7.2: Results from counting radioactivity for three calibration discs, using different counting programmes.

	DPM[1]	Programme A	Programme B	Programme A[2]
Sample 1	4352	6300	5058	4902
Sample 2	4292	6073	5040	4954
Sample 3	4420	6404	5178	4984
Mean	4355	6259	5092	4947

1. Raw scintillation count (Channels 1 - 1000), unadjusted for quench. Total
2. With channel 1 re-defined as channels 1 - 400, not 1 - 670.

Assuming a 98% recovery from the sample oxidiser, Programme A overestimated the activity of the samples by 28%, and programme B by 4%. However, when programme A was re-run with the counting window for channel 1 re-defined, the values obtained were almost exactly as expected.

It is concluded that neither programme is ideal for the present batch of samples. Programme A substantially over-estimates DPM, but gives acceptable accuracy if a counting window of channels 0 - 400 is used. However, this counting window is not ideal for ^{14}C. Programme B slightly overestimates counts and again uses a counting window which is not ideal for ^{14}C samples. It appears that programme A was originally determined for a counting window of channels 0 - 400, perhaps for counting ^{3}H samples, but in the process of handing down the programme, someone has changed the counting window for ^{14}C without running a new set of standard samples to determine new co-efficients for the cubic equation.

Samples from Experiment 6 had already been counted using programme A prior to running this calibration test, and could not be recounted due to colour changes and evaporation from vials during storage. Therefore, for presentation in Section 8.4, values obtained using programme A were adjusted by multiplying by a constant (0.78). This constant is based on the mean for the three calibration discs of 5000 DPM nominal activity counted using programme A (Table A7.2), and assumes 98% recovery of radiocarbon by the sample oxidiser. A linear conversion for samples of differing activities is felt to be appropriate because of the linear relationship between counts obtained using programme A and counts obtained using programme B (Figure A7.2).

BIBLIOGRAPHY

Arosteguy, J. C. 1982. *The dynamics of herbage production and utilsation in swards grazed by cattle and sheep.* Ph D. Thesis, University of Edinburgh. 303pp.

Atkinson, D. 1984. Spatial and temporal aspects of root distribution as indicated by the use of a root observation laboratory. In: *Ecological interactions in soil* Ed. by A. H. Fitter, D. Atkinson, D. J. Read & M. B. Usher. British Ecological Society special publication No. 4. Blackwell Scientific Publications, Oxford. pp. 43-65.

Auda, H., Blaser, R. E., Brown, R. H. 1966. Tillering and carbohydrate contents of orchard grass as influenced by environmental factors. *Crop Science 6:* 139-143.

Awopetu, J. A. 1979. *Patterns of tiller formation, regrowth and changes in percentage water-soluble carbohydrate during the reproductive phase of different grass types.* M. Sc. Thesis, University of Wales. (Cited by Davies *et al.*, 1981.)

Baker, H. K. 1957. Studies on the root development of herbage plants III. The influence of cutting treatments on the root stubble and herbage production of a perennial ryegrass sward. *Journal of the British Grassland Society 12:* 197-208.

Barber, S. A. 1971. Effect of tillage practice on corn (*Zea mays* L.). Root distribution and morphology. *Agronomy Journal 63:* 724-726.

Barber, S. A., Mackay, A. D. 1986. Root growth and phosphorus and potassium uptake by two corn genotypes in the field. *Fertilizer Research 10:* 217-230.

Barker, D. J., Zhang, D. M., Mackay, A. D. 1988. Root distribution in a low fertility hill country sward grazed by sheep. *New Zealand Journal of Experimental Agriculture 16:* 73-76.

Barry, T. N. 1976. Effects of intraperitoneal injections of DL-methionine on the voluntary intake of sheep fed sole diets of hay, silage and pasture differing in digestibility. *Journal of Agricultural Science, Cambridge. 86:* 141-149.

Belgrave, B. R., Watt, P. C., Brock, J. L., Wewala, S., Sedcole, J. R. 1990. A survey of farmer knowledge and use of pasture cultivars in New Zealand. *New Zealand Journal of Agricultural Research 33:* 199-211.

Bell, A.D. 1976. The vascular pattern of Italian Ryegrass (*Lolium multiflorum*) Lam.) 3. The leaf trace system and tiller insertion in the adult. *Annals of Botany 40*: 241-250.

Bircham, J. S., 1981. The effects of change in herbage mass on herbage growth, senescence and net production rates in a continuously stocked mixed species sward. pp 85-87 In: C. E. Wright Ed. *Plant physiology and herbage production.* British Grassland Society, Occasional symposium No. 13. British Grassland Society, Hurley.

Bircham, J. S, Hodgson, J. 1983. The influence of sward condition on rates of herbage growth and senescence in mixed swards under continuous stocking management. *Grass and Forage Science 38:* 323-331.

Bohm, W. 1979. *Methods of studying root systems.* Volume 33: Ecological studies - analysis and synthesis. Eds: W. D. Billings, F. Golley, O. L. Lange, J. S. Olson. Springer-Verlag. 188pp.

Booysen, P. de V., Tainton, N. M., Scott, J. D. 1963. Shoot apex development in grasses and its importance in grassland management. *Herbage Abstracts 33:* 209-213.

Bragg, P. L., Rubino, P., Henderson, F. K. G., Fielding, W. J., Cannell, R. Q. 1984. A comparison of the root and shoot growth of winter barley and winter wheat, and the effect of an early application of chlormequat. *Journal of Agricultural Science, Cambridge 103:* 257-264.

Brougham, R. W., 1955. A study in the rate of pasture growth. *Australian Journal of Agricultural Research 6:* 804-812.

Brougham, R. W. 1956a. The rate of growth of short-rotation-ryegrass pastures in late autumn, winter, and early spring. *New Zealand Journal of Science and Technology 38a:* 78-87.

Brougham, R. W. 1956b. Effect of intensity of defoliation on regrowth of pasture. *Australian Journal of Agricultural Research 7:* 377-387.

Brougham, R. W. 1957. Pasture growth rate studies in relation to grazing management. *Proceedings of the New Zealand Society of Animal Production 17:* 46-55.

Brougham, R. W. 1958. Interception of light by the foliage of pure and mixed stands of pasture plants. *Australian Journal of Agricultural Research 9:* 39-52.

Brougham, R. W. 1959. The effect of frequency and intensity of grazing on the productivity of a pasture of short rotation ryegrass and red and white clover. *New Zealand Journal of Agricultural Research 2:* 1232-1248.

Brougham, R. W. 1960a. The effects of frequent hard grazings at different times of the year on the productivity and species yield of a grass-clover pasture. *New Zealand Journal of Agricultural Research 3:* 125-136.

Brougham, R. W. 1960b. The relationship between the critical leaf area, total chlorophyll content and maximum growth rate of some pasture and crop plants. *Annals of Botany 24:* 463-474.

Brougham, R. W. 1970. Frequency and intensity of grazing and their effects on pasture production. *Proceedings of the New Zealand Grassland Association 32:* 137-152.

Brouwer, R. 1983. Functional equilibrium: sense or nonsense? *Netherlands Journal of Agricultural Science 31:* 335-348.

Brown, J. M., Fontendo, W. C., Cassel, D. K., Johnson, G. A. 1987. Computed tomographic analyses of water distribution in three porous foam media. *Journal of the Soil Science Society of America 51:* 1121-1125.

Brown, R. H., Cooper, R. B., Blaser, R. E. 1966. Effects of leaf age efficiency. *Crop Science 6:* 206-209.

Browse, J. A., Haselmore, R. M., Thaine, R. 1981. The effects of closing and cutting dates on the yield and nutritive value of pasture for conservation. *Proceedings of the Agronomy Society of New Zealand 11:* 51-55.

Butler, B. M. 1986. *The effect of grazing intensity and frequency during spring and early summer on sward characteristics of a ryegrass-white clover pasture.* M. Agr. Sci. Thesis, Massey University. 300pp.

Butler, B, M., Matthew, C., Heerdegen, R. 1990. The greenhouse effect - what consequences for seasonality of pasture production. *Weather and Climate 10:* 55-60.

Caradus, J. R., Evans, P. S. 1977. Seasonal root formation of white clover, ryegrass, and cocksfoot in New Zealand. *New Zealand Journal of Agricultural Research 20:* 337-342.

Carman, J. G. 1982. A non-destructive stain technique for investigating root growth dynamics. *Journal of Applied Ecology 19:* 873-879.

Carton, O. T., Brereton, A. J., O'Keeffe, W. F., Keane, G. P. 1989. Effect of turnout date and grazing severity in a rotationally grazed reproductive sward. 1. Dry matter production. *Irish Journal of Agricultural Research 28:* 153-163.

Casal, J. J., Deregibus, V. A., Sanchez, R. A. 1985. Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. Vegetative and reproductive plants as affected by differences in red/far-red irradiation. *Annals of Botany 56:* 553-559.

Chapman, D. F., Clark, D. A., Land, C. A., Dymock, N. 1983. Leaf and tiller growth of *Lolium perenne* and *Agrostis* spp. and leaf appearance rates of *Trifolium repens* in set-stocked and rotationally grazed hill pastures. *New Zealand Journal of Agricultural Research 26:* 159-168.

Chatfield, C., Collins, A. J. 1980. *Introduction to multivariate analysis.* Chapman & Hall, London, New York. 246pp.

Clark, D. A., Lambert, M. G., Chapman, D. F. 1982. Pasture management and hill country production. *Proceedings of the New Zealand Grassland Association 43:* 205-214.

Clemence, T.G.A.; Hebblethwaite, P.D., 1984. An appraisal of ear, leaf and stem $^{14}CO_2$ assimilat

Clifford, P. E. 1977. Tiller bud suppression in reproductive plants of *Lolium multiflorum* Lam. Cv. Westerwoldicum. *Annals of Botany 41:* 605-615.

Clifford, P. E., Langer, R. H. M. 1975. Pattern and control of distribution of ^{14}C-assimilates in reproductive plants of *Lolium multiflorum* Lam. var. *Westerwoldicum*. *Annals of Botany 39:* 403-411.

Clifford, P. E., Marshall, C., Sagar, G. R., 1973. The reciprocal transfer of radiocarbon between a developing tiller and its parent shoot in vegetative plants of *Lolium multiflorum* Lam. *Annals of Botany 37:* 777-785.

Cochrane, T. 1989. *Quantitative analysis of root structures. Getting down to grass roots with image analysis*. B. Sc. (Hons.) Thesis, Massey University. 45pp.

Cochrane, T., Matthew, C., Apperley, M. D., Ngan, P. M. 1990. Plant root length diameter determination using an image analysis thinning algorithm. *Proceedings of the Agronomy Society of New Zealand 20:* 77-82.

Cole, J. W. L., Grizzle, J. E. 1966. Applications of multivariate analysis of variance to repeated measurements experiments. *Biometrics 22:* 810-828.

Colvill, K.E., Marshall, C., 1984. Tiller dynamics and assimilate partitioning in Lolium perenne with particular reference to flowering. *Annals of Applied Biology 104*: 543-557.

Cooley, W. W., Lohnes, P. R. 1971. *Multivariate data analysis*. John Wiley & Sons, New York. 364pp.

Cumberland, G. L. B., Honore, E. N. 1970. An evaluation of Mangere ryegrass. *New Zealand Agricultural Science 4(4):* 21-24.

Danckwerts, J.E., Gordon, A.J.; 1987. Long-term partitioning, storage and re-mobilisation of ^{14}C assimilated by *Lolium perenne* (cv. Melle). *Annals of Botany 59:* 55-66.

Davidson, R. L. 1969. Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. *Annals of Botany 33:* 561-569.

Davidson, R. L. 1978. Root systems - the forgotten component of pastures. Chapter 6 In: J. R. Wilson Ed. *Plant relations in pastures*, CSIRO Melbourne.

Davies, A. 1974. Leaf tissue remaining after cutting and regrowth in perennial ryegrass. *Journal of Agricultural Science, Cambridge 82:* 165-172.

Davies, A. 1977. Structure of the grass sward. In: B. Gilsenan Ed. *Proceedings of an International Meeting on Animal Production from Temperate Grassland.* An Foras Taluntais, Dublin. pp 36-44.

Davies, A. 1981. Tissue turnover in the sward. Chapter 9 in: J. Hodgson, R. D. Baker, A. Davies, A. S. Laidlaw & J. D. Leaver Eds. *Sward measurement handbook*. British Grassland Society, Hurley. pp179-208.

Davies, A., 1988. The regrowth of grass swards. Chapter 3 In: M.B. Jones and A. Lazenby Eds. *The Grass Crop.* Chapman & Hall, London. pp 85-127.

Davies, A., Evans, M. E., Exley, J. K. 1983. Regrowth of perennial ryegrass as affected by simulated leaf sheaths. *Journal of Agricultural Science, Cambridge, 101:* 131-137.

Davies, A., Evans, M.E., Pollock C.J. 1989. Influence of date of tiller origin on leaf extension rates in perennial and Italian ryegrass at 15 °C in relation to flowering propensity and carbohydrate status. *Annals of Botany 63*: 377-384.

Davies, A., Evans, M. E., Sant, F. I. 1981. Changes in the type and rate of production of ryegrass tillers in the post-flowering period in relation to seasonal growth. pp 73 - 76 In C. E. Wright Ed. *Plant physiology and herbage production.* British Grassland Society, Occasional symposium No. 13. British Grassland Society, Hurley.

Davies, A., Thomas, H. 1983. Rates of leaf and tiller production in young spaced perennial ryegrass plants in relation to soil temperature and solar radiation. *Annals of Botany 57:* 591-597.

Deinum, B. 1985. Root mass of grass swards in different grazing systems. *Netherlands Journal of Agricultural Science 33:* 377-384.

Department of Statistics, 1989. *Agricultural statistics.* Government Printer, Wellington, 64pp.

Deregibus, V. A., Sanchez, R. A., Casal, J. J. 1983. Effects of light quality on tiller production in *Lolium* spp. *Plant Physiology 72:* 900-902.

De Willigen, P., Van Noordwijk, M. 1987. *Roots, plant production and nutrient use efficiency.* Ph D Thesis, Agricultural University, Wageningen, The Netherlands. 282pp.

Drew, M. C., Saker, L. R. 1980. Assessment of a rapid method, using soil cores, for estimating the ammount and distribution of crop roots in the field. *Plant and Soil 55:* 297-305.

Eccles, W. J. 1988. *A study on the relationship between soil oxygen concentration, soil moisture, and growth of prairie grass, under different soil types.* B. Agr. Sci. (Hons.) Thesis, Massey University. 69pp.

Etter, A. G. 1951. How Kentucky bluegrass grows. *Annals of the Missouri Botanical Garden 38:* 293-375.

Evans, M. W., Grover, F. O. 1940. Developmental morphology of the growing point of the shoot and inflorescence in grasses. *Journal of Agricultural Research 61:* 481-520.

Evans, P. S. 1970. Root growth of *Lolium perenne* L. 1. Effect of plant age, seed weight, and nutrient concentration on root weight, length, and number of apices. *New Zealand Journal of Botany 8:* 344-356.

Evans, P. S. 1971a. *The effects of defoliation and shading on root growth of Lolium perenne* L. Ph D Thesis, Massey University, 114pp.

Evans, P. S. 1971b. Root growth of *Lolium perenne* L. 2. Effects of defoliation and shading. *New Zealand Journal of Agricultural Research 14:* 552-562.

Evans, P. S. 1973. The effect of repeated defoliation to three different levels on root growth of five pasture species. *New Zealand Journal of Experimental Agriculture 16:* 31-34.

Evans, P. S. 1976. The effect of management on root activity. In: G. F. Wilson Ed. *Dairy Farming Annual, Massey University 28:* 123-127.

Evans, P. S. 1978. Plant root distribution and water use patterns of some pasture and crop species. *New Zealand Journal of Agricultural Research 21:* 261-265.

Fairley, R. I. 1985. Grass root production in restored soil following open cast mining. pp.81-85 In: Fitter, A. H. Ed. *Ecological interactions in soil.* Blackwell, London.

Feeney, G. J., Hester, D. D. 1967. Stock market indices: a principal component analysis. In: *Risk aversion and portfolio choice.* Edited by D. Hester & J. Tobin. Wiley, New York. pp 118-130. Cited by Jolliffe, 1986.

Fitter, A. H. 1976. Effects of nutrient supply and competition from other species on root growth of *Lolium perenne* in soil. *Plant and Soil 45:* 177-189.

Gandar, P. W., Hughes, K. A. 1988. Kiwifruit root systems 1. Root-length densities. *New Zealand Journal of Experimental Agriculture 16:* 35-46.

Garwood, E. A. 1965. *Some factors which influence the root growth of herbage species.* M. Sc. Thesis. University of Reading.

Garwood, E. A. 1967a. Seasonal variation in appearance and growth of grass roots. *Journal of the British Grassland Society 22:* 121-129.

Garwood, E. A. 1967b. Studies on the roots of grasses. *Annual report of the Grassland Research Institute, Hurley, 1966.* pp 72-79.

Garwood, E. A. 1968. Some effects of soil-water conditions and soil temperature on the roots of grasses and clover 2. Effects of variation in the soil-water content and in soil temperature on root growth. *Journal of the British Grassland Society 23:* 117-128.

Garwood, E. A. 1969. Seasonal tiller populations of grass and grass/clover swards with and without irrigation. *Journal of the British Grassland Society 24:* 333-344.

Gibbs, R. J. 1986. *Changes in soil structure under different cropping systems.* Ph D thesis, Lincoln College, Canterbury, 261pp.

Gill, J. L. 1986. Repeated measurement: sensitive tests for experiments with few animals. *Journal of Animal Science 63:* 943-954.

Gillingham, A. G. 1982. Topographic and management effects on dung distribution by grazing sheep. *Proceedings of the New Zealand Grassland Association 43:* 161-170.

Grant, S. A., Barthram, G. T., Torvell, L., King, J., Elston, D. A. 1988. Comparison of herbage production under continuous stocking and intermittent grazing. *Grass and Forage Science 43:* 29-39.

Grant, S. A., King, J., Barthram, G. T., Torvell, L. 1981. Responses of tiller populations to variation in grazing management in continuously stocked swards as affected by the time of year. pp 81 - 84 In C. E. Wright Ed. *Plant physiology and herbage production.* British Grassland Society, Occasional symposium No. 13. British Grassland Society, Hurley.

Hainsworth, J. M., Aylmore, L. A. G. 1986. Water extraction by single plant roots. *Journal of the Soil Science Society of America 50:* 841-848.

Hampton, J.G.; Hebblethwaite, P.D.; Clemence, T.G.A.; 1987. The effect of lodging on ^{14}C-assimilate distribution after anthesis in *Lolium perenne* cv S24 grown for seed. *Grass and Forage Science 42:* 121-127.

Harris, A. J., Boyd, A. F., Anderson, L. B., Vartha, E. W., Ryan, D. L. 1977. Performance of four ryegrass cultivars under cutting. *New Zealand Journal of Experimental Agriculture 5:* 391-395.

Harris, W. 1973. Ryegrass genotype-environment interactions in response to density, cutting height, and competition with white clover. *New Zealand Journal of Agricultural Research 16:* 207-222.

Harris, W., Pandey, K. K., Gray, Y. S., Couchman, P. K. 1979. Observations on the spread of perennial ryegrass by stolons in a lawn. *New Zealand Journal of Agricultural Research 22:* 61-68.

Hay, M. J. M., Chapman, D. F. 1984. Observations on the distribution of white clover stolons in hill swards. *Proceedings of the New Zealand Grassland Association 45:* 124-130.

Hay, M. J. M., Chapman, D. F., Hay, R. J. M., Pennell, G. G. L., Woods, P. W., Fletcher, R. H., 1987. Seasonal variation in the vertical distribution of clover stolons in grazed swards. *New Zealand Journal of Agricultural Research 30:* 1-8.

Haylock, J. A. 1987. *Pasture condition and soil desiccation as influences on tunnel-related erosion.* M. Phil Thesis, Massey University. 195pp

Head, G. C. Estimating seasonal changes in the quantity of white unsuberised root on fruit trees. *Journal of Horticultural Science 41:* 197-206.

Hendrickson, A. H., Veihmeyer, F. J. 1931. Influence of dry soil on root extension. *Plant Physiology 6:* 567-576.

Hilder, E. J. 1964. The distribution of plant nutrients by sheep at pasture. *Proceedings of the 5th biennial conference, Australian Society of Animal Production:* 241-248.

Hitch, P. A., Sharman, B. C. 1971. The vascular pattern of festucoid grass axes, with particular reference to nodal plexi. *Botanical Gazette 132:* 38-56.

Hodgson, J. 1990. *Grazing Management. Science into practice.* Longman scientific & technical, Harlow, England. 203pp.

Hodgson, J., Ollerenshaw, J. H. 1969. The frequency and severity of defoliation of individual tillers in set-stocked swards. *Journal of the British Grassland Society 24:* 226-237.

Hodgson, J., Wade, M. H. 1978. Grazing management and herbage production. Proceedings British Grassland Society winter meeting, 1978. Grazing, sward production and livestock output.

Hoogendoorn, C. *Studies on the effects of grazing regime on sward and dairy cow performance.* Ph. D. Thesis. Massey University.

Hughes, T. P., 1983. Late spring grazing management. *Proceedings of the Lincoln College Farmer's Conference 33:* 18-21.

Hunt, W. F. 1989. Grazing management effects on perennial ryegrass and white clover tiller populations. *Proceedings XVI International Grassland Congress, Nice, France:* 1055-1056.

Hunt, W. F., Easton, H. S. 1989. Fifty years of ryegrass research in New Zealand. *Proceedings of the New Zealand grassland Association 50:* 11-23.

Hunt, W. F., Field, T. R. O. 1978. Growth characteristics of perennial ryegrass. *Proceedings of the New Zealand Grassland Association 40:* 104-113.

Hunt, W. F., Halligan, G. 1981. Growth and developmental responses of perennial ryegrass grown at constant temperature. I. Influence of light and temperature on growth and net assimilation. *Australian Journal of Plant Physiology 12:* 69-76.

Hunt, W. F., Thomas V. J. 1985. Growth and developmental responses of perennial ryegrass sown at constant temperature. II. Influence of light and temperature on leaf, tiller, and root appearance. *Australian Journal of Plant Physiology 12:* 69-76.

Jackson, D. K. 1976. Influence of patterns of defoliation on sward morphology. pp 51 - 60 In: J. Hodgson & D. K. Jackson Eds. *Pasture utilisation by the grazing animal.* British Grassland Society occasional symposium No. 8, The British Grassland Society, Hurley.

Jacques, W. A. 1937. The effect of different rates of defoliation on the root development of certain grasses. *New Zealand Journal of Science and Technology 19:* 441-450.

Jacques, W. A. 1943. Root development in some New Zealand pasture plants. II Perennial ryegrass, (*Loium perenne*), (*Dactylis glomerata*), and white clover (*Trifolium repens*). *New Zealand Journal of Science and Technology A25:* 91-117.

Jacques, W. A. 1956. Root development in some common New Zealand pasture plants. IX The root replacement pattern in perennial ryegrass (*Lolium perenne*). *New Zealand Journal of Science and Technology 38A:* 160-165.

Jacques, W. A., Edmond, D. B. 1952. Root development in some common New Zealand pasture plants. V. The effect of defoliation and root pruning on cocksfoot (*Dactylis glomerata*) and perennial ryegrass (*Lolium perenne*). *New Zealand Journal of Science and Technology A34:* 231-248.

Jacques, W. A., Schwass, R. H. 1956. Root development in some common New Zealand pasture plants. VII. Seasonal root replacement in perennial ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*) and tall fescue (*Festuca arundinacea*). *New Zealand Journal of Science and Technology 37A:* 569-583.

Jeffay, H., Alvarez, J. 1961. Liquid scintillation counting of carbon-14. Use of ethanolamine-ethylene glycol monomethyl ether-toluene. *Analytical Chemistry 33:* 612-615.

Jewiss, O. R. 1966. Morphological and physiological aspects of the growth of grasses during the vegetative phase. In: *The growth of cereals and grasses.* Proceedings 12th Easter School in agricultural science, Nottingham University. Ed. by F. L. Milthorpe & J. D. Ivins. Butterworths, London. pp39-55.

Jewiss, O. R. 1972. Tillering in grasses - its significance and control. *Journal of the British Grassland Society 27:* 65-82.

Jewiss, O. R. 1981. Shoot development and number. Chapter 5 In: *Sward measurement handbook.* Ed. by J. Hodgson, R. D. Baker, A. Davies, A. S. Laidlaw & J. D. Leaver. British Grassland Society, Hurley. 277pp.

Jolliffe, I. T. 1986. *Principal component analysis.* Springer-Verlag, New York. 271pp.

Kays, S., Harper, J. L. 1974. The regulation of plant and tiller density in a grass sward. *Journal of Ecology 62:* 97-105.

Klepper, E., Belford, R. K., Rickman, R. W. 1984. Root and shoot development in winter wheat. *Agronomy Journal 76:* 117-122.

Korte, C. J. 1981. Studies of late spring grazing management in perennial ryegrass dominant pasture. Ph D. Thesis, Massey University. 176pp.

Korte, C. J. 1986. Tillering in 'Grasslands Nui' perennial ryegrass swards 2. Seasonal pattern of tillering and age of flowering tillers with two mowing frequencies. *New Zealand Journal of Agricultural Research 29:* 629-638.

Korte, C. J., Harris, W. 1987. Stolon development in grazed 'Grasslands Nui' perennial ryegrass. *New Zealand Journal of Agricultural Research 30:* 139-148.

Korte, C. J., Watkin, B. R., Harris, W. 1982. Use of residual leaf area index and light interception as criteria for spring-grazing management of a ryegrass dominant pasture. *New Zealand Journal of Agricultural Research 25:* 309-319.

Korte, C. J., Watkin, B. R., Harris, W. 1984. Effects of timing and intensity of spring grazings on reproductive development, tillering, and herbage production of perennial ryegrass dominant pasture. *New Zealand Journal of Agricultural Research 27:* 135-149.

Korte, C. J., Watkin, B. R., Harris, W. 1987. Tillering in 'Grasslands Nui' perennial ryegrass swards 3. Aerial tillering in swards grazed by sheep. *New Zealand Journal of Agricultural Research 30:* 9-14.

Lambert, M. G., Clark, D. A., Grant, D. A., Costall, D. A. 1986. Influence of fertiliser and grazing management on North Island moist hill country. 2. Pasture botanical composition. *New Zealand Journal of Agricultural Research 29:* 1-10.

Langer, R. H. M., 1956. Growth and nutrition of timothy (*Phleum pratense*) I. The life history of individual tillers. *Annals of Applied Biology 44:* 166-187.

Langer, R. H. M. 1963. Tillering in herbage grasses. *Herbage Abstracts 33:* 141-148.

Langer, R. H. M. 1979. *How grasses grow.* The Institute of Biology's studies in biology No. 34. Edward Arnold.

Lantinga, E. A. 1985. *Productivity of grasslands under continuous and rotational grazing.* Ph D Thesis, Wageningen University. 109 pp.

LaTour, S. A., Miniard, P. W. 1983. The misuse of repeated measures analysis in marketing research. *Journal of Marketing Research 20:* 45-77.

Lewis, E. R. 1977. *Network models in population biology.* Springer-Verlag, Berlin. 402pp.

L'Huillier, P. J. 1987. Tiller appearance and death of of *Lolium perenne* in mixed swards grazed by dairy cattle at two stocking rates. *New Zealand Journal of Agricultural Research 30:* 15-22.

L'Huillier, P. J., Aislabie, D. W. 1988. Natural reseeding in perennial ryegrass/ white clover dairy pastures. *Proceedings of the New Zealand Grassland Association 49:* 111-115.

L'Huillier, P. J., Bryant, A. M. 1987. Influence of spring grazing management on pasture composition and density and dairy cow performance. *Proceedings of the 4th animal science congress of the Asian-Australasian Association of Animal Production Societies:* 141.

Lonsdale, W. M., Watkinson, A. R. 1982. Light and self-thinning. *New Phytologist 90:* 431-445.

Lonsdale, W. M., Watkinson, A. R. 1983. Tiller dynamics and self-thinning in grassland habitats *Oecologia 60:* 390-395.

Mackie-Dawson, L. A., Atkinson, D. 1991. Methodology for the study of roots in field experiments and the interpretation of results. pp 25-47 In: D. Atkinson Ed. *Plant root growth, an ecological perspective.* British Ecological Society special publication No. 10. Blackwell Scientific Publications, Oxford.

Manly, B. F. J. 1986. "*Multivariate statistical methods: a primer.*" Chapman and Hall, London. 159pp.

Matthew, C. 1991. "Late control" - what is it, and why should it work? *Massey University Dairyfarming Annual 43:* 37-42.

Matthew, C., Mackay, A. D., Chu, A. C. P., 1986. Techniques for measuring the root growth of a perennial ryegrass (*Lolium perenne* L.) pasture under contrasting spring managements. *Proceedings of the Agronomy Society of New Zealand 16:* 59-64.

Matthew, C., Tillman, R. W., Hedley, M. J., Thompson, M. C. 1988. Observations on the relationship between soil fertility, pasture botanical composition, and pasture growth rate; for a North Island Lowland pasture. *Proceedings of the New Zealand Grassland Association 49:* 141-144.

Matthew, C.; Xia, J. X.; Chu, A. C. P.; Mackay, A. D.; Hodgson, J; 1991. Relationship between root production and tiller appearance rates in perennial ryegrass (*Lolium perenne* L.) pp 281 - 290 In: D. Atkinson Ed. *Plant root growth, an ecological perspective.* British Ecological Society special publication No. 10. Blackwell Scientific Publications, Oxford.

Matthew, C., Xia, J.X., Hodgson, J., Chu, A.C.P., 1989. Effect of late spring grazing management on tiller age profiles and summer-autumn pasture growth rates in a perennial ryegrass (*Lolium perenne* L.) sward. *Proceedings XVI International grasslands congress, Nice, France:* 521-522.

Matyac, C. A., Cofer, G. P., Bailey, J. E., Johnson, G. A. 1989. In situ observations of root-gall formation using nuclear magnetic resonance imaging. *Journal of Nematology 21:* 131-134.

McMeekan, C. P. 1960. Grazing management. *Proceedings of the VIIIth International Grasslands Congress:* 21-26.

McMeekan, C. P., Walshe, M. J. 1963. The inter-relationships of grazing method and stocking rate in the efficiency of pasture utilization by dairy cattle. *Journal of Agricultural Science, Cambridge, 61:* 147-163.

McMichael, B. L. and Taylor, H. M. 1987. Applications and limitations of rhizotrons and minirhizotrons. Chapter 1 In: Taylor, H. M. Ed. *Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics.* American Society of Agronomy special publication No. 50. 143pp.

Mitchell, K. J. 1953. Influence of light and temperature on the growth of ryegrass (Lolium spp.) II. The control of lateral bud development. *Physiologia Plantarum 6:* 425-443.

Mitchell, K. J. 1954. Influence of light and temperature on the growth of ryegrass (Lolium spp.). III. Pattern and rate of tissue formation. *Physiologia Plantarum 7:* 51-65.

Mitchell, K. J., Coles, S. T. J., 1955. Effects of defoliation and shading on short-rotation ryegrass. *New Zealand Journal of Science and Technology 36A:* 586-604.

Mitchell, K. J., Glenday, A. C. 1958. The tiller population of pastures. *New Zealand Journal of Agricultural Research 1:* 305-318.

Mwebaze, S. 1986. Factors affecting the regrowth of Matua prairie grass (*Bromus willdenowii* Vahl.). Dip. Agr. Sci. Thesis, Massey University. 49pp.

Neuteboom, J. H., Lantinga, E. A. 1989. Tillering potential and relationship between leaf and tiller production in perennial ryegrass. *Annals of Botany 63:* 265-270.

Newman, E. I. 1966. A method of estimating the total length of root in a sample. *Journal of Applied Ecology 3:* 139-145.

Ong, C.K. 1978a. The physiology of tiller death in grasses. (1) The influence of tiller age, size and position. *Journal of the British Grassland Society 33:* 197-203.

Ong, C.K. 1978b. The physiology of tiller death in grasses. (2) The causes of tiller death in a grass sward. *Journal of the British Grassland Society 33:* 204-211.

Ong, C. K., Marshall, C. 1979. The growth and survival of severely shaded tillers in *Lolium perenne* L. *Annals of Botany 43:* 147-155.

Parmenter, G. A., Boswell, C. C. 1983. Effect of number and timing of winter grazings on winter and spring production. *New Zealand Journal of Experimental Agriculture 11:* 281-287.

Parsons, A. J. 1981. Carbon exchange and assimilate partitioning. Chapter 10 in: J. Hodgson, R. D. Baker, A. Davies, A. S. Laidlaw & J. D. Leaver Eds. *Sward measurement handbook*. British Grassland Society, Hurley. pp179-208.

Parsons, A. J., Collett, B., Lewis, J. 1984. Changes in the structure and physiology of a perennial ryegrass sward when released from a continuous stocking management: implications for the use of exclusion cages in continuously stocked swards. *Grass and Forage Science 39:* 1-9.

Parsons, A. J., Leafe, E. L., Collett, B., Stiles, W. 1983a. The physiology of grass production under grazing I. Characteristics of leaf and canopy photosynthesis in continuously grazed swards. *Journal of Applied Ecology 20:* 117-126.

Parsons, A. J., Leafe, E.L., Collett, B. Penning, P. D., Lewis, J. 1983b. The physiology of grass production under grazing II. Photosynthesis, crop growth and animal intake of continuously-grazed swards. *Journal of Applied Ecology 20:* 127-139.

Parsons, A. J., Robson, M. J. 1981a. Seasonal changes in the physiology of S24 perennial ryegrass (*Lolium perenne* L.). 2. Potential leaf and canopy photosynthesis during the transition from vegetative to reproductive growth. *Annals of Botany 47:* 249-258.

Parsons, A. J., Robson, M. J. 1981b. Seasonal changes in the physiology of S23 perennial ryegrass (*Lolium perenne* L.). 3. Partition of assimilates between root and shoot during the transition from vegetative to reproductive growth. *Annals of Botany 48:* 733-744.

Parsons, A. J., Robson, M. J. 1982. Seasonal changes in the physiology of S23 perennial ryegrass (*Lolium perenne* L.). 4. Comparison of the carbon balance of the reproductive crop in spring and the vegetative crop in autumn. *Annals of Botany 50:* 167-177.

Parsons, A. J., Johnson, I. R., Harvey, A. 1988a. Use of a model to optimize the interaction between frequency and severity of intermittent defoliation and to provide a fundamental comparison of the continuous and intermittent defoliation of grass. *Grass and Forage Science 43:* 49-59.

Parsons, A. J., Johnson, I. R., Williams, J. H. H. 1988b. Leaf age structure and canopy photosynthesis in rotationally and continuously grazed swards. *Grass and Forage Science 43:* 1-14.

Pasumarty, S. V. 1987. *Role of photosynthates in flower development in white clover.* M. Sc. Thesis, Massey University.

Persson, H. 1980. Spatial distribution of fine root growth, mortality and decomposition in a young Scots pine stand in Central Sweden. *Oikos 34:* 77-87.

Riddet, W. 1951. Advantages of rationed grazing. *Dairyfarming Annual 6:* 75-83.

Roadley, J. D. 1985. Dairying with border dyke irrigation. *Proceedings of the New Zealand Grassland Association 46:* 163-165.

Roberts, E. A., Raison, J. M. 1983. An analysis of the moisture content of soil cores in a designed experiment. *Biometrics 39:* 1097-1105.

Roberts, R. A., Hunt, I. V. 1936. The effect of shoot cutting on the growth of root and shoot of perennial ryegrass (*Lolium perenne* L.) and of timothy (*Phleum pratense* L.) *Welsh Journal of Agriculture 12:* 158-174.

Robson, M. J., Ryle, G. J. A., Woledge, J. 1988. The grass plant - its form and function. In: M. B. Jones & A. Lazenby Eds. *The grass crop. The physiological basis of production.* Chapman and Hall, London, New York. pp. 25-83.

Rogers, H. H., Bottomley, P. A. 1987. In situ nuclear magnetic resonance imaging of roots: influence of soil type, ferromagnetic particle content, and soil water. *Agronomy Journal 79:* 957-965.

Rowell, J. G., Walters, D. E. 1976. Analysing data with repeated observations on each experimental unit. *Journal of Agricultural Science, Cambridge. 87:* 423-432.

Ryle, G. J. A. 1970. Distribution patterns of assimilated ^{14}C in vegetative and reproductive shoots of *Lolium perenne* and *L. temulentum. Annals of Applied Biology 66:* 155-167.

Sangakkara, U. R., Roberts, E., Watkin, B. R. 1982. Grass species used and pasture establishment practices in central New Zealand. *New Zealand Journal of Experimental Agriculture 10:* 359-364.

Schnyder, H., Nelson, C. J. 1988. Diurnal growth of tall fescue leaf blades. I. Spatial distribution of growth, deposition of water, and assimilate import in the elongation zone. *Plant Physiologist 86:* 1070-1076.

Schuurman, J. J., Knot L. 1974. The effect of nitrogen on the root and shoot development of *Lolium multiflorum* var. *westerwoldicum. Netherlands Journal of Agricultural Science 22:* 82-88.

Sheard, R. W., Winch, J. E. 1966. The use of light interception, grass morphology and time as criteria for the harvesting of timothy, smooth brome and cocksfoot. *Journal of the British Grassland Society 21:* 231-237.

Sheath, G. W. 1981. *Lotus pedunculatus* - An agricultural plant? *Proceedings of the New Zealand Grassland Association 42:* 160-168.

Sheath, G. W., Bircham, J. S. 1983. Grazing management in hill country: pasture production. *Proceedings of the Ruakura Farmer's Conference 35:* 41-45.

Sheath, G. W., Hay, R. J. M., Giles, K. H. 1987. Managing pastures for grazing animals. Chapter 5 In: *Livestock feeding on pasture.* New Zealand Society of Animal Production, Occasional publication No. 10. pp 65 - 74.

Sheath, G. W., Webby, R. W., Pengelly, W. J. 1984. Management of late spring-early summer pasture surpluses in hill country. *Proceedings of the New Zealand Grassland Association 45:* 199-206.

Silsbury, J. H. 1964. Tiller dynamics, growth, and persistency of *Lolium perenne* L. and of *Lolium rigidum* Gaud. *Australian Journal of Agricultural Research 15:* 9-20.

Silsbury, J. H. 1970. Leaf growth in pasture grasses. *Tropical Grasslands 4:* 17-36.

Simon, J. G., Lemaire, G. 1987. Tillering and leaf area index in grasses in the vegetative phase. *Grass and Forage Science 42:* 373-380.

Smith, M. E., Dawson, A. D., Short, W. D. 1976. Increasing hill country production without increasing costs. *Proceedings of the Ruakura Farmers Conference 28:* 22-26.

Smucker, A. J. M., McBurney, S. L., Srivastava, A. K. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agronomy Journal 74:* 500-503.

Soper, K., Mitchell, K. J. 1956. The developmental anatomy of perennial ryegrass (*Lolium perenne* L.). *New Zealand Journal of Science and Technology A37:* 484-504.

Steel, R. G. D., Torrie, J. H. 1981. *Principles and procedures of statistics, a biometrical approach.* McGraw-Hill, Japan. 633pp.

Steen, E. 1983. The net stocking method for studying quantitative and qualitative variation with time of grass roots. pp 63 - 74 In W. Bohm, L. Kutschera & E. Lichtenegger Eds. *Root ecology and its practical application*. Proceedings of an international symposium, Gumpenstein, 1982.

Steen, E. 1984. Variation of root growth in a grass ley studied with a mesh bag technique. *Swedish Journal of Agricultural research 14:* 93-97.

Steen, E. 1985. Root and rhizome dynamics in a perennial grass crop during an annual growth cycle. *Swedish Journal of Agricultural Research 15:* 25-30.

Stuckey, I. H. 1941. Seasonal growth of grass roots. *American Journal of Botany 28:* 486-491.

Suckling, F. E. T. 1975. Pasture management trials on unploughable hill country at Te Awa. III. Results for 1959 - 69. *New Zealand Journal of Experimental Agriculture 3:* 351-456.

Tainton, N. M. 1974. Effects of different grazing rotations on pasture production. *Journal of the British Grassland Society 29:* 191-202.

Tallowin, J. R. B. 1981. An interpretation of tiller number changes under grazing. pp 77-80 In *Plant physiology and herbage production.* Ed. by C. E. Wright. British Grassland Society, Occasional symposium No. 13. British Grassland Society, Hurley.

Taylor, H. M. and Klepper, E. 1975. Water uptake by cotton root systems: An examination of assumptions in the single root model. *Soil Science 120:* 57-67.

Tennant, D. 1975. A test of a modified line intersect method of estimating root length. *Journal of Ecology 63:* 995-1001.

Thom, E. R., Sheath, G. W., Bryant, A. M. 1989. Seasonal variations in total nonstructural carbohydrate and major element levels in perennial ryegrass and paspalum in mixed pasture. *New Zealand Journal of Agricultural Research 32:* 157-165.

Thomas, H. 1980. Terminology and definitions in studies of grassland plants. *Grass and Forage Science 35:* 13-23.

Thornley, J. H. M., 1972. A balanced quantitative model for root: shoot ratios in vegetative plants. *Annals of Botany 36:* 431-441.

Troughton, A. 1956. Studies on the growth of young grass plants with special reference to the relationship between the shoot and root systems. *Journal of the British Grassland Society 11:* 56-65.

Troughton, A. 1957. *The underground organs of herbage grasses*. Bulletin No. 44. Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire. 163pp.

Troughton, A. 1978. The influence of reproductive development upon the root system of perennial ryegrass and some effects upon herbage production. *Journal of Agricultural Science, Cambridge. 91:* 427-431.

Troughton, A. 1981a. Length of life of grass roots. *Grass and Forage Science 36:* 117-120.

Troughton, A. 1981b. Root mass and distribution. Chapter 8 In *Sward measurement handbook*. Ed. by J. Hodgson, R. D. Baker, A. Davies, A. S. Laidlaw & J. D. Leaver. British Grassland Society, Hurley. 277pp.Ed. by J. Hodgson, R. D. Baker, A. Davies, A. S. Laidlaw & J. D. Leaver. British Grassland Society, Hurley. 277pp.

Ward, K. J., Klepper, E., Rickman, R. W., Allmaras, R. R. 1978. Quantitative estimation of living wheat-root lengths in soil cores. *Agronomy Journal 70:* 675-677.

Warembourg, F. R., Paul, E. A. 1977. Seasonal transfers of assimilated ^{14}C in grassland: plant production and turnover, translocation and respiration. pp 133-140 In J. K. Marshall Ed. *The below ground ecosystem: a synthesis of plant related processes.* Range Science Department science series No. 26, Colorado State University.

Weaver, J. E., Voigt, J. W. 1950. Monolith method of root sampling in studies of succession and degeneration. *Botanical Gazette 111:* 286-299.

Weaver, J. E., Zink, E. 1946. Length of life of roots of ten species of perennial range and pasture species. *Plant Physiology 21:* 201-207.

Wilson, J. B. 1988. A review of evidence on the control of shoot: root ratio, in relation to models. *Annals of Botany 61:* 433-449.

Woledge, J. 1972. The effect of shading on the photosynthetic rate and longevity of grass leaves. *Annals of Botany 36:* 551-561.

Woledge, J. 1977. The effects of shading and cutting treatments on the photosynthetic rate of ryegrass leaves. *Annals of Botany 41:* 1279-1286.

Wolf, G. L. 1986. Vitalism and proton relaxation. *Investigative radiology 21:* 427-428.

Xia, J. X. 1991. *The effects of defoliation on tissue turnover and pasture production in perennial ryegrass, prairie grass and smooth bromegrass pasture.* Ph. D. Thesis. Massey University. 173pp.

Yoda, K., Kira, T. Ogawa, H., Hozumi, H. 1963. Self thinning in overcrowded pure stands under cultivated and natural conditions. *Journal of Institute of Polytechnics, Osaka City University, Series D, 14:* 107-129.