Chapter 1

Introduction

In dairying regions of subtropical Australia, the majority of milk production comes from pastures based on tropical grasses. As these tropical grasses typically decline in growth and quality during the cooler months (late autumn to mid-spring), temperate pasture species are sown to prevent a shortage of forage during this time. The ryegrass (*Lolium*) species are the most widely sown temperate grass species, and under favourable management and climate, are capable of producing high yields of quality forage (over 25 t dry matter (DM)/ha) (Fulkerson *et al.* 1993a).

Annual (*L. temulentum* L. or *L. rigidum* Gaudin) and biennial (*L. multiflorum* L.) ryegrass genotypes are generally sown in monoculture at high seeding rates (35 to 40 kg ryegrass/ha) direct into a tropical grass base each year, and fertilised with 50 to 60 kg nitrogen (N)/ha/month. Perennial ryegrass (*L. perenne* L.) genotypes are generally sown at lower seeding rates as a mixed pasture with white clover (*Trifolium repens* L.), and only require strategic applications of N fertiliser in winter and spring of the establishment year, after which the clover is relied upon to supply adequate N for this pasture. The benefits of a perennial, over an annual, pasture include a longer growing season and reduced cost of pasture re-establishment. In addition, the inclusion of a legume increases pasture quality, and reduces the need for fertiliser N input, which in turn reduces fertiliser costs, and leads to less soil acidification. However, perennial ryegrass pastures have not persisted more than 2 years in the subtropics, and this has led to the majority of dairy farmers opting to re-establish shorter-lived annual or biennial ryegrass pastures each year (Lambourne 1985; Fulkerson *et al.* 1993a).

This lack of persistence of perennial ryegrass in a subtropical environment is believed to be associated with severe stress over summer, and in particular during the first summer following autumn sowing, when there is a substantial loss of ryegrass plants. The causal factors associated with death of ryegrass plants over the summer include vigorous

competition from tropical grasses which invade the sown pasture, lack of ryegrass root development (Fulkerson *et al.* 1993b), and low levels of plant energy reserves (Fulkerson and Lowe 1994). However, the relative importance of each of these factors on plant survival, and their mechanism of action, has not yet been defined.

Fulkerson *et al.* (1993b) and Fulkerson and Slack (1994a) showed that survival of perennial ryegrass plants over the first summer was influenced by pasture management techniques, including seedbed preparation, irrigation and defoliation, with defoliation interval having the largest single effect on plant persistence and productivity. Defoliating ryegrass plants when they had completed their regrowth cycle based on expansion of 3 leaves/tiller, resulted in less tropical grasses invading the pasture and more ryegrass plants surviving summer, than when pasture was defoliated more frequently.

White clover also suffers problems with persistence in the subtropics, with its lifespan limited to only 3 or 4 years from time of sowing. There is some evidence from studies on white clover in kikuyu pasture that the low persistence of white clover in this environment may be in part due to a gradual buildup of nematodes and perhaps other pathogens in soil (Fulkerson and Reeves 1996; Fulkerson and Slack 1996a). Further research is necessary to clearly identify the factors contributing to poor persistence of white clover.

The studies reported in this thesis aimed to confirm the importance of defoliation interval under grazing, and to determine the mechanism by which defoliation affects the survival of perennial ryegrass and white clover. The initial strategy was to determine the critical time of defoliation on subsequent plant survival over summer. The observed association between ryegrass survival and tiller and root growth, and water soluble carbohydrate (WSC) reserves, was then studied further in the glasshouse, in order to determine the mechanism of action. Inducing plants to become dormant over summer, or to regenerate from seed the following autumn, was then evaluated as an alternative to managing plants to survive summer. Lastly, the effect of defoliation on the white clover component of mixed perennial ryegrass/white clover pasture was studied.

Chapter 2

Review of literature

2.1 Introduction

2.1.1 History of dairy pastures in subtropical Australia

The northern dairying region of Australia includes the subtropical region, which extends along the coast from Taree in New South Wales (32°S) to Rockhampton in Queensland (23°S), and the tropical region, which is north of Rockhampton (Plate 1). In New South Wales, the tropical grass kikuyu (*Pennisetum clandestinum* Hochst. ex. Chiov.) forms the base species in 30% of dairy pastures, and accounts for 70% of milk produced in the summer-autumn period (Anon 1992a).
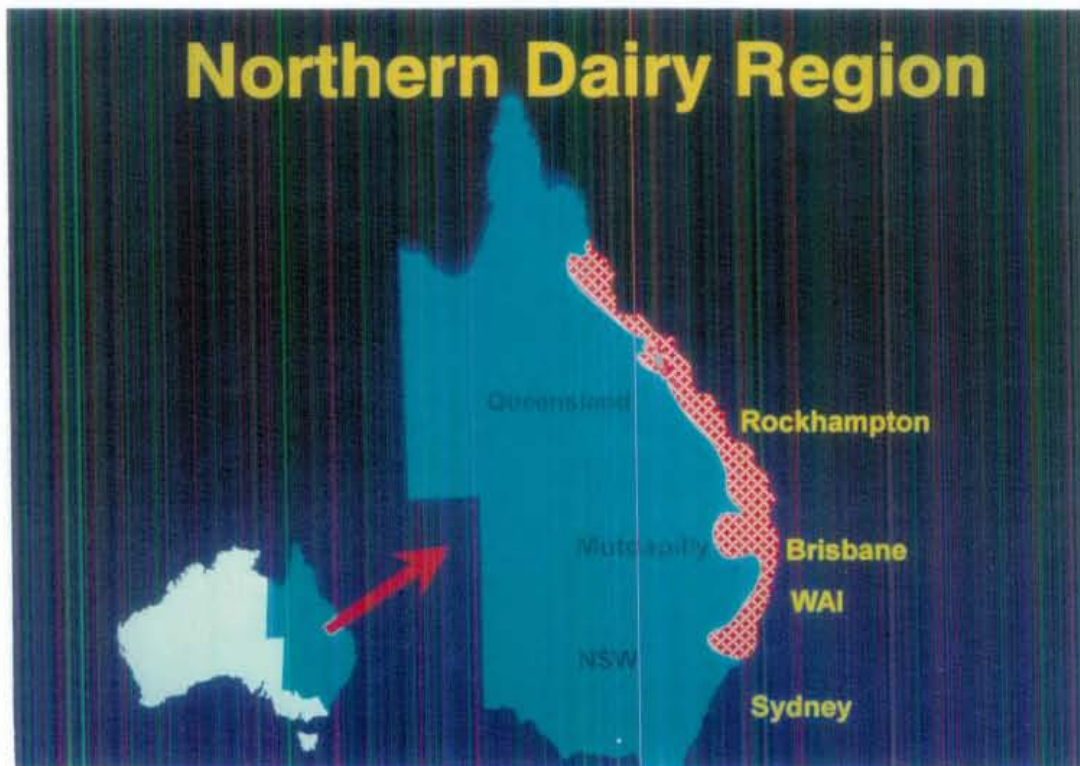

Plate 1. The subtropical dairy region of eastern Australia.

However, kikuyu, and other summer-growing tropical grasses (paspalum (*Paspalum dilatatum* Poiret), narrow-leaf carpet grass (*Axonopus affinis* Chase), setaria (*Setaria sphacelata* (Schunach.) Stapf & C.E. Hubbard), rhodes grass (*Chloris gayana* Kunth.)), are almost dormant from late autumn to mid-spring, and the resultant decline in productivity and quality of these grasses during this period results in a shortage of feed. Temperate pastures are the most common, least expensive forage source used to fill this winter/spring feedgap in subtropical dairy systems (Lambourne 1985; Fulkerson *et al.* 1993a). This is illustrated in Plate 2.
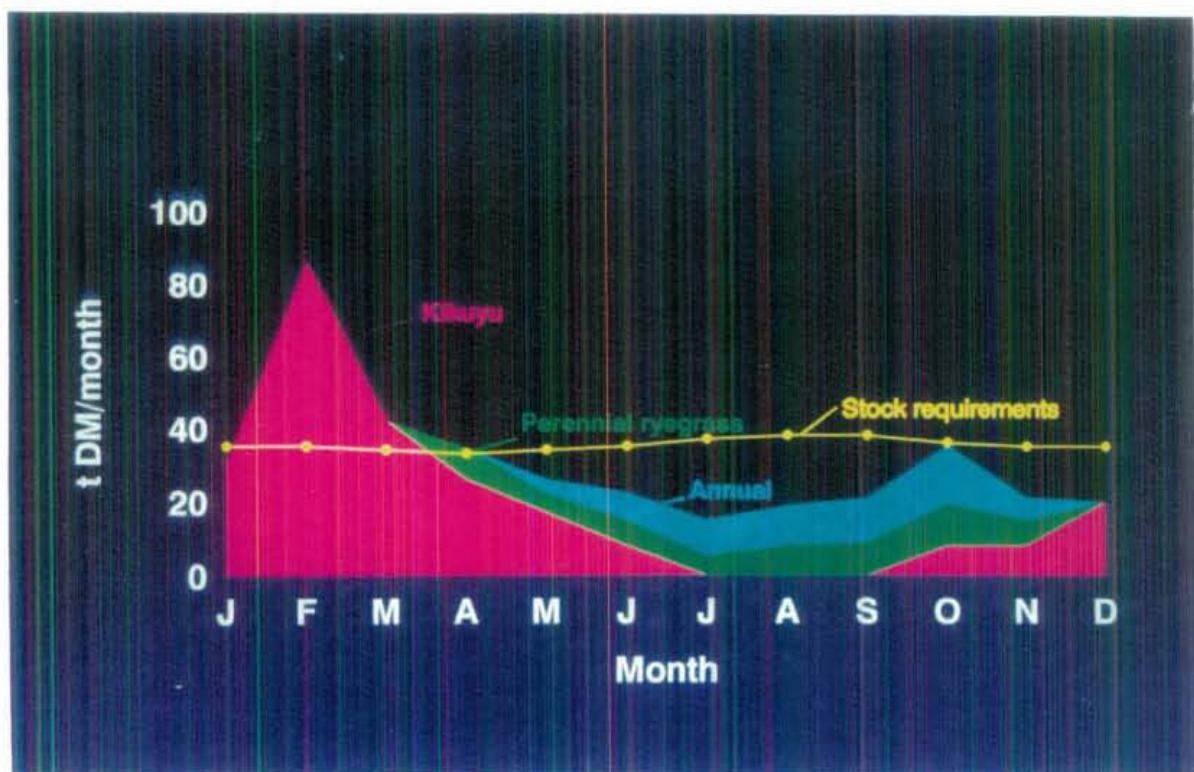

Plate 2. Pasture availability and feed requirements (t DM/month) on a typical dairy farm in subtropical Australia in the early 1990s. Pasture comprises 14 ha of ryegrass (6 ha perennial, 8 ha annual) and 50 ha of kikuyu. The requirements are for 77 milkers and 45 replacement cows (Fulkerson *et al.* 1993a).

From Plate 2, it is clear that temperate species do not adequately fill the feedgap due to a lack of adequate irrigation capacity on many farms - the deficit is usually made up by

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concentrates and/or loss of body condition in cows.

Many temperate grasses have been trialed as pasture species in the subtropics, including ryegrasses, fescues (*Festuca* spp.), bromes and prairie grass (*Bromus* spp.), cocksfoot (*Dactylis glomerata* L.) and phalaris (*Phalaris tuberosa* L.) (Lowe and Bowdler 1984, 1995). Of these, the fescues proved the highest yielding and most persistent under cutting, however under subsequent grazing trials, poor acceptance by animals led to low adoption rates by farmers. The bromes, cocksfoot and phalaris genotypes exhibited uniform poor persistence and in some cases, poor yield, while ryegrass genotypes varied in persistence and production, generally exhibiting high production under adequate N fertiliser and irrigation, and minimal competition from tropical grasses (Lowe and Bowdler 1995). Of a range of legumes, the white clover cultivar 'Haifa' out-yielded all other white clover cultivars, as well as red clover (*Trifolium pratense* L.) and lotus (*Lotus pedunculatus* Cav.) cultivars, throughout summer, autumn and winter (Lowe and Bowdler 1984). Lucerne (*Medicago sativa* L.) has also yielded well under subtropical conditions, but in the past, cultivars have proven susceptible to aphids, nematodes and diseases (K.F. Lowe, personal communication).

Temperate pastures for dairy production in subtropical Australia fall into 3 main categories - annual pasture, perennial pasture, and perennial legume/tropical grass pasture.

Annual pastures are generally monocultures of annual or biennial ryegrass, annual clovers (persian (*Trifolium resupinatum* L.), subterranean (*Trifolium subterraneum* L.) or berseem (*Trifolium alexandrinum* L.)), or oats (*Avena sativa* L.). These pastures are generally sown at high seeding rates (20 kg clover/ha, 35 to 40 kg ryegrass/ha, 70 kg oats/ha) direct into a tropical grass pasture base each year (Fulkerson *et al.* 1993a).

Perennial temperate pastures consist of perennial ryegrass and white clover, and may be sown at high or low seeding rates. At high seeding rates (30 to 40 kg/ha combined seed), the high ryegrass plant density prevents clover making a significant contribution to N fertility in the first year, and pasture is usually fertilised with 50 to 60 kg N/ha/month. At lower seeding rates (15 to 20 kg/ha combined seed), N fertiliser is only applied

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strategically in the winter and spring of the establishment year (Fulkerson *et al.* 1993a). The third pasture type consists of legumes (white clover or lotus) sown into a kikuyu pasture base (Fulkerson and Slack 1996a).

Annual pastures are faster establishing and higher producing during their shorter growing season than perennial pastures, however the time of sowing (generally March to April) is likely to coincide with periods of heavy rainfall, and so establishment may be unreliable. Annual ryegrass pastures sown in March or April are ready to graze from May onwards, but quality declines from October due to stem elongation and formation of seed-heads, and plants die soon afterwards. Thus, a feedgap still exists between the decline in tropical pasture DM from March onwards (see Plate 2) and the time when annual temperate pastures are ready to be grazed.

In contrast, perennial ryegrass remains largely vegetative into November and December, thereby retaining a higher forage quality, and can be very productive until early summer, provided adequate soil moisture is available (Lambourne 1985). In addition to this extended period of pasture production, perennial ryegrass/white clover pasture offers additional advantages over annual ryegrass pasture. Firstly, cost of re-establishment is reduced, as pastures are only resown every 2 to 3 years. Secondly, the inclusion of a legume is possible, and this increases overall pasture quality, and reduces the need for fertiliser N input, which in turn reduces fertiliser costs, and decreases the likelihood of soil acidification, which can result when excess nitrate leaches through soil following heavy rainfall (Fulkerson *et al.* 1993a).

Although total yields of perennial ryegrass/white clover pastures can be high (Table 2.1), these pastures generally persist for only 2 years, are over-sown in the third year (usually with annual ryegrass), then re-established in the fourth year. As a consequence, throughout most of Queensland, and about 80% of northern New South Wales, dairy farmers sow annual ryegrass pasture (Fulkerson and Lowe 1994).

Table 2.1. Total annual yield (t DM/ha) of irrigated winter forage crops, from experiments conducted in south east Queensland (K.F. Lowe and T.M. Bowdler, unpublished data).

Pasture or Crop	Yield (t DM/ha)
Persian clover	15
Annual ryegrass (400 kg N/ha)	29
Perennial ryegrass (400 kg N/ha)	19
Perennial ryegrass/persian clover (100 kg N/ha)	17
Perennial ryegrass/white clover (100 kg N/ha)	18
Fescue (400 kg N/ha)	25
Lucerne	23
Oats (300 kg N/ha)	16

The lack of persistence of perennial ryegrass in the subtropics is linked to a loss of substantial numbers of ryegrass plant during the first summer after sowing. Factors associated with death of ryegrass plants over the summer include vigorous competition from tropical grasses (mainly kikuyu and paspalum) which invade the sown pasture sward (Lowe and Bowdler 1984; Fulkerson *et al.* 1993b), lack of ryegrass root development (Fulkerson *et al.* 1993b), and low plant energy reserve levels (Fulkerson and Lowe 1994), and each of these factors will be discussed further in later sections.

White clover also suffers problems with persistence in the subtropics, with a lifespan limited to 3 or 4 years, and this appears to be related to a gradual buildup of plant-parasitic nematodes and perhaps other plant pathogens in soil to levels detrimental to plant survival (Fulkerson and Reeves 1996; Fulkerson and Slack 1996a), although further research is necessary to identify causal factors involved.

Plates 3 and 4 show a first and third year perennial ryegrass/white clover dairy pasture in the subtropics, and illustrate the decline in density of perennial ryegrass plants over this time.

Plate 3. A first year perennial ryegrass/white clover dairy pasture.

Plate 4. A third year perennial ryegrass/white clover dairy pasture, showing severe decline of ryegrass, and dominance of clover, tropical grasses and weeds.

2.1.2 The subtropical environment for pasture growth

Dairy regions in subtropical Australia are described as having a hot, wet summer and autumn, and a cool, dry winter and spring (Lambourne 1985; Fulkerson *et al.* 1993a).

In terms of temperature, the main difference between subtropic and temperate areas is the higher average maximum and minimum temperatures, especially during the cooler months of the year, and less frosts in the subtropics. During the cooler months (late autumn to mid-spring) average temperatures are optimal for growth of temperate grasses (see section 2.2), and this is confirmed by the high pasture DM yields obtained. However, for the remainder of the year, the often high temperatures are detrimental to ryegrass growth. In addition, humidity is consistently higher throughout the year in the subtropics than in temperate areas, and this places additional stress on temperate species, as well as providing an environment conducive to spread of disease, particularly leaf or crown rust (*Puccinia coronata*) (Fulkerson *et al.* 1993a).

Average annual rainfall in the subtropical dairy areas of Australia ranges from 700 to 2,500 mm, varying with distance from the coast and elevation above sea level. Rainfall is summer-dominant, with about two-thirds of total rainfall occurring between November and April (Eyles and Cameron 1985; Lambourne 1985). Rainfall may average 100 to 150 mm/month in the wettest months (February and March), and 25 to 50 mm/month in the driest months (August and September) (Lambourne 1985; Fulkerson *et al.* 1993a). Waterlogging in summer and drought in spring are two relatively common features of subtropical dairy regions in Australia which can mitigate against pasture persistence.

Although the average annual rainfall in the subtropics is somewhat high and seasonal, it is relatively unreliable on a world basis, with much of it associated with infrequent storms with high levels of rainfall, compared to the comparatively reliable and more frequent rainfall events of many temperate regions. Due to higher overall temperatures than temperate areas, evaporation rates in the subtropics are also higher. In subtropical dairying regions of Australia, it is only between January and March that moisture is unlikely to restrict growth of temperate pasture (Mawson and White 1971). Thus, irrigation is an essential requirement to maintain productivity and persistence of temperate pastures in

subtropical Australia (Lambourne 1985, see also section 4.2.3).

Frost may occur between May and October, with frequency increasing with distance from the coast and elevation above sea level. In general, coastal areas average 20, and elevated areas 50, frost days/year, however frosts are generally not severe enough to cause damage to temperate pasture plants (Fulkerson et al. 1993a).

Soils in dairying areas of subtropical Australia range from arable clays to infertile duplex soils, with a large range of fertility levels. Availability of irrigation is the greatest restriction to location of dairy farms, so most farms are situated along waterways, on predominantly alluvial clay and loamy soils. The pH of soils in subtropical Australia is generally neutral to slightly acidic (pH 4.5 to 5 in $CaCl_2$) (Fulkerson and Lowe 1994), and this may compromise productivity and persistence of temperate pastures, which generally are not tolerant of an acidic soil environment (Cornish 1987).

The following sections describe the morphology of ryegrass and white clover plants, along with their physiological responses to variation in climate and management.

2.2 The ryegrass plant

2.2.1 Origin and distribution

Perennial ryegrass is a temperate perennial grass native to Europe, temperate Asia and North Africa (Balasko et al. 1995), and was the first grass cultivated for the purposes of grazing and hay making by farmers in the United Kingdom in the 16th century (Barnard and Frankel 1964). Perennial ryegrass is now widely distributed across North and South America, Europe, Australia and New Zealand, and is one of the world's primary pasture grasses (Barnard and Frankel 1964).

2.2.2 Ryegrass plant structure

The ryegrass plant is composed of a number of tillers, each bearing leaves and each having its own root system. Tillers are thus capable of acting as independent units, however,

being interconnected at the base, they are also capable of sharing water, nutrients and photosynthate (carbohydrates produced via photosynthesis) (Marshall and Sagar 1965; Gifford and Marshall 1973; Danckwerts and Gordon 1987).

Grass tillers are composed of nodes from where the leaves originate. In its vegetative state, the internodes are extremely short and are all located at the base of the tiller; leaves and tillers are initiated from these 'growing points' (apical meristems), located close to the soil surface and thus beyond the reach of grazing animals. It is only during reproduction, when growing points are elevated to form the seed-head, that they are at risk of being removed by grazing (Langer 1979). Thus what appears to be a solid tiller 'stem' is actually a collection of leaf sheaths tightly rolled inside one another.

Studies in New Zealand (Brock and Thomas 1991; Brock and Fletcher 1993; Brock et al. 1996) have shown that perennial ryegrass and cocksfoot plants exhibit a clonal pattern of growth similar to white clover (see section 3.2), with continual growth from apices accompanied by regular death of older basal stems, leading to plant fragmentation. After several years, what appeared to be large single perennial ryegrass plants were actually many small independent plants. Spring was the period during which fragmentation peaked, causing an increase in the population of small plants relative to larger plants (Brock et al. 1996).

Fragmentation of ryegrass plants is thus a vegetative means of propagation, which appears to take place during spring from the fourth or fifth year after sowing, (J.L. Brock, personal communication). In the subtropics, where perennial ryegrass plants usually persist for less than 3 years, there is probably little opportunity for this vegetative propagation, and indeed, it has been observed that ryegrass plants still remaining after 3 years had not fragmented (W.J. Fulkerson, unpublished data). In the following sections, growth of leaves, tillers and roots will be examined in more detail, and the role of plant energy reserves in their growth will be discussed.

2.2.2.1 Leaf. Ryegrass is termed a '3-leaf' plant, as each tiller maintains around 3 live leaves, the oldest leaf (first to emerge) beginning to senesce as the youngest fourth leaf

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emerges (Alberda 1966a; Davies 1969; Simons *et al.* 1972; Robson 1973a; Fulkerson and Slack 1994b). By inference then, leaf lifespan is related to leaf number, and evidently the factors which affect lifespan also affect the leaf appearance interval (Li; the time taken for one leaf to fully expand) to a similar extent. Thus, one leaf lifespan coincides with 3 Li's, but there is some variation, as described below:

1. Under favourable conditions, the number of live leaves/tiller remains relatively constant (Hunt and Brougham 1967; Robson 1973a), but leaf appearance and death are not precisely correlated throughout the year (Marshall 1987). For example, Vine (1983) observed in perennial ryegrass grown in field plots in the United Kingdom, that leaf death exceeded leaf appearance from late summer to autumn, while from late winter to spring, leaf appearance exceeded death. This seasonal variation has also been observed in Australia by W.J. Fulkerson (unpublished data).

2. An extreme of this spring variation is found during reproductive development when the tiller can have up to 6 live leaves.

The Li is influenced by temperature and to a lesser extent light, being longest at low temperatures and low light intensity (Mitchell 1953a; Silsbury 1970). Soil temperature appears to be more important than air temperature in determining leaf growth (Peacock 1975), and this is to be expected given the proximity of the growing point to the soil surface. Response to temperature varies with plant development stage (Marshall 1987), with emerging leaves of reproductive tillers being more responsive to temperature than leaves of vegetative tillers.

Li increases rapidly (i.e. plant growth rate declines) below about 10°C, although there is still some growth at 5°C. Above 25°C growth also declines and may cease above 30 to 35°C (Mitchell 1956; Cooper and Tainton 1968). Leaf function (photosynthesis) has a similar optimum temperature range to growth. Murata and Iyama (1963) showed that both perennial and annual ryegrasses photosynthesised most rapidly when temperatures were between 10 and 15°C. Rate of photosynthesis declined slowly at lower temperatures, but declined rapidly at temperatures greater than about 35°C. Thus for ryegrass, the optimum temperature range for overall plant growth and function lies between 13 and 25°C

(Mitchell 1956; Evans *et al.* 1964; Spedding 1971).

Nutrient supply generally has little effect on Li in most grasses (Langer 1979). Although Davies (1969) reported a decrease in Li with addition of N fertiliser, this was offset by reduced longevity of those leaves. On the other hand, moisture stress is reported to have caused an increase in Li of perennial ryegrass in the field (Barker *et al.* 1985). In a controlled environment, Van Loo (1992) found that low water potential (-1.3 MPa) caused a 12% reduction in rate of leaf appearance (i.e. Li increased) compared to normal water potential (0 MPa), but this effect had disappeared after 2 to 3 weeks, when the plants had adjusted to the lower water potential.

Temperature and light generally have little effect on leaf size (length and width), although leaves produced under higher temperatures or low light conditions are usually thinner than leaves produced under more optimal temperature and light (Silsbury 1970). Leaf size is regulated more by nutrient supply and moisture availability, with an increase in size occurring with increasing N supply (Ryle 1964; Langer 1979) and under adequate moisture availability (Turner and Begg 1978). In addition to the effect of nutrients and water on leaf size, it is reported that in grasses, successive emerging leaves are greater in size than previous leaves, until maximum leaf size is reached around about the time ceiling yield of the pasture is attained (Langer 1979).

In a comparison of leaf development between grasses grown under identical conditions in the field, Gao and Wilman (1994) found that biennial ryegrass genotypes had higher rates of leaf appearance and extension, and achieved greater total leaf size, than did perennial genotypes. Similarly, in a glasshouse study, Fulkerson *et al.* (1994) showed that biennial ryegrass genotypes had greater DM production than perennials, but only under appropriate defoliation regimes (defoliating when 3 new leaves/tiller had fully expanded).

2.2.2.2 Tiller. Sites of tiller production are initiated at the same rate as leaves (i.e. the potential for tiller initiation is one tiller/Li), and the environment has little effect upon this, although subsequent development and emergence of tillers is highly dependent on environmental conditions (Marshall 1987). As tillers arise from buds in leaf axils, the

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potential rate of tillering (tiller emergence) depends on the rate of leaf appearance (Jewiss 1966), and the number of tillers which actually form per Li is termed 'site filling' (Davies 1974; Van Loo 1992). Mitchell (1953a) and Ryle (1964) found that in perennial ryegrass, a tiller does not appear until there is at least one fully-expanded leaf above it on the stem. Adverse environmental conditions such as extremes of temperature, suppress tillering more than leaf production (Jewiss 1966), and under such conditions tiller buds accumulate, having the potential to develop later if environmental conditions improve (Marshall 1987). In the United Kingdom, Ryle (1964) found that when grown under natural conditions, site filling of perennial ryegrass plants was around 60%. Tiller (and leaf) development depend on current, rather than previous, environmental conditions. Plants can re-adjust tillering within several days to compensate for changes in the environment, to re-establish an equilibrium (Mitchell 1953a).

Although the optimum temperature for tillering is similar to that for leaf growth, tillering is more responsive to changes in light intensity and mineral nutrition (Langer 1979). Tillering is highest in full light; lowering light intensity results in a concomitant reduction in the rate of tiller emergence (Mitchell 1953a, b; Auda et al. 1966). Tillering responds positively to soil N status, and to a lesser extent, to phosphorus and potassium (Langer 1966). As with leaf expansion, tiller emergence is reduced by moisture stress (Turner and Begg 1978).

The emerging tiller is reliant on the parent tiller for nutrients, water and photosynthate until it develops its own roots and leaves. In a minisward of perennial ryegrass, Colvill and Marshall (1981) noted that the daughter tiller became independent from its parent when it reached a dry weight of around 25 mg, which was equivalent to the time taken to expand 2 new leaves/tiller and produce adventitious roots.. However, there was still transfer of WSC from parent to daughter until the daughter was more than 50 mg DM. This supply of WSC ceased when the parent tiller was stressed by low levels of light, water or nutrients, or by heat or the physical damage caused by grazing animals. The mortality rate of young tillers under these conditions was high (Kays and Harper 1974; Ong 1978; Ong and Marshall 1979; Colvill and Marshall 1984). It is only when daughter tillers become completely self-supporting that they are capable of producing their own

daughter tillers (Jewiss 1966).

The perenniality of ryegrass depends on its capacity to replace dying tillers (Colvill and Marshall 1984; Marshall 1987), and in field studies in the United Kingdom, Colvill and Marshall (1984) noted a peak in tiller initiation around flowering in spring, and claimed that these tillers were particularly important for perennation. In the subtropics, replacement of dying tillers is vital for perenniality, as seedling recruitment from perennial ryegrass cultivars is minimal (Fulkerson et al. 1993a; Lowe and Bowdler 1995), however there is a paucity of information regarding seasonal trends in tillering in this environment.

In a temperate environment, few perennial ryegrass tillers live for longer than one year (Jewiss 1966; Langer 1979), and there are marked seasonal trends in tiller production. Studies in the United Kingdom (Colvill and Marshall 1984; Bullock et al. 1994) and New Zealand (L'Huillier 1987; Brock and Thomas 1991; Brock et al. 1996) have reported that peak initiation of perennial ryegrass tillers is from spring to early summer. However, under subtropical conditions, McKenzie (1997) noted that tillering of perennial ryegrass was most rapid in the cooler months, with a peak in early spring. This is probably due to temperatures during a typical subtropical summer being above-optimal for tillering, whereas in the cooler months, high radiation levels and mild temperatures (Fulkerson et al. 1993a) would promote rapid tillering and growth.

In temperate areas, the flush of tillering in spring has been attributed to an improvement in climatic conditions from that prevailing during winter (Colvill and Marshall 1984), while the continuation of this flush into early summer has been associated with resumption of tillering from vegetative growing points at the base of tillers which become reproductive (Colvill and Marshall 1984; Matthew et al. 1991).

Tillers may increase in length through internode elongation when they become reproductive. The upper 4 or 5 internodes elongate to produce a flowering stem, and the active growing point, which will develop into a seed-head, is elevated from its usual position close to ground level up into the pasture canopy (Langer 1979). Removal of the growing point by defoliation results in the death of the reproductive tiller, but does not

affect vegetative growing points at the base of the tiller, and in fact encourages their emergence (Langer 1979, see also section 2 3.3).

The induction of reproductive development in ryegrasses is reviewed by Heide (1994). Most perennial temperate grasses have a dual induction requirement for flowering; a primary induction brought about by low temperatures ('vernalisation') and/or short day length, and a secondary induction brought about by a transition to longer days and higher temperatures. In ryegrasses, the requirements for reproductive induction vary from obligatory in most perennial genotypes, through intermediate to slight in biennial genotypes, to none in many annual genotypes (Heide 1994). In addition, the absolute values of temperature and daylength vary considerably between perennial ryegrass ecotypes from different latitudes. Evans (1960) found that perennial ryegrass does not flower if vernalised for less than 2 weeks, and since the most effective vernalising temperatures lie between 0 and 10°C, this explains the negligible seed set in perennial ryegrass in the subtropics with its mild winters, while annual and biennial ryegrasses flower prolifically (Lambourne 1985).

It has previously been noted in field trials in the subtropics that less than 10% of perennial ryegrass tillers become reproductive (W.J. Fulkerson, unpublished data), whereas figures quoted from field trials in temperate regions range from 17% (Brock et al. 1996) to 25% (Colvill and Marshall 1984), to 50% (Waite and Boyd 1953; Thom 1991) of total tillers reproductive. Apart from variations in climate and ryegrass cultivars, these differences in the extent of reproductive development may be brought about by defoliation pressure. For example, the 50% of reproductive tillers reported by Waite and Boyd (1953) was under conditions of uninterrupted growth, whereas the 17% reported by Brock et al. (1996) was under conditions of grazing, which presumably removed growing points and caused death of tillers.

The process of stem elongation has also been noted at times other than flowering, and appears to be a response by heavily-shaded tillers to find more favourable growing niches (Brock and Fletcher 1993). This process has been extensively studied in New Zealand, where it often results from either physical burial of tillers by wormcastings and stock

treading, or through shading of tillers by other plants in the pasture (Mathew *et al.* 1989; Brock and Thomas 1991; Brock and Fletcher 1993).

During reproductive development, some vegetative tiller buds may remain 'dormant' until either the inflorescence of the parent tiller has emerged, or the elevated growing point has been physically removed (Marshall 1987). This is the concept behind the practice of 'topping' pastures (defoliating to remove reproductive tillers) to encourage more vegetative growth in dairy pastures.

Tiller buds may also remain dormant in the short term under unfavourable climatic conditions and then form daughter tillers at a later date when conditions are more conducive to growth (Marshall 1987).

A third type of tiller dormancy is evident in some Mediterranean ecotypes of perennial ryegrass, phalaris and cocksfoot, and is a physiological adaptation by the plant to avoid the stress of a hot, dry summer. This summer dormancy involves the death of most above-ground plant tissue, and the formation of dormant growing points in tiller bases, which resume growth in autumn as climatic conditions improve (Laude 1953; Hoen 1968; McWilliam 1968). In temperate Australia, Silsbury (1961) found that summer-dormant cultivars of perennial ryegrass were more persistent than existing commercial cultivars, due to better summer survival. Summer dormancy may be one means of improving the persistence of perennial ryegrass in the subtropics (see section 4.1.1c).

2.2.2.3 Root. Grasses have 2 root systems, consisting of seminal roots, which are the primary roots of the plant as it germinates from seed, and adventitious or nodal roots, which arise from nodes at, or just below, ground level. Although seminal roots amount to less than 5% of total root DM in grasses during the first year and only have a lifespan of several months, they are highly branched compared to adventitious roots, and able to exploit a larger volume of soil (Langer 1979). As roots arise from tillers, and lifespan of tillers is less than one year (Jewiss 1966; Langer 1979), it follows that roots of perennial ryegrass follow an annual pattern of replacement.

In perennial ryegrass, the optimum temperature for root growth is lower than for growth of leaves or tillers, at around 12°C (Davidson 1969), and rooting follows marked seasonal trends. Stuckey (1941) reported that for perennial ryegrass grown at Rhode Island, root growth was most rapid in early spring and late autumn, while low soil temperatures in winter and high soil temperatures in summer caused root growth to slow, with many roots dying in late summer. Few new roots were produced during stem elongation and flowering, although elongation continued in earlier-formed roots. Therefore it appears that in addition to the seasonal effects associated with temperature, seed development also takes precedence over root production.

Garwood (1967) found that the mean lifespan of perennial ryegrass roots ranged from 61 to 188 days, with roots produced in autumn living longest. Troughton (1981) found that roots of undefoliated perennial ryegrass plants lived for 365 days, but the mean life of roots was reduced to 191 days when the plants were defoliated.

As well as being reduced by high temperatures, root growth is also reduced by low light intensity and by defoliation, and it has been postulated that the mechanism by which root growth is retarded is through reduced supply of WSC from the tiller (Brouwer 1966; Langer 1979), and this will be discussed further in section 2.3.4.

Temperate grasses are generally less tolerant of low pH soils than tropical grasses, and subsoil acidity is one factor which may restrict the rooting depth of grasses *per se*, and also by increasing the availability of toxic cations such as manganese and aluminium (Cornish 1987). Rooting may also be restricted by low levels of soil nutrients. It is generally accepted that nutrient deficiency reduces root numbers by retarding root branching, while increasing root extension (Oswalt *et al.* 1959; Brouwer 1966), except in the case of potassium deficiency, which can decrease root extension (Cornish 1987). However, increasing the supply of nutrients, especially N, usually increases root diameter (Oswalt *et al.* 1959; Evans 1970) and decreases root extension, and can lead to surface rooting (Oswalt *et al.*1959; Cornish 1987; Fulkerson *et al.* 1993b).

Moisture stress can also cause an immediate reduction in root extension (Cornish 1987), although in the long term, roots of plants infrequently irrigated tend to achieve greater soil depth than roots of regularly irrigated plants; consequently frequent irrigation may also encourage surface rooting (Langer 1990).

The rooting depth of ryegrass plants will thus vary with climate and management, and also soil type. In the United Kingdom, Garwood and Sinclair (1979) reported that perennial ryegrass was effectively utilising water to a soil depth of 800 mm, while in New Zealand, Evans (1978) reported that perennial ryegrass had the potential to extract water to a soil depth of 1,300 mm. These reports contrast to studies in the subtropics, where observations by Fulkerson and Lowe (1994) showed that greater than 85% of perennial ryegrass root DM was confined to the top 50 mm of soil, and total rooting depth was no greater than 200 mm. This was reported for 2 different sites, on both heavy clay and lighter krasnozem soil types.

Any factor which retards root growth has a carry-over effect on the rest of the plant's growth, due to restriction in uptake of water and nutrients (Davidson and Milthorpe 1966b; Clement et al. 1978; Langer 1979), and a decrease in competitive ability with other plants. For example, Roush and Radosevich (1985) found that the competitive ability of 4 weed species was stronger when relatively more DM was invested in roots. Poor rooting depth could also contribute to loss of plants through 'sod pulling' (physical removal of plants from soil by grazing stock) (Fulkerson et al. 1993a). Lack of persistence of perennial ryegrass appears to be causally linked to poor root development (Hughes and Jackson 1974; Arcioni et al. 1980, 1985a, b; Fulkerson et al. 1993b).

2.2.2.4 Reserves. The results of early research into plant reserve substances has been comprehensively reviewed by Weinmann (1948), May (1960) and White (1973). In these reviews, 'reserves' are defined as carbohydrate and nitrogenous (N) compounds which are formulated by the plant and stored for future use in providing energy for maintenance and growth processes. It is generally accepted that carbohydrates are the predominant reserves, although N reserves may also contribute. The situations under which N substances become important as reserves have not yet been defined, although a recent

review of a role for N reserves in forage plants has been provided by Volenec et al. (1996).

Carbohydrate reserves are non-structural carbohydrates, and include the monosaccharides glucose and fructose, the disaccharides sucrose and maltose, and the polysaccharides fructosan and starch (Smith 1972). Plants contain high concentrations of polysaccharides, while the monosaccharides and disaccharides are metabolic intermediates, and are usually found in lower concentrations. Grasses of temperate origin predominantly accumulate fructosans as reserve carbohydrate, while grasses of tropical origin and all legumes predominantly accumulate starch (Smith 1973). The carbohydrate reserves in ryegrasses are mainly fructosan, with some sucrose, glucose and fructose (Weinmann 1948; Waite and Boyd 1953; Smouter et al. 1995; Prud'homme et al. 1992), and will be referred to hence as WSC. N reserves include proteins, amino acids and nitrates, but compared to our knowledge of WSC reserves, there is a dearth of knowledge regarding the identification and function of N reserves.

In ryegrasses, the majority of reserves are stored in the tiller bases, or stubble, of the plant, with a small amount stored in the roots. In biennial ryegrass grown in New Zealand, Vartha and Bailey (1980) measured WSC levels up to 47% of DM in stubble, to 27% of DM in leaf, and to less than 11% of DM in roots. In general, WSC concentration is higher in more mature plant tissue (tiller and leaf bases) than in younger tissue (tiller and leaf tips) (Smith 1973). Accumulation of WSC by plants is determined by the balance between photosynthesis (source) and respiration (sink), and absolute levels depend on plant growth rate and stage of development, and on prevailing environmental conditions (White 1973).

Accumulation of WSC is greatest in full light (Alberda 1957, 1965, 1966a; Auda et al. 1966; Deinum 1966), which promotes photosynthesis, and under cool to mild temperatures (Alberda 1957, 1965, 1966a; Auda et al. 1966; Colby et al. 1966; Deinum 1966; Smith and Jewiss 1966), which reduce respiration. Thus in New Zealand, Kingsbury (1965) reported that the seasonal concentration of WSC in perennial ryegrass was greatest in early winter after a bright, sunny afternoon, whereas Vartha and Bailey (1980) noted that the WSC content in biennial ryegrass fell markedly during a period of dull, cloudy weather. Baker and Jung (1968a) grew timothy (*Phleum pratense* L.), bromegrass (*Bromus inermis*

Leysser), cocksfoot and Kentucky bluegrass (*Poa pratensis* L.) in a controlled environment, and showed that high night temperatures (up to 18°C) decreased WSC more than high day temperatures (up to 35°C). This is because increasing day temperature, if below optimum for grass growth, causes an increase in both photosynthesis and respiration, whereas increasing night temperature, or increasing day temperature if above optimum for grass growth, only increases respiration (White 1973).

WSC levels are lower when plants are actively growing than when growth is slow, due to WSC demand (growth) exceeding WSC supply (photosynthesis). Hence WSC concentration is generally highest in winter provided there is sunshine (Blaser *et al.* 1966; Colby *et al.* 1966; Pollock and Jones 1979; Thom *et al.* 1989). Also in winter, WSC loss through respiration would be minimal, due to low temperatures (Murata and Iyama 1963; Auda *et al.* 1966). The addition of N fertiliser, by promoting plant growth, initially lowers WSC concentration in grasses (Carroll 1943; Sprague and Sullivan 1950; Alberda 1965, 1966a; Auda *et al.* 1966; Colby *et al.* 1966; Smith and Jewiss 1966; McGrath 1992).

Variation in WSC with season is due partly to environmental effects on plant processes, and partly to plant development stage. For example, WSC concentration increases as plants mature, and the proportion of stem material increases (Smith 1973). In a field study in the United Kingdom, Waite and Boyd (1953) showed that WSC accumulated in stubble of perennial ryegrass plants during stem elongation, then decreased during seed-head development, due to conversion to starch, and storage in the seed. This was also observed in perennial ryegrass by Sullivan and Sprague (1943), in biennial ryegrass by Griffith (1992), and in cocksfoot by Sprague and Sullivan (1950). Also, within the regrowth cycle, Fulkerson and Slack (1994b) recorded an increase in WSC in perennial ryegrass from approximately 4% at the 1-leaf stage, to 15% at the 3-leaf stage.

Moisture stress also influences WSC concentration, causing an increase in some cases (Julander 1945; Blaser *et al.* 1966), and a decrease in others, depending on the degree of stress and also on the stage of plant growth (White 1973). Presumably, moisture stress initially increases WSC by reducing plant growth (hence WSC use), however after a period of time, leaves would begin to die, so WSC buildup through photosynthesis would

decline.

WSC concentration also fluctuates diurnally in the leaf. Waite and Boyd (1953) in the United Kingdom, Kingsbury (1965) in New Zealand and Fulkerson *et al.* (1994) in Australia, reported diurnal variation in WSC of perennial ryegrass plants, which reached a peak in mid-afternoon, then declined during the night. The rate of increase in WSC throughout a sunny day was about ½%/hour (Fulkerson *et al.* 1994). Similar diurnal fluctuations in WSC have been reported in the United States for lucerne (Curtis 1944; Holt and Hilst 1969) and tall fescue (*Festuca arundinacea* Schreb.), bromegrass and Kentucky bluegrass (Holt and Hilst 1969). However, unlike seasonal fluctuations of WSC in perennial ryegrass, which are brought about largely by a change in fructosan content (Waite and Boyd 1953), diurnal fluctuations in WSC are due mainly to changes in the sucrose content (Waite and Boyd 1953; Kingsbury 1965).

The importance of stored reserves is in supplying energy for the continued growth of a plant when current supply of energy (through photosynthesis) is inadequate to meet the demands of growth and maintenance (White 1973). Thus in theory, reserves should sustain plant growth during interruptions to photosynthetic activity such as defoliation, and reduction or cessation of light interception (shading by companion plants, cloud cover or night), and this will be further discussed in section 2.3.1. Also, in those plants which undergo summer dormancy, reserves would be crucial in keeping the dormant buds alive, and in providing energy for regrowth of these buds following the breaking of dormancy.

There is also evidence that reserves play a role in development of tolerance by plants to periods of stress such as heat, frost and drought (Weinmann 1952). The premise for a role of reserves in tolerance to heat, frost or drought is that reserves are present in plant cells as solutes, hence are osmotically active, and through the processes of polymerisation and depolymerisation, could rapidly change the osmotic pressure of the cell, and therefore confer tolerance to injury by these stresses (Pontis and Del Campillo 1985). The term 'hardening' refers to the process of exposure of a plant to below- or above-optimal temperatures or to moisture stress for a short period, which induces reserve buildup and 'conditions' the plant to survive longer periods of temperature or moisture stress (Pollock

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et al. 1993).

A number of researchers have noted a buildup of reserves in plants when hardened by low temperatures (Rosa 1921; Eagles and Williams 1992; Bredemeijer and Esselink 1994) or moisture stress (Julander 1945; Barlow *et al.* 1976; Horst and Nelson 1979; Korte and Chu 1983; Barker *et al.* 1985; Thomas 1991; Volaire and Thomas 1995). Although Julander (1945) reported that a buildup of WSC reserves under drought conditions appeared to confer an advantage in survival of grasses through a subsequent period of high temperatures, Sullivan and Sprague (1949) reported that high temperatures caused a decrease in WSC reserves, presumably due to higher respiratory load, although high levels of N reserves were noted. More recently, the production and buildup of specific proteins, labelled 'heat-shock' proteins, in response to heat stress has been reported (Pollock 1992; Pollock *et al.* 1993; DiMascio *et al.* 1994; Bettany 1995a, b).

A direct role of reserves in protecting a plant against the effects of heat, frost or drought has not been conclusively proven. For example, Eagles and Williams (1992) were not convinced of a direct role of WSC in improving tolerance of perennial ryegrass to low temperatures, concluding instead that accumulation of WSC reserves during cold hardening was simply a consequence of reduced growth. Thomas (1991) reported that under conditions of moisture stress, perennial ryegrass accumulated WSC, but this had no effect on osmotic potential. Volaire (1994a) reported that drought-resistant cocksfoot cultivars accumulated more WSC than drought-susceptible cultivars, whether or not plants were subjected to drought, and concluded that WSC accumulation during drought was not simply an adaptive factor by plants to drought. In a detailed analysis of factors affecting drought survival of cocksfoot in the United Kingdom, Volaire and Thomas (1995) found that relative to drought-affected cultivars, drought-resistant cultivars had a higher WSC concentration in tillers, displayed greater osmotic adjustment in leaf bases, and also had denser roots at depth in soil. Thus in this study, WSC buildup was only one possible factor involved in drought tolerance.

The real benefit of reserves stored during times of stress may not be so much their role in protecting the plant against direct effects of the stress, but in promoting rapid recovery

after the stress. For example, a number of studies have shown 'compensatory' growth of grasses following release from drought (Corleto and Laude 1974; Horst and Nelson 1979; Korte and Chu 1983; Barker *et al.* 1985), in which rates of leaf elongation and tillering were more rapid than in plants which were not subjected to drought.

2.2.3 Response to defoliation

Plant regrowth following defoliation varies with defoliation severity - regrowth is generally slower under frequent and close defoliation than when defoliation interval is increased and/or greater amounts of herbage are left behind. Under intermittent grazing practice leading to optimal utilisation of pasture, little residual leaf area (RLA) remains and regrowth of perennial ryegrass has been observed to conform to the pattern shown in Figure 2.1 (W.J. Fulkerson, unpublished data).
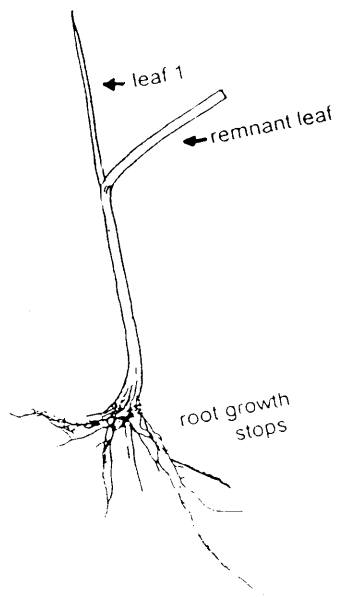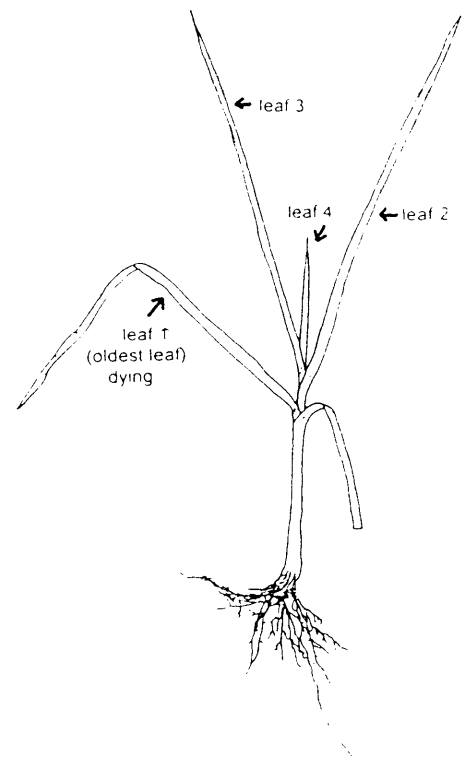
A. Regrowth of remnant leaf, and emergence of first new leaf.
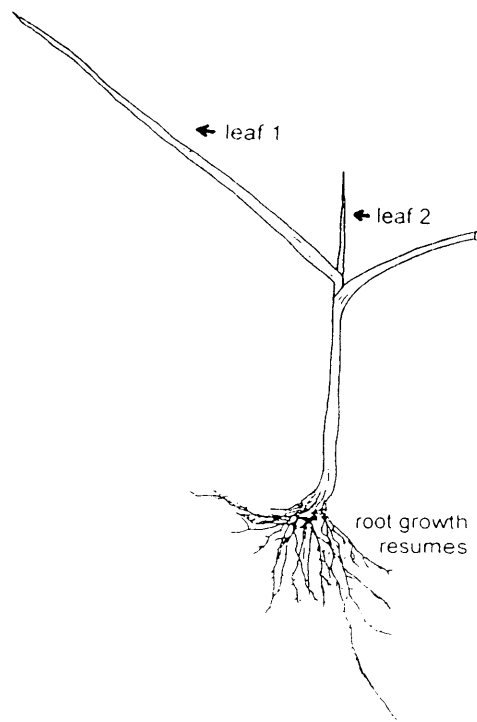
B. First new leaf fully emerged, and second new leaf beginning to emerge.
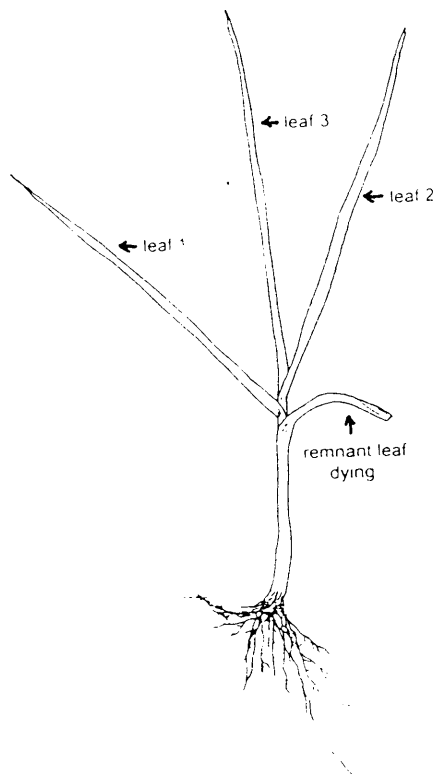
C. The 3-leaf stage - 3 new leaves fully emerged.

D. The onset of senescence occurs with the emergence of the fourth new leaf.

Figure 2.1. Regrowth of a ryegrass tiller following defoliation.

After defoliation, root growth stops almost immediately (Crider 1955; Evans 1973), and the 'remnant leaf' (that leaf which was emerging from the stubble sheath when defoliation took place) begins to expand. Before the remnant leaf has fully expanded, the first of the new leaves emerges (Figure 2.1(A)). Root growth resumes around the time of expansion of the second new leaf (Fulkerson and Slack 1994b) (Figure 2.1(B)), and the leaf emergence process continues until 3 new leaves have fully expanded (Figure 2.1(C)). It is at this 3-leaf stage that the remnant leaf begins to senesce, and the emergence of any further leaves is balanced by the death of the oldest leaf on the plant (Alberda 1966a; Davies 1969; Simons et al. 1972) (Figure 2. (D)).

The response of the ryegrass plant to defoliation is discussed in more detail in the following sections as it affects growth of leaves, tillers and roots.

2.2.3.1 Reserves. Following defoliation, photosynthesis (hence WSC production) is reduced or ceases altogether, depending on the severity of defoliation, and uptake of N also rapidly declines. However, regrowth and ongoing respiration, although reduced following defoliation, both require energy (Clement et al. 1978). This demand is met by current photosynthate and/or mobilisation of stored reserves. Reserves are mobilised predominantly from the stubble; using labelled ^{14}C in biennial ryegrass, Marshall and Sagar (1965) found that defoliation did not result in mobilisation of WSC from roots, even when plants were completely defoliated and kept in the dark (to prevent photosynthesis).

As leaf material is the source of current photosynthate, increasing severity of defoliation (reduced RLA) presumably increases reliance of regrowth on stored reserves, both WSC and N. To illustrate this, Booysen and Nelson (1975) pre-treated tall fescue plants to obtain high or low stubble WSC levels (12 vs. 6%, respectively), then defoliated both groups of plants to leave either zero, or two-thirds RLA. Subsequent regrowth was found to increase with both high WSC and high RLA, with RLA contributing more to growth rate and total plant size after 20 days than WSC. Where RLA was low, regrowth was initially at the expense of WSC reserves, irrespective of WSC level. Studies with cocksfoot (Ward and Blaser 1961) and timothy (Smith 1974), in which WSC levels and RLA were manipulated in a similar manner, reported almost identical findings.

Furthermore, Grant *et al.* (1981) observed under both grazing and cutting that regrowth of perennial ryegrass was increased with increasing RLA. However, in all these studies, the effect of RLA was not absolutely separated from WSC; 'low' RLA plants had little or no RLA, and appeared to rely on WSC for regrowth, whereas 'high' RLA plants had both RLA *and* WSC. It is thus apparent that both RLA and reserves contribute energy to regrowth of grasses, and that severity of defoliation probably affects the dependence of regrowth on reserves.

Given that regrowth appears to be supported by plant reserves, it follows that an optimum level of reserves should exist, below which regrowth would be retarded. A positive correlation between plant WSC levels prior to defoliation, and regrowth capacity following defoliation, has been obtained for perennial ryegrass (Alberda 1957; Davies 1965; Alberda 1966b; Gonzalez *et al.* 1989; Fulkerson and Slack 1994b, 1995), cocksfoot (Ward and Blaser 1961; Davidson and Milthorpe 1966a), tall fescue (Booysen and Nelson 1975; Volenec 1986), timothy (Smith 1974), red grass (*Themeda triandra* (R. Br.) Stapf) (Danckwerts 1993), and African lovegrass (*Eragrostis curvula* (Schrad.) Nees) (Bartholomew and Booysen 1969). Research by Davies (1965) and Alberda (1966b) indicated that, while regrowth of perennial ryegrass appeared retarded when stubble WSC concentrations were below about 15%, there was no further increase in regrowth when stubble WSC concentrations were much above this level. It appears that a stubble WSC concentration of around 15% may represent a critical level for perennial ryegrass regrowth, although this hypothesis has not been tested. No research to date has been conducted to determine if an optimum level of stubble N exists for regrowth.

There has been some debate regarding the relative importance of WSC and N on regrowth. For example, Alberda (1966b) pre-treated perennial ryegrass plants for a short period, which altered WSC content but had no effect on the content of organic N compounds. Plants were defoliated to a stubble height of 50 mm, and leaf regrowth of 'high WSC' plants (22% WSC in stubble) was almost double that of 'low WSC' plants (9% WSC) after 2 weeks (18 vs. 10 g DM/plant respectively). Sullivan and Sprague (1943) and Gonzalez *et al.* (1989) have similarly observed that regrowth of perennial ryegrass following defoliation induced a change in WSC, but not N, levels within the stubble.

However, other researchers have concluded that N compounds also play an important part in regrowth. Davidson and Milthorpe (1966b) concluded in a study with cocksfoot that the change in stubble WSC following defoliation accounted for net respiratory losses and regrowth, except when defoliation was severe, in which case even high levels of WSC could not meet the carbon (C) usage, and other substances, presumably N compounds, must have been mobilised for respiration and regrowth. Also, Dilz (1966) varied fertiliser N and defoliation frequency to obtain different concentrations of WSC and N in perennial ryegrass prior to defoliation, and reported that regrowth was more strongly correlated to N levels in the form of crude protein, than to WSC levels. More recently, research by Ourry et al. (1988; 1989a, b) has highlighted the importance of N reserves in regrowth. However, to date, the reliance of regrowth on reserves, and the relative contribution of WSC and N, have not been conclusively proven. N reserves may play a more prominent role in regrowth when WSC buildup is prevented by frequent or close defoliation, for example.

The importance of WSC reserves for regrowth appears limited to the time during which photosynthesis is unable to provide substrate for regrowth. When pastures are well-utilised (little RLA remaining), the initial rapid depletion of WSC and N in the stubble during the first few days following defoliation coincides with regrowth of the remnant leaf and the first new leaf to expand. As subsequent new leaves emerge and expand, and root growth and function continue, WSC and N levels may gradually return to pre-defoliation levels (see review by White 1973). Gonzalez et al. (1989) and Prud'homme et al. (1992) observed that loss of stubble WSC in perennial ryegrass occurred during the first 6 days of regrowth, after which levels gradually rose, attaining values observed prior to defoliation after 28 days. From these and other studies (Grant et al. 1981; Danckwerts and Gordon 1987; Fulkerson and Slack 1994b), it appears that the period of association between stubble WSC and regrowth of perennial ryegrass is limited to 3 to 6 days after defoliation. Similar results have been obtained by Volenec (1986) for tall fescue (4 days), by Davidson and Milthorpe (1966a, b) for cocksfoot (2 to 4 days), by Danckwerts (1993) for red grass (3 days), by Richards and Caldwell (1985) for wheatgrasses (*Agropyron* spp.) (3 days) and by Bartholomew and Booysen (1969) for African lovegrass (one week).

As with WSC reserves, the period of reliance of perennial ryegrass on N reserves seems limited to the first few days after defoliation (Ourry *et al.* 1988; 1989a, b). Ourry *et al.* (1989b), using labelled ^{15}N, found that within the first week following defoliation, more than 70% of protein N in the regrowing leaves came from organic N mobilised from stubble and roots, but this had declined to less than 45% after 2 weeks of regrowth. It was concluded from these studies that during the early phase of regrowth (less than 6 days), N supply for regrowth appears highly dependent on N reserves, thereafter, assimilation of mineral N takes place from the soil.

Given that high levels of WSC prior to defoliation are associated with development of a larger initial leaf area than low levels of WSC, it seems reasonable to assume that, by virtue of a larger photosynthetic area, these plants would contain more photosynthate than would be present in plants with low WSC. Hence, the benefit of high WSC levels for regrowth would not just be confined to the initial regrowth phase. In support of this hypothesis, isotopic labelling with both ^{14}C (Danckwerts and Gordon 1987) and ^{15}N (Ourry *et al.* 1989b) has shown that previously stored C and N continue to appear in regrowing leaf tissue for several weeks after defoliation. Fulkerson and Slack (1995) reported that perennial ryegrass plants defoliated once at 3 leaves/tiller had higher WSC content and greater subsequent regrowth over a full 3-leaf regrowth cycle (30 days), than similar plants defoliated 3 times at 1 leaf/tiller. Similarly, Danckwerts (1993) concluded in a study with red grass pre-treated to obtain high and low reserve levels, that high levels of reserves were associated with initially greater regrowth (over 3 days), but that regrowth after 12 days was still greater, possibly due to the greater contribution by current photosynthate. In cocksfoot, Ward and Blaser (1961) reported that high stubble WSC levels were associated with greater initial regrowth following defoliation than low WSC levels (8 vs. 2% WSC, respectively), and DM differences were still present after 25 days, even though leaf area was now contributing to growth.

A different finding was reported by Davies (1965), who varied WSC levels in perennial ryegrass by short-term exposure to darkness and raised temperatures, then defoliated the plants and concluded that, although subsequent regrowth was associated with WSC levels, once growth was fully re-established, growth rate was independent of WSC level. A

possible explanation for this discrepancy is that in the studies by Fulkerson and Slack (1995), Danckwerts (1993) and Ward and Blaser (1961), defoliation was used as a pre-treatment rather than shading.

The period of reliance of perennial ryegrass on reserves post-defoliation could be expected to increase if the plant was unable to effectively photosynthesise, as would be the case with shading (Alberda 1957; Ludlow 1978), either from competing plants or tillers within the canopy, or from cloud cover. Likewise, if the regrowing leaf was removed by re-defoliation, regrowth would be expected to be retarded in response to depleted reserves. Fulkerson (1994) re-defoliated perennial ryegrass plants at 3, 6, or 3 and 6 days post-defoliation, to 2 different stubble heights (20 and 50 mm), and found that at the extreme treatment - defoliation at 3 and 6 days to 20 mm height - 70% of plants died. At 50 mm height, re-defoliation reduced total regrowth by 55, 78 and 95% for plants re-defoliated at 3, 6 or 3 and 6 days, respectively. Grant et al. (1981) also reported that as defoliation severity increased, so did the decline in perennial ryegrass WSC levels, and subsequent regrowth. Thus the capacity of perennial ryegrass to regrow appears to be reduced by frequent defoliation which prevents reserve buildup by removing photosynthetic area, and by close defoliation which removes part of the main reserve storage organ (stubble).

It appears that preferential allocation of WSC may exist in regrowth, and it would seem logical in a survival context for re-establishment of the photosynthetic canopy to assume a higher priority than root growth and daughter tiller initiation and development. Under this hypothesis, secondary sinks such as the roots and tillers would be deprived of reserves until the needs of the strongest sink - the leaves - are met. ^{14}C tracer studies in perennial ryegrass (Danckwerts and Gordon 1987), biennial ryegrass (Marshall and Sagar 1965; Gifford and Marshall 1973), fescue (Johansson 1993) and barley (Hordeum vulgare L.) (Ryle and Powell 1975) indicate that defoliation induces an increase in WSC allocated to initiate and maintain leaf regrowth, at the expense of WSC allocated to initiate and maintain roots and tillers, and replenish stubble stores. N reserves also appear preferentially allocated to leaf regrowth, at the expense of allocation to secondary sinks, in much the same pattern as WSC (Richards 1993). For example, Ourry et al. (1988) showed that when perennial ryegrass was defoliated, N was mobilised from the stubble and roots,

and used exclusively in leaf regrowth. It was not until after 6 days that N allocation to root growth and tillering resumed.

Although a number of studies have indicated that a preferential allocation of reserves may exist, no effort has been made to quantify this in perennial ryegrass by observing the sequential regrowth of leaves, roots and tillers following defoliation, and reserve allocation to each component. Also, at low reserve levels, it follows that reserve allocation to (and hence regrowth of) secondary sinks would be minimal, to the obvious detriment of plant survival. From the point of view of plant persistence, there exists a need to investigate whether a priority for reserve allocation does exist, and if the priority changes with low WSC.

2.2.3.2 Leaves. Davies (1960) reported a useful plant-related indicator of optimal defoliation time for *Lolium* species, as being the time to full expansion of 3 leaves/tiller, or before the fourth leaf emerges and senescence of the oldest leaf begins. In the subtropics, Fulkerson and Slack (1994b) showed that perennial ryegrass grown in a glasshouse followed an exponential growth pattern up to 3½ leaves/tiller, after which leaf senescence began. Thus, although the maximum ceiling yield of DM occurs after 3 leaves have expanded, in a grazing or harvesting situation, senescence represents a waste of herbage; therefore for optimum *utilisation* of ryegrass, maximum defoliation interval should be at 3 to 3½ leaves/tiller.

There is some debate regarding the optimum defoliation intensity for perennial ryegrass. Although regrowth may be increased with increased RLA following lax defoliation (Ward and Blaser 1961; Smith 1974; Booysen and Nelson 1975), the leaves left behind are generally older and less photosynthetically active than younger leaves (Gifford and Marshall 1973; Woledge 1977; Gay and Thomas 1995). Davies (1974) showed that removal of older (expanded) leaves of perennial ryegrass had no effect on the relative growth rate of the plant, and even increased the relative growth rate in some cases, suggesting that older leaves are less efficient in producing and/or translocating photosynthate than younger leaves. The relative growth rates (g/g/day of top growth) measured in this study were 0.92 for undefoliated plants, 0.91 for plants where all fully

expanded leaves were defoliated, and 0.64 for plants from which all leaves were removed. These results support those of Davidson and Milthorpe (1966a), who found in cocksfoot that regrowth of new leaf tissue was unaffected by the removal of older expanded leaves, although regrowth was reduced when all leaf tissue was removed. Also, as leaves have a limited lifespan, these residual leaves will die before the next defoliation, again representing wasted herbage. As such, the loss of available DM through increasing post-defoliation residue is not compensated for by the increased growth rate (Fulkerson and Slack 1995).

In the study by Davies (1974), whenever some leaf remained after defoliation, relative growth rate was maintained at a level not far below that of undefoliated plants, which suggested that plants have the capacity to compensate for loss of leaf tissue by increasing the activity of the remaining leaves. Supporting this hypothesis, Gifford and Marshall (1973) reported that compared to undefoliated biennial ryegrass plants, the remaining leaves of partially defoliated plants showed an increase in the rate of photosynthesis, as well as an increase in the proportion of WSC exported to regrowing leaves. It was concluded that the enhanced demand for WSC caused by defoliation delayed the normal decline in photosynthetic rate with age of remaining leaves. This response, termed compensatory photosynthesis, has also been observed in regrowing leaves of perennial ryegrass following defoliation (Woledge 1977). However, in the latter study, when regrowing plants were shaded, no compensatory photosynthesis was observed, and it was concluded that for perennial ryegrass, any change in the photosynthetic capacity of leaves during regrowth is a result of changes in the light environment of the developing leaves.

In a study with barley, Ryle and Powell (1975) found that defoliation which retained the 2 oldest leaves actually resulted in a decrease in the rate of photosynthesis of these older leaves by 5 or 10% compared to that of similar leaves on undefoliated plants, however this was offset by an increase in the export of WSC from leaves to meristems during the first 48 hours following defoliation, which declined as new leaves regrew from the shoot apex. The reason for a difference in photosynthetic response between this study and those reporting compensatory photosynthesis is unclear, however repeated defoliation in the study of Ryle and Powell (1975) may be a factor. It can be concluded then, that for

grasses, defoliation may result in compensatory photosynthesis of regrowing/remaining leaves, and this may be induced either by changes in the light environment, by source-sink or hormonal changes in the plant, or by a combination of these.

Hunt and Brougham (1967) found that where repeated lax defoliation of perennial ryegrass left enough herbage to intercept 95% of incident light, the amount of green leaf and the number of tillers initiated progressively declined, while the proportion of dead material increased, indicating the need for periodic close defoliation to renew the photosynthetic efficiency of the grass sward and to prevent shading of tiller bases. Furthermore, in a grazing study conducted over 2 years in New Zealand, Campbell (1969) found that at all times in both years, the highest pasture utilisation (kg DM/ha of perennial ryegrass and white clover) was obtained from the grazing treatment which left the lowest residual stubble mass. In this study, a stocking rate of 38 ewes/ha on a 28 day grazing interval produced a mean of 14,685 kg DM/ha, while a stocking rate of 25 ewes/ha on a 7 day grazing interval produced only 12,286 kg DM/ha, despite leaving over twice as much stubble mass after each grazing.

Following defoliation, leaf regrowth must arise from either existing active meristematic tissue, or from axillary buds in the tiller base, the former process being faster than the latter (Hyder 1972). Depending on their stage of growth, leaves are affected differently by defoliation. Older leaves will be nearing, or have reached full size, and if defoliated will not be capable of further regrowth. In younger leaves not yet fully expanded, growth of the sheath may continue after defoliation, exposing the defoliated tip (Langer 1979). However, Davies (1974) found that the first new leaf produced after defoliation weighed less than a similar new leaf produced by undefoliated plants; a single defoliation to remove all leaves resulted in a 30% reduction in leaf DM compared to leaf DM from undefoliated plants (16 vs. 23 mg, respectively). This difference reflected the production of narrower, rather than shorter, leaves by defoliated plants. Madison (1962) also reported that defoliation acted to decrease leaf width. A reduction in leaf length with increasing defoliation severity has been reported for perennial ryegrass by Grant et al. (1981), under both grazing and cutting. In this study, decreasing defoliation from 60 to 20 mm stubble height led to a decrease in leaf extension rate by 2 mm/tiller/day after one week of

regrowth, and by 1 mm/tiller/day after 4 weeks.

The Li (time taken for one leaf to fully expand), has been found to be largely independent of defoliation imposed. Glasshouse studies conducted by Fulkerson and Slack (1994b, 1995) found that the Li of perennial ryegrass plants was unaffected by defoliation interval (defoliating at either 1 or 3 leaves/tiller), however plants defoliated to either 20 or 50 mm stubble height took 3 to 4 days longer to regrow to 3 leaves/tiller than plants defoliated to 120 mm stubble height (Fulkerson and Slack 1995). Although Davies (1974) reported that the Li of regrowing perennial ryegrass increased following removal of progressively more leaf from plants in a glasshouse study in the United Kingdom, this difference was confined to the first week of regrowth. Even then, they were not large; the mean number of leaves appearing per tiller over a 12 day period was 2.1 vs. 1.4 for undefoliated and completely defoliated plants, respectively. Grant et al. (1981) recorded a decrease in Li under close, compared to lax, grazing of perennial ryegrass, but this effect was due to improved light penetration into the sward, and differences had disappeared after 3 weeks.

2.2.3.3 Tillers. The effect of defoliation on ryegrass tillers depends on whether the tillers are vegetative or reproductive. In elongating reproductive tillers, defoliation may remove the growing point (elevated during stem elongation), causing death of the primary tiller. However, experimental evidence suggests that as elongating reproductive tillers inhibit growth of their lateral buds through production of auxins, removal of the growing point by defoliation may stimulate growth of these lateral buds, resulting in an increase in daughter tiller production (Clifford 1977).

A high mortality rate of young perennial ryegrass tillers, commonly observed at the time of stem elongation, has been attributed, in part, to increased competition for WSC from the developing seed-head, as supply of WSC to young daughter tillers by the parent tiller is minimal at this time (Ong et al. 1978; Colvill and Marshall 1984). Increased shading from elongating parent tillers during stem elongation would also increase the mortality of young tillers, shading per se is recognised as a major factor in death of tillers (Kays and Harper 1974; Ong and Marshall 1979; Parsons et al. 1984).

Thus, defoliation not only provides a more favourable light environment which enhances tiller initiation and survival, but during stem elongation, removal of the developing seed-head has been claimed by Matthew *et al.* (1991) to allow WSC, accumulating in the stem for use in seed production, to be diverted to daughter tiller initiation and development. In a glasshouse study, Matthew *et al.* (1991) found that defoliation of the reproductive tiller at the late stem elongation to early seed-head stage increased the number and size of daughter tillers subsequently formed. If the seed-head was allowed to develop, or the parent tiller was removed entirely, there was a reduction in the number and size of daughter tillers formed. However, the benefits of this practice have not been shown under grazing conditions. Nevertheless, frequent (3 times at the 1-leaf stage), compared to infrequent (once at the 3-leaf stage) defoliation causes a decrease in tiller DM, and this was associated with a decrease in stubble WSC during the full 3-leaf regrowth cycle (Fulkerson and Slack 1995); this may negate any positive effect of removing the seed-head. Under field conditions in the subtropics, it has been observed that frequent defoliation of perennial ryegrass in winter and spring causes an increase in tiller density in late spring (W.J. Fulkerson, unpublished data), but these tillers were smaller than under infrequent defoliation (at or near the 3-leaf stage), and less survived the summer (Fulkerson *et al.* 1993b).

The observation that a low WSC status is associated with increased tiller mortality in perennial ryegrass (Alberda 1957, 1966b; Colvill and Marshall 1984), timothy (Colby *et al.* 1974) and cocksfoot (Volaire 1995) indicates that daughter tillers assume a lower priority for WSC allocation when the parent tiller is subjected to stress which reduces WSC (e.g. severe defoliation, low light levels). Furthermore, increased daughter tiller mortality is associated with reproductive development (Ong *et al.* 1978; Colvill and Marshall 1984), thus seed production also takes precedence over vegetative daughter tiller development. In further support of this hypothesis, Ong and Marshall (1979) observed that in partially-shaded plants, unshaded tillers supplied shaded tillers with WSC, but when the whole plant was shaded, the smallest tillers died, as export of WSC from larger tillers ceased. Similarly, Ong (1978) found that when perennial ryegrass plants were deprived of light or nutrients, the smallest tillers were sacrificed first, presumably so that the more mature parent tillers could survive.

The optimum time to defoliate perennial ryegrass would appear to coincide with the 3-leaf stage of regrowth. Defoliation at this interval leads to subsequent production of large tillers, with a high WSC content, which are presumably better able to sustain daughter tiller initiation and survival than the smaller tillers of more frequently-defoliated plants.

2.2.3.4 Roots. Following defoliation, root growth (elongation and branching) ceases until after top growth is well advanced (Crider 1955). The time taken for roots to resume growth is increased by more severe defoliation (decreased interval and height). To illustrate this, Evans (1973) showed that when young plants of perennial ryegrass, cocksfoot and timothy were defoliated every second day to a stubble height of 25 mm, complete or near complete cessation of root elongation occurred, while elongation of roots continued when plants were defoliated every second day to stubble heights of 50 and 100 mm, albeit at reduced rates compared to undefoliated plants. In this same experiment, when defoliation height was reduced from 50 to 25 mm, the percentage of dead roots was 9 times higher in perennial ryegrass, 2.5 times higher in cocksfoot, and 10 times higher in timothy. Similarly, Hodgkinson and Baas Becking (1977) found that root death doubled as defoliation height was reduced from 40 mm to 5 mm in wallaby grass (*Danthonia caespitosa* Gaudin). Also, Jacques and Edmond (1952), found that the initiation of new roots decreased in direct proportion to increasing severity (decreased interval and height) of defoliation in perennial ryegrass and cocksfoot.

Concomitant with a reduction in root growth of perennial ryegrass in response to defoliation is a decline in root respiration rate and uptake of nutrients (Clement *et al.* 1978). When perennial ryegrass plants grown in flowing nutrient solution were defoliated to 50 mm stubble height, Clement *et al.* (1978) observed that root respiration began to decline within hours, and was substantially reduced after 24 hours. In the same experiment, the rate of nitrate uptake declined within 30 minutes of defoliation, and within 2 hours had declined to less than 40% of the uptake rate prior to defoliation. Nitrate uptake rate remained negligible for the next 7 days, and did not increase until a positive daily whole-plant C balance had been re-established.

Crider (1955) reported that following defoliation of rhodes grass, the time taken for roots to begin regrowth varied in proportion to the percentage of foliage removed; a single defoliation removing 90% of top growth resulted in complete cessation of root growth for 17 days, while removal of 80% of top growth resulted in cessation of root growth for 12 days. Root growth was unaffected by removal of 40% or less of top growth, even under repeated defoliation.

The effect of defoliation on weakening the root system has been shown to be a cumulative process (Crider 1955; Evans 1971; Hodgkinson and Baas Becking 1977). This explains the observations of Fulkerson et al. (1993b) that following defoliation of perennial ryegrass in winter and spring at 1 or 3 leaves/tiller, no difference in total root DM between defoliation intervals was found in late spring, however by autumn of the second year, root DM in the top 50 mm of soil under the previous frequent defoliation regime was reduced to nearly half that of root DM under the infrequent defoliation regime.

The reduction of the grass root system through severe defoliation may be due to a shortage of WSC for root growth. Evans (1972) reported that defoliating perennial ryegrass plants to a stubble height of 25 mm resulted in a decrease in root WSC levels and an associated suppression of root elongation, compared to undefoliated plants. This suppression was largely countered by addition of sugar to the root medium, indicating that WSC depletion was the main cause of suppression of root elongation. In red and white clover, Evans (1973) found that root growth was less inhibited than in grasses under comparable defoliation, and concluded that reserve substances stored in the clover taproots may have been used to sustain lateral root extension. Similarly, in a study where wallaby grass, saltbush species (Atriplex spp.) and lucerne underwent defoliation to remove all foliage, Hodgkinson and Baas Becking (1977) found that lucerne root extension, longevity and branching was least affected by complete defoliation, and also concluded this was due to the availability of carbohydrates from the taproot. Thus it appears that root regrowth assumes a lower priority for WSC allocation than leaf regrowth (Nielsen and Humphries 1966). Further support of this hypothesis is provided by Jacques and Edmond (1952) in a study of the effects of defoliation on perennial ryegrass and cocksfoot. They found that as frequency and/or closeness of defoliation increased, so too did the tendency for plants to

produce new leaves before root growth resumed. There is a need to investigate the mechanism by which frequent or close defoliation reduces root growth.

In the subtropics, the action of defoliation in restricting root growth and function may partly explain why less perennial ryegrass plants survive summer under frequent, compared to infrequent, winter and spring defoliation. The smaller root system resulting from frequent defoliation would presumably place these plants at greater risk of sod pulling, as well as making the surviving plants more vulnerable to environmental stresses in summer. Research by Thom *et al.* (1986) in New Zealand indicated that from late winter to autumn, frequent, compared to infrequent grazing (14 vs. 28 day intervals) more than doubled the loss of perennial ryegrass plants from sod pulling (17 vs. 7% of total plants lost, respectively). Although sod pulling is regarded as a factor contributing to perennial ryegrass plant losses in subtropical dairy pastures in Australia (Fulkerson *et al.* 1993a), quantification of plant losses has no. been reported. Again, as with leaf and tiller dynamics, the optimum defoliation regime for maximum root development of perennial ryegrass in the subtropics would appear to be around the 2½- to 3-leaf stage of regrowth.

2.2.4 Limitations to growth and persistence

In subtropical Australia, the higher temperatures and radiation levels in the cooler months compared to temperate areas, lead to higher growth rates of temperate pasture, but during the warmer months, lead to increased stress and disease incidence, which may depress pasture growth rates and increase plant mortality (Fulkerson *et al.* 1993a).

2.2.4.1 High temperature. Temperatures above about 35°C, when prolonged, are lethal to most temperate grass species (Downes 1970; Beard 1973; McWilliam 1978). However, measurement of air temperature alone is not always an accurate indication of the stress suffered by the plant, as temperature of plant tissue may be greater than air temperature due to absorption of solar radiation (Levit. 1980). In addition, if the soil is exposed, temperature at the base of the plant may also be much higher than in the canopy. Julander (1945) observed a soil temperature of 51.5°C at the surface, and 48.5°C at 25 mm depth when the corresponding air temperature was around 36°C.

The degree of injury to a plant from high temperature increases in proportion to relative humidity, as plants derive a degree of cooling from increased transpirational rates at lower relative humidities (Beard 1973). Thus in the subtropics, where summer temperatures are often above 30°C and frequently coincide with relatively high humidity, the temperature stress experienced by perennial ryegrass plants may be substantially greater than in temperate areas. This would be particularly relevant following defoliation which exposes soil to solar radiation.

Older plant tissue is generally more heat tolerant than younger tissue (Lange 1965), hence the effect of high temperatures during a period of active plant growth (especially regrowth), would be particularly deleterious. To illustrate this, Wehner and Watschke (1981) subjected 4 cultivars of perennial ryegrass to different levels of N fertiliser and water, then exposed plants for 30 minutes to temperatures ranging from 41 to 49°C, and found that all plants were injured at 41°C, with complete death of most plants at 47°C. However, plants receiving high levels of N and water, which stimulated growth, were less heat tolerant than plants receiving lower rates of N and water. Also, Julander (1945), in a study with 4 grass species, found greater death of plants which were watered and defoliated prior to exposure to high temperature (48°C), than of unwatered, undefoliated plants, and concluded the lower reserve levels in defoliated and watered plants was a major factor in their lack of heat hardiness.

In addition to the difference in heat hardiness as plants or plant tissue ages, there are also genotypic differences. For example, Mitchell (1956) found that perennial ryegrass was less sensitive to high temperature (up to 35°C), than biennial ryegrass.

As temperature increases above the optimal range for grasses, one of the first morphological effects observed is an increase in the rate of root maturation (Beard 1973), and death of some of the root system (Stuckey 1941; Sullivan and Sprague 1949), along with cessation of root initiation (Beard 1973). This is illustrated in perennial ryegrass by Sullivan and Sprague (1949), where plants were exposed to temperatures ranging from 10 to 32°C; as temperature increased, roots were the first part of the plant to suffer injury and death.

After roots, the next discernible effect of high temperatures is a reduction in leaf size and rate of appearance (Baker and Jung 1968a; Mitchell 1953a, 1956). Reducing the rate of leaf appearance also reduces the number of potential tiller sites (Davies 1974), explaining the observation by Smith and Jewiss (1966) that timothy grown at 29.5/21°C (day/night) had fewer tillers/plant than when grown at 18.5/10°C. Alberda (1957) also found in perennial ryegrass that resumption of tillering following defoliation, enhanced at a temperature regime of 20/3°C, was inhibited at a regime of 20/28°C. Higher temperatures not only reduce tiller initiation, but also reduce expansion of tillers already initiated (Ryle 1964), and may also hasten flowering or maturity of temperate grasses (Smith and Jewiss 1966; Smith 1970). Mitchell (1956) found that there was a wide range of temperature over which growth of individual tillers of perennial ryegrass was unaffected - a decrease in growth was not observed until temperature was less than 13°C, or greater than 29°C.

The mechanism by which heat stress reduces growth may be direct, through disruption of protein or nucleic acid synthesis, or indirect, through depletion of reserve levels.

Pollock et al. (1993) noted that once temperature was increased beyond a certain point, a sudden decline in the plant's ability to synthesise proteins occurred, and growth ceased. In response to high temperatures, plants produce heat-shock proteins (see section 2.2.4), and as temperatures rise to injurious levels, the presence of these heat-shock proteins is associated with development of a degree of thermotolerance by the plant (Bettany 1995a). In general, a heat-shock response is induced by a 10°C rise from the optimum temperature for growth, and in annual ryegrass, Pollock et al. (1993) reported induction of heat-shock proteins at 35°C. However both the production of heat-shock proteins, and related acquisition of thermotolerance by the plant, last less than 48 hours, and prolonged high temperature does not result in a continuation of this response (Pollock 1992; Pollock et al. 1993; Bettany 1995b).

Another direct effect of high temperatures on plants is a reduction in nucleic acids. Baker and Jung (1970) subjected timothy, cocksfoot, bromegrass and Kentucky bluegrass plants to temperatures of up to 35°C in a glasshouse, and found the more heat-sensitive species (timothy and Kentucky bluegrass) contained less ribonucleic acid and deoxyribonucleic

acid per g tissue, than the more heat-tolerant cocksfoot and bromegrass. This reduction of nucleic acids would ultimately reduce plant growth and function.

Despite the fact that older plant tissue is more resistant to injury from exposure to high temperatures than younger tissue, the temperature optimum for plant growth declines with age of plant tissue, due to the increasing importance of dark respiration (McWilliam 1978). As opposed to respiration in daylight (photorespiration or 'growth' respiration), dark respiration (respiration in absence of light) is mainly associated with protein turnover in older plant cells, and repair and maintenance of cell structures (Robson 1981; Lambers 1985). On average, 40 to 60% of WSC produced via photosynthesis each day is consumed by the respiration process, even under the most favourable conditions (Robson 1973b; Lambers 1985; Danckwerts and Gordon 1987), and dark respiration exhibits an exponential increase with increasing temperature (Murata and Iyama 1963; McWilliam 1978).

In confirmation of this, high temperature has been shown to decrease reserve levels in storage tissues of temperate grasses (Alberda 1957, 1965; Blaser et al. 1966; Deinum 1966; Smith and Jewiss 1966; Baker and Jung 1968a, b), - this is because the temperature optimum for photosynthesis is lower than for respiration, so as temperature increases, WSC buildup from photosynthesis slows and loss from respiration increases. If this situation continues long enough, plant reserve levels may become exhausted (Beard 1973); this is illustrated in a study by Sullivan and Sprague (1949), where high temperature following defoliation of perennial ryegrass adversely affected regrowth. In this study, plants were stunted, the roots were discoloured and dead in some cases, and stubble WSC was less than 5% following 40 days of exposure to temperatures of 32/27°C (day/night), compared to plants regrown at 15/10°C (day/night).

Death of plants through exhaustion of plant reserves may be one reason behind the observed decrease in the number of perennial ryegrass plants during the summer months in subtropical Australia (Fulkerson et al. 1993a), and this is termed 'C starvation' (W.J. Fulkerson, personal communication). The theory behind C starvation in perennial ryegrass is that high summer temperatures would lower WSC levels, while the frequent

cloud cover at this time would retard photosynthesis (Evans *et al.* 1964; Ludlow 1978), and therefore hinder WSC buildup in the plant (W.J. Fulkerson, unpublished data).

Baker and Jung (1968a) found that night temperature was more important than day temperature in causing a decline in the level of reserves in timothy, cocksfoot, bromegrass and Kentucky bluegrass - in general, WSC decreased as night temperature increased from 2 to 19°C. Similarly, Alberda (1957) found that following defoliation, under a day/night temperature regime of 20/3°C, regrowth of perennial ryegrass was rapid and stubble WSC was replenished to original levels within 3 weeks. However, under a temperature regime of 20/28°C, regrowth was slower, some plants died, and after 7 weeks, stubble WSC levels had not been replenished to any degree.

2.2.4.2 Lack of root development. A major limitation to persistence of perennial ryegrass in the subtropics appears to be lack of root development - more than 80% of root DM is in the top 50 mm of soil, and penetration is to a maximum of about 200 mm depth (Fulkerson *et al.* 1993b; Fulkerson and Lowe 1994). This lack of root development has been observed for both perennial and annual ryegrass genotypes, when grown on all major soil types on which subtropical dairy pastures are grown in Australia (W.J. Fulkerson, personal communication).

The problems resulting from this poor rooting of ryegrass include a limited volume of soil from which to extract water and nutrients, making plants susceptible to periods of low moisture availability or poor soil nutrition. Shallow rooting may also lead to increased loss of plants through sod pulling by grazing stock, and damage to plants through 'pugging' (soil compaction caused by movement of stock over wet soil).

It is unclear why ryegrass roots are restricted to the soil surface in the subtropics, but a number of factors are known to impinge on the root activity of grasses. Firstly, defoliation reduces rooting (see section 2.3.4). However, the distribution of roots through the soil profile is unchanged by defoliation - greater than 80% of root DM is still found in the top 50 mm of soil (W.J. Fulkerson, unpublished data).

Secondly, application of N fertiliser may result in surface rooting (Oswalt *et al.* 1959; Fulkerson *et al.* 1993b), and this has been reported to increase sod pulling of perennial ryegrass (Tallowin *et al.* 1986). A third factor implicated in the poor rooting of ryegrass is the high temperature experienced in the subtropics. Davidson (1969) showed that the temperature optimum for root growth of perennial ryegrass grown in a glasshouse was 12.5°C, indicating that for many months of the year, prevailing temperatures in the subtropics would restrict root growth.

Fourthly, pathogens are believed to affect root development. Few studies have been conducted in the subtropics to determine the effects of pathogens on root integrity of perennial ryegrass. Fungal analysis of roots from sod-pulled perennial ryegrass plants showed high levels of *Rhizoctonia* fungus (W.J. Fulkerson, unpublished data), indicating the possible involvement of this fungus in weakening the root system. However, it may also be that the roots had been injured by other pathogens, and thus *Rhizoctonia* fungus infection may not be a causative factor in sod pulling. Little is known about the effects of other root pathogens, such as insect larvae, on perennial ryegrass roots under subtropical conditions.

Soil physical and chemical properties may also contribute to poor rooting in ryegrass. Physical properties of soil such as bulk density and the formation of hardpans (compact soil layer beneath the surface) both restrict roots, and while this may have some bearing on pastures grown on the clay flood plains, the pattern of surface rooting is also observed on the much less dense krasnozem loams. Chemical properties such as nutrient availability and pH also impinge on rooting, however seedbed preparation practices and subsequent soil tests indicate that neither of these factors should have limited root growth in the study by Fulkerson *et al.* (1993b).

The observation that roots of perennial ryegrass grown in potting mix, in both outdoor and glasshouse conditions, easily reach 300 to 400 mm depth (W.J. Fulkerson, unpublished data), confirms that these plants are capable of deeper rooting, and indicates the need for detailed studies on soil physical and chemical properties, and also root pathogens, to explain why roots do not reach this depth in the field.

2.2.4.3 Competition from tropical grasses. Reduced persistence of perennial ryegrass in the subtropics has been closely associated with invasion of pasture by unsown tropical grasses, which germinate during the warmer months of the year (Jones *et al.* 1968; Fulkerson *et al.* 1993b; Fulkerson and Slack 1994a). Even in a temperate environment, pastures sown with temperate species are prone to invasion by vigorous summer-growing species (Hutchinson 1970). It is unclear whether tropical grass invasion is a consequence of loss of ryegrass plants, or a cause of it, or both, although it is believed that death of ryegrass plants provides gaps in the pasture which tropical grasses are quick to colonise (Fulkerson *et al.* 1993a).

Tropical grasses are characterised by faster growth rates both above and below ground at temperatures above about 20°C (Colman and Lazenby 1970; Downes 1970; Wilson and Ford 1971), have higher rates of photosynthesis, lose less C through respiration, and use water more efficiently than temperate grasses. Both Murata and Iyama (1963) and Downes and Hesketh (1968) measured average rates of photosynthesis in tropical grasses nearly double that of temperate grasses, without the photorespiratory loss of CO_2. In a later experiment, Downes (1970) noted that tropical grasses produced double the leaf DM of temperate grasses per unit of water lost through transpiration, indicating greater efficiency in water use. In addition, the activity of CO_2-fixing enzymes is higher in tropical than temperate grasses, further indicating that tropical grasses are more efficient in the process of photosynthesis.

In addition to greater growth and activity above ground, tropical grasses also exhibit greater root growth than temperate species in the subtropics. In a comparison of kikuyu and perennial ryegrass plants of similar age, Fulkerson and Lowe (1994) observed kikuyu had a more extensive root system than perennial ryegrass; roots of kikuyu extended to at least 400 mm soil depth, while those of perennial ryegrass did not penetrate any deeper than 200 mm. Between 350 and 400 soil depth, kikuyu root DM was comparable to that of perennial ryegrass at 100 mm depth. In addition to a more extensive root system, Colman and Lazenby (1970) found that tropical grasses at higher temperatures (greater than 20°C) exhibited higher root activity (as determined by increased N uptake) than temperate grasses.

In a study of 4 annual weed species, Roush and Radosevich (1985) determined that the relative competitive ability of a species was strongly related to growth rate. Thus tropical grasses, by growing faster and larger than perennial ryegrass during the warmer months in the subtropics, utilise a disproportionate amount of available resources and hence are more competitive. Simply having a deeper root system gives tropical grasses a competitive advantage over temperate grasses in situations where soil nutrients may be limiting, and rainfall may be infrequent or severe, as is characteristic of the subtropics (Fulkerson and Lowe 1994).

To illustrate the magnitude of competition from tropical grasses on perennial ryegrass, in New Zealand, Thom *et al.* (1986) reduced the competition to perennial ryegrass plants from paspalum by clipping potentially shading leaves of paspalum in early summer. As well as reducing competition for light and space, clipping would, through retarding the root system, reduce competition from paspalum for water and nutrients, and a combination of these factors led to a 230 to 300% increase in ryegrass tillers/plant, and a 3-fold reduction in ryegrass plant deaths over summer.

2.2.4.4 Effect of plant pathogens. Leaf or crown rust is the most damaging disease of perennial and annual ryegrasses in the subtropics (Fulkerson *et al.* 1993a; Fulkerson and Lowe 1994). In New Zealand, Lancashire (1974) found that rust infestation of a perennial ryegrass/white clover pasture decreased ryegrass DM yield by 15% under a 10 day grazing rotation throughout summer and autumn, but by 45% under a 40 day rotation. However, if rust was negligible, the longer grazing interval resulted in 150% higher DM than grazing at 10 day intervals and indicates the penalty of having to graze frequently to overcome the spread of rust. The importance of rust in the subtropics was highlighted by Lowe *et al.* (1993), who found that the performance of annual ryegrass under field conditions was highly and positively correlated with rust resistance, however to date, there are no rust resistant perennial ryegrass cultivars commercially available, although cultivars exhibit differing degrees of tolerance to infection (Lowe and Bowdler 1995). Without N and moisture stress, the effect of rust on ryegrass can be minimal (Fulkerson *et al.* 1993a; Lowe and Bowdler 1995). Although more frequent defoliation reduces rust infestation, care must be taken when using this as a management tool, as frequent defoliation may be

detrimental to the plant (see section 2.3).

Sarathchandra *et al.* (1995) found that growth of perennial ryegrass in New Zealand was unaffected by any of the common plant-parasitic nematodes present in soil, and although several fungal species such as *Fusarium* were found to be associated with plant roots, this had no apparent affect on plant production or survival. However, the fungus *Conidaea fertilis*, associated with plant roots, adversely affected DM production. In the subtropics, relatively little is known regarding the effects of other pathogens besides rust on perennial ryegrass, as few studies have been conducted to identify potential pathogens in temperate pastures under irrigation.

2.2.4.5 Edaphic limitations. Edaphic limitations to growth of sown temperate pasture in the subtropics include pH, moisture and nutrient levels, and compaction.

Soil pH in the subtropics is generally neutral to slightly acidic, and the leaching of applied N fertiliser may reduce pH even more (Reason *et al.* 1989). On a heavy clay soil, Fulkerson and Slack (1993b) found that increasing soil pH from 4.6 to 5.8 (measured in $CaCl_2$) increased DM yield of perennial ryegrass and white clover by a mean of 23% over a 2 year period.

Lowe *et al.* (1992) reported that soil compaction from pugging resulted in a reduction of annual ryegrass yields which lasted well into the next growing season. Similarly, W.J Fulkerson and K. Slack (unpublished data) showed that prolonged waterlogging (4 to 5 weeks) reduced pasture growth by 18%, but if cows were allowed to graze under these conditions, regrowth following grazing was reduced by 73%, indicating the need to keep stock off waterlogged pasture.

Losses of the major nutrients phosphorus and potassium in a dairy system may be considerable, and unless replaced, will lower soil fertility levels, and eventually affect the productivity and persistence of ryegrass plants.

The main losses of phosphorus and potassium take place through removal of animal products (milk, sale of livestock), transfer to unproductive areas (excretion in laneways, dairy area), and leaching and runoff during heavy rainfall (Parfitt 1980; Williams *et al.* 1990). In addition, phosphorus may be immobilised in the dung, in plant residues or bound to soil particles, and therefore be unavailable for plant uptake (Parfitt 1980).

The following is an approximation of fertility losses in a dairy system in temperate Australia (E.J. Havilah, personal communication); figures will vary with changes in climate and soil type, and in stocking rate and animal productivity. Based on a stocking rate of 2.5 cows/ha milking area, at an average production per lactation of 5,000 L, it was calculated that 40 kg of phosphorus/ha/year and 75 kg potassium/ha/year needed to be applied to replace nutrient loss via export in products.

2.2.4.6 N fertiliser application. The addition of N fertiliser to grass generally promotes leaf growth (Langer 1966) and tillering (Wilman and Fisher 1996), although it may have adverse environmental consequences. In subtropical Australia, Fulkerson *et al.* (1993b) and Lowe *et al.* (1995) reported that application of N fertiliser through winter and spring improved survival of perennial ryegrass plants over summer, however these studies were conducted under cutting; results may be altered under grazing conditions.

N fertiliser application has previously been observed to decrease WSC content of grasses (see section 2.2.4), probably through stimulating new growth and hence use of WSC (Auda *et al.* 1966). In a study in the United States with 17 grass species including perennial and biennial ryegrass, Carroll (1943) found that the decline in WSC levels with increased application of fertiliser N, was associated with reduced plant survival if the plant was subsequently stressed by low soil moisture, and high or low temperatures. Lucanus *et al.* (1960) obtained similar results with both perennial and biennial ryegrass in New Zealand - plant survival under conditions of low soil moisture and high temperature was much less when soil N was high, and this has also been found by Baker and Jung (1968b) with 4 grass species in the United States. Thus, although the decrease in WSC with addition of N fertiliser may only be transient (as the increase in leaf area should increase further production of WSC), in times of stress such as drought, application of N may

prejudice plant survival.

Application of fertiliser N also stimulates root growth (Brouwer 1966), particularly in the upper layer of soil (Oswalt *et al.* 1959; Fulkerson *et al.* 1993b), and this would presumably increase damage to roots through soil compaction, exacerbate the effect of low soil nutrient or water levels, and make plants more prone to sod pulling. To illustrate this, in a field study in the United Kingdom, Tallowin *et al.* (1986) found sod pulling of perennial ryegrass was highly and positively correlated with an increase in the rate of applied N.

Finally, applications of N fertiliser may reduce soil pH. On the heavy clay soils in subtropical Australia, this reduction in pH is small and pasture growth is unlikely to be affected (Fulkerson and Lowe 1994), however on the more porous krasnozem soils, pH may be reduced to levels which retard pasture growth (Awad and Edwards 1977).

2.3 The clover plant

2.3.1 Origin and distribution

White clover is a temperate legume, whose origins have been traced back to Eurasia, North America and possibly Africa (Frame and Newbould 1986). It is grown principally as the legume component of mixed grass/clover pasture, and is the most common legume component of perennial pastures in all temperate countries (Ostrowski 1972). White clover is generally not grown in monoculture due to difficulties in keeping out weeds, low annual herbage production compared to mixed pasture, and concern with bloat and possible reproductive problems in grazing livestock, due to phyto-oestrogen levels in the herage (Frame and Newbould 1986).

2.3.2 Clover plant structure

White clover generally has a prostrate growth habit, and initially develops a branching stolon structure radiating outward from a central taproot. Lateral spread is from growing points at the tips of stolons, and each stolon is segmented by nodes, from which arise trifoliate leaves, borne upon petioles. Nodes are also capable of producing adventitious

roots, and are the sites at which axillary buds can grow out to form a stolon branch or a flower (Carlson *et al.* 1985; Frame and Newbould 1986).

White clover exhibits a strong clonal growth pattern, with expansion of the plant from growing points eventually balanced by death of the taproot and older stolons, leading to plant breakup similar to that in ryegrass (Figure 2.2, see also section 2.2). Also similar to ryegrass, breakup of white clover in temperate areas reaches a peak in spring (Brock *et al.* 1988; Pinxterhuis *et al.* 1993; Brock and Hay 1996). However, whereas breakup of ryegrass plants occurs some 4 or 5 years after sowing, breakup of white clover may take place from the first or second year after sowing (Carlson *et al.* 1985; J.L. Brock, personal communication).
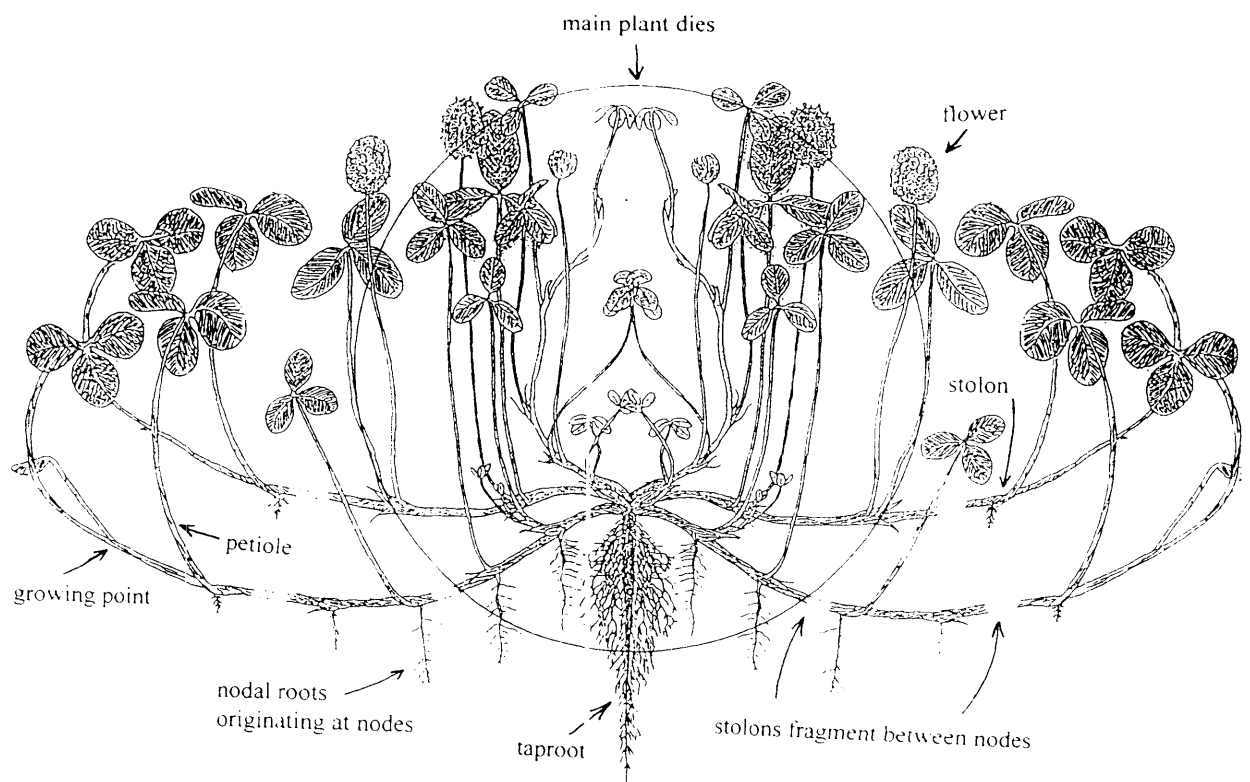
main plant dies

flower

stolon

petiole

growing point

nodal roots originating at nodes

stolons fragment between nodes

taproot

Figure 2.2. The physiological process of stolon breakup in a white clover plant.

Perennation of white clover into a second year may be by survival of stolon fragments following breakup, and/or recruitment of seedlings from seed set in the first year. In New

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Zealand, Watson *et al.* (1993) found that recovery of white clover following summer was much more rapid when regeneration was from stolon fragments than from seed. Under irrigated conditions, perennation has been shown to depend overwhelmingly on stolon survival (Hollowell 1966; Fulkerson and Slack 1996a), whereas under dryland conditions, seed production and seedling recruitment assumes greater importance (Hutchinson 1970; Jones 1982; Garden 1988; Archer and Robinson 1989).

Under irrigated conditions, propagation of white clover from seed is regarded as a long-term strategy for perennation, whereas propagation through stolon breakup is regarded as a more immediate survival strategy. This is because seed production may vary greatly each year with seasonal changes, and is naturally lower under high grazing pressure when most flowers may be removed. Also, a high proportion of seed set by white clover is 'hard' (protected by an impermeable coat and therefore will not germinate until the coat is eroded by weather or insect damage, for example). Field studies in subtropical (Jones and Evans 1977; Fulkerson and Slack 1996a) and temperate (Archer and Rochester 1982) environments found greater than 80% of white clover seed to be hard. Therefore development and replacement of stolons has a greater influence on the perenniality of white clover than seedling recruitment (Hollowell 1966; Archer and Robinson 1989; Clark and McFadden 1997). In theory, then, more stoloniferous white clover types (characteristic of small-leafed genotypes) may have greater potential to persist than the less stoloniferous, large-leafed types (Frame and Newbould 1986).

In the following sections, growth of leaves, stolons and roots will be examined in more detail, and the role of plant energy reserves in their growth will be discussed.

2.3.2.1 Leaves and petioles. The trifoliate leaves of white clover are initiated from the nodes along the stolon, and have a lifespan of 20 to 40 days from time of emergence (Brougham 1958a, b). This short leaf lifespan, together with continual movement of growing points outward from the taproot, eventually results in large areas of leafless stolons (Carlson *et al.* 1985). Field studies in New Zealand (Brock *et al.* 1988) have shown that at any given time of the year, only the youngest 3 or 4 nodes back from the growing point of a stolon bear live leaves.

Leaf number and rate of development are dependent on temperature, whereas leaf size is mainly influenced by the light environment within the pasture canopy, in particular the balance between red and far-red radiation (Brougham et al. 1978). White clover grows well at temperatures between 18 and 30°C, with an optimum temperature for general growth and function around 25°C, although it will still grow at temperatures as low as 5°C or as high as 40°C (Mitchell 1956; Brougham et al. 1978). The optimal temperature for growth declines with a fall in light intensity (Frame and Newbould 1986), as white clover is a sun-loving plant, with even moderate shading detrimentally affecting growth and function (Brougham 1958b; Ostrowski 1972; Brougham et al. 1978). However, when shaded by companion plants in a sward, white clover petioles are able to elongate, pushing leaves up into a more favourable light environment (Brougham 1958b).

A review by Frame and Newbould (1986) concluded that white clover is more sensitive than most grasses to low pH, with growth retarded below a pH of about 5. White clover is also more sensitive than grasses to deficiencies of soil phosphorus or potassium (Brougham et al. 1978; Frame and Newbould 1986), due to a less fibrous root system than grasses (Evans 1977; Caradus 1980).

2.3.2.2 Stolons. As previously mentioned, individual nodes along a stolon are the sites at which stolons branch and flowers and adventitious roots may be initiated, although flower production at a node eliminates the potential for a stolon branch to form, and vice-versa (Frame and Newbould 1986). The requirements for flowering in white clover are more diverse than in ryegrass. Some white clover genotypes require a period of vernalisation, while others require a defined photoperiod to flower (Brougham et al. 1978).

Studies in New Zealand have concluded that persistence of white clover into a second year is achieved through attaining a high density of growing points in the first year (Brock and Hay 1996), and as this occurs through repeated branching of stolons, extensive study has taken place on the stolon branching process and factors which influence it. Robin et al. (1994a) found that when nodes on a stolon were subjected to a low red: far-red light ratio (as would occur under shading within a pasture canopy), the appearance rate of branches was delayed, resulting in fewer branches than on stolons receiving a higher red: far-red

ratio. Apart from this effect of light quality on branching, Newton *et al.* (1992) reported that in grazed pasture, by the time axillary buds at nodes were mature enough to grow out and form branches, over half had lost their capacity to do so (i.e. they had become non-viable, dormant, or had formed a flower), whereas in the glasshouse, nearly all buds eventually formed branches. Further study revealed that, of a number of factors investigated, defoliation had the largest single effect on reducing bud viability (Newton and Hay 1996); the effects of defoliation on the white clover plant will be discussed further in section 3.3.

Brock and Hay (1996) stated that the period and extent of stolon breakup in New Zealand pasture can be modified by varying environmental conditions. Thus, the pattern of stolon breakup in subtropical areas, where a different climatic pattern exists, may be different to that in temperate areas. Support for this hypothesis can be found in previous studies on white clover grown under dryland conditions in the subtropics (Irwin and Jones 1977; Jones 1982; Garden 1988). Stolon death reached a peak during the first summer, and was associated with the loss of the primary taproot, and development of nodal roots (Jones 1980; Garden 1988). However, this peak in stolon death was attributed to moisture stress and pathogen damage, and the physiological process of stolon breakup was not measured. Given the importance of stolon breakup to perennation of white clover under irrigation, there exists a need to further investigate the timing and extent of breakup in the subtropics.

2.3.2.3 Roots. The white clover seedling develops an extensive seminal taproot system (Frame and Newbould 1986), up to 1 m length in some environments (Brougham *et al.* 1978). However, this root system is short-lived (Carlson *et al.* 1985), usually lasting less than one year, and stolons eventually depend on nodal roots (Hollowell 1966). Studies in New Zealand by Chapman (1983) and Brock *et al.* (1988) reported that rooted stolons had faster growth rates and lower mortality levels than unrooted stolons. Also in New Zealand, a field study by Newton and Hay (1994) found that less than half of stolon nodes developed roots, and this was influenced by grazing pressure. Under rotational grazing with sheep, 40% of nodes developed roots, compared to 29% of nodes under set stocking. Thus, overgrazing of white clover may decrease persistence through disruption of the formation of nodal roots.

Initiation of nodal roots appears to require relatively high humidity, with root initiation in a glasshouse trial inhibited at a relative humidity of 85% or less (Stevenson and Laidlaw 1985), thus under dry conditions, rooting may be minimal. Chapman (1983) found that most nodal roots were initiated within 4 to 8 weeks from emergence of the node on the stolon. Once initiated, the survival of nodal roots is greater in the presence of a stolon branch at the same node (Newton and Hay 1994).

In New Zealand pasture, Brock et al. (1988) and Newton and Hay (1994) noted that nodal root formation reached a maximum in early spring prior to breakup. The death of the taproot soon after this peak in nodal rooting has led to speculation that establishment of nodal roots renders the taproot 'redundant', and death occurs soon after. However, Hollowell (1966) prevented nodal rooting of white clover in both the field and the glasshouse by elevating stolons, and reported that the initial taproot lived only slightly longer than taproots from plants in which nodal rooting was allowed to take place.

Legume roots form nodules through a symbiotic relationship with *Rhizobium* bacteria, enabling conversion of atmospheric N to nitrates for use by the plant (Raven et al. 1986). In a review, Brougham et al. (1978) quoted that up to 680 kg N/ha of atmospheric N may be fixed by white clover under favourable conditions of climate and management. Levels were substantially reduced under dryland conditions. The process of nodulation requires molybdenum, and prefers a soil pH close to neutral (Ostrowski 1972). The main taproot rarely forms nodules (Moustafa et al. 1969), highlighting the importance of nodal roots, not only in perennation, but in N fixation, which gives clover a competitive advantage over grasses under conditions of low soil mineral N availability.

Investigating the root systems of 18 white clover varieties grown in the field in New Zealand, Caradus (1977) found that large-leafed clover genotypes develop a more well-defined taproot system compared to more prostrate, small-leafed types, which may render small-leafed white clover genotypes less vulnerable to loss of the taproot following breakup.

2.3.2.4 Reserves. Compared to grass species, the white clover plant is generally lower in WSC (up to 15% of clover plant DM), but higher in total N compounds (up to 5% of DM) (Smith 1973; Frame and Newbould 1986). The carbohydrate reserves of white clover are predominantly starch, with some sucrose and hexose sugars (Smith 1973; Hay *et al.* 1989a). Starch, a polysaccharide, is largely water-insoluble, and so most white clover reserves are not WSC. For the purpose of white clover reserves, starch, along with sucrose and hexose sugars, will be referred to hence as total non-structural carbohydrates (TNC).

As with perennial ryegrass, storage of TNC in white clover is mainly in the basal portion of the plant, in this case the stolons and roots. In a tracer study using ^{14}C, Danckwerts and Gordon (1989) found that around 10% of the total ^{14}C assimilated by leaves was invested in long-term storage in stolons and roots. In that study, the observation that relative growth rate of plants was either constant, or increased throughout the experiment, confirmed that the C buildup observed was not due to a reduction in growth, but was in fact actual storage.

Due to the marked clonal growth pattern of white clover, where continual growth of stolons is balanced by death of older portions of the plant, the pattern of TNC storage differs with position (hence age) of the node from the growing point. In a field study in New Zealand, Hay *et al.* (1989a) noted that nodes numbered 4 to 10 back from the growing point (i.e. progressively older), dominated as the major storage zone for starch. Nodes numbered greater than 9 from the growing point were increasingly involved in transport of TNC (as sucrose), and decreasingly involved with both storage of TNC (as starch), and meristematic activity (where hexose sugars are high).

As with grasses, the concentration of TNC in white clover is markedly influenced by current rates of photosynthesis (as a source) and growth (as a sink), and follow a diurnal pattern of buildup from daylight to some time in the afternoon, then depletion until daylight the following day. Thus, as with WSC, TNC are decreased through a decline in light intensity, and also through an increase in temperature, due to greater respiratory load (Smith 1973).

Whereas the diurnal change in TNC is due to a change in both sucrose and starch fractions (Smith 1973), seasonal changes in TNC are dominated by variation in the starch fraction only (Hay *et al.* 1989a). In Europe, Murphy (1982) observed that stolon starch levels decreased in late winter (probably due to an increase in growth as climatic conditions improved), then increased along with an accompanying increase in spring growth and hence photosynthetic activity. In a more detailed study of stolon TNC fractions, Hay *et al.* (1989a) noted that under New Zealand conditions, starch abruptly declined in mid-spring, due to breakup and subsequent decay of stolons. This was followed by a buildup of starch in autumn, probably due to both an increase in photosynthetic activity, as stolon fragments would be actively growing, and a decrease in respiration due to falling temperatures. To complete this seasonal pattern, stolon starch gradually declined over winter, probably due to continued growth utilising TNC but cloudy, dull weather preventing TNC buildup.

The role of reserves in sustaining plant growth during interruptions to photosynthetic activity such as defoliation, will be further discussed in section 3.3.1.

Similar to perennial ryegrass, white clover reserves have been implicated in development of tolerance by the plant to periods of stress, and in particular to frost. Collins and Rhodes (1995) reported that stolon TNC was an important factor in white clover survival over winter in the United Kingdom. N reserves also appear to play an important role, as in a controlled environment, Sandli *et al.* (1993) found that frost hardiness of white clover was enhanced by increasing N fertiliser supply, and was positively correlated with total N content of stolons. They also noted a significant increase in stolon TNC content during cold hardening. However, as is the case with perennial ryegrass (section 2.2.4), the buildup of reserves during periods of stress (heat, frost or drought) may not be an adaptive mechanism by which plants combat the stress, but simply an accumulation of reserves through decreased growth caused by the stress.

2.3.3 Response to defoliation

As with ryegrass, regrowth of white clover following defoliation is generally slower when plants were severely defoliated. For example, Hartwig *et al.* (1994), found that white clover plant growth was unaffected by removal of 40% or less of leaf area, but removal of

85 to 100% of leaf area depressed growth for several days. Overall however, clover is considered to be more resistant to defoliation than erect grass types such as ryegrass, due to stolons and roots, being the sites of reserve storage, generally escaping defoliation (Danckwerts and Gordon 1989) and to the greater photosynthetic area remaining after defoliation (Garden 1988). Also, clover has the ability to adapt to repeated defoliation through morphological adaptation by decreasing plant height (Frame and Newbould 1986, see also section 3.3.2). The plant will readily resume normal growth type if defoliation pressure is relaxed (Brougham et al. 1978).

The response of the white clover plant to defoliation is discussed in the following sections as it affects growth of leaves and petioles, stolons and roots.

2.3.3.1 Reserves. Defoliation of white clover leads to a reduction in supply of current photosynthate (which is exacerbated as defoliation severity increases), and the plant draws on a limited supply of stored reserves for subsequent regrowth (Danckwerts and Gordon 1989), resulting in a decline in TNC and N in all remaining plant parts, especially the stolon (Gallagher et al. 1994). Stolon reserves are preferentially used and only when they have been depleted are root reserves mobilised (Danckwerts and Gordon 1989). White clover plants only partly defoliated (removal of all but 2 leaves) regrew faster than those totally defoliated, implying that as with grasses, both RLA and reserves contribute energy to regrowth of white clover (see section 2.3.1), and hence severity of defoliation may affect the dependence of regrowth on reserves.

Gordon et al. (1986) found that severe defoliation of white clover (removal of up to 70% of shoot DM) caused TNC levels in root nodules to approximately halve within 24 to 48 hours; the rate of decline, and time to subsequently return to pre-defoliation levels, were both increased by increasing severity of defoliation. To elaborate, removal of 45% of shoot DM caused starch content to halve within 2 days, followed by a gradual recovery back to pre-defoliation levels within one week, whereas removal of 70% of shoot DM caused starch content to halve within one day, and levels still did not reach pre-defoliation levels after 2 weeks. Other studies have also measured a decline in white clover TNC proportional to severity of defoliation (Baur-Hoch et al. 1990; Kang and Brink 1995;

Lawson et al. 1995; Frankow-Lindberg 1997; Singh and Sale 1997a) - decreases in stolon TNC of up to 93% have been recorded one week after defoliation (Murphy 1982), coinciding with regrowth.

Compared to ryegrass, few studies have tried to link high levels of reserves prior to defoliation of white clover with proportionally greater regrowth. Baur-Hoch et al. (1990) reported higher regrowth rates in white clover with high TNC levels prior to defoliation, compared to plants with lower TNC levels. To date, no attempt has been made to elucidate critical TNC levels, probably due to the main reserve storage areas in white clover, the stolons and roots, generally being safe from removal by defoliation. Also, defoliation, unless severe, will not remove all leaves of clover plants, and regrowth may thus rely less on reserves than does regrowth of the more erect grass species.

Similar to regrowth of ryegrass, N reserves are also mobilised following defoliation of white clover. Using labelled ^{15}N, Corre et al. (1996) showed that defoliation of white clover led to about 55% of root N, and 70% of stolon N, being mobilised to support regrowth during the first 6 days following defoliation. Thereafter, most of the shoot N was derived from N taken up by the roots, and the contribution of reserves to regrowth declined. Similarly, Gordon et al. (1986) found that severe defoliation of white clover (removal of up to 70% of shoot DM) caused a 20% decline in soluble protein content and a 50% decline in leghaemoglobin (the major protein in white clover nodules) within the first 4 to 7 days after defoliation. With uninterrupted regrowth, protein and leghaemoglobin returned to pre-defoliation levels after about 16 days, whereas under continual defoliation, leghaemoglobin levels were depleted after 14 days. This pattern appears to be similar across forage legumes, as Kim et al. (1991, 1993) reported that in lucerne, approximately 40 and 60% of total N in roots and crowns, respectively, was mobilised to supply N to shoot regrowth following defoliation, and Culvenor and Simpson (1991) found that defoliation of subterranean clover caused a 37% reduction in root N within 5 days of defoliation, to supply regrowing shoots. The reported decline in soluble protein by Corre et al. (1996) from roots and stolons within the first 6 days of regrowth (32%), was similar to that reported from the stubble of perennial ryegrass (33%) 4 days after defoliation (Ourry et al. 1988).

Thus as with ryegrass, defoliation of white clover decreases TNC and N reserve levels, coinciding with regrowth, followed by a gradual buildup to pre-defoliation levels as regrowth progresses. The period of reliance of white clover on reserves appears of similar duration to ryegrass (less than one week), and may actually be less, due to clover generally retaining greater photosynthetic area than ryegrass following defoliation to any given height. However, under conditions of shading or prolonged defoliation, clover reserves would presumably assume greater importance for regrowth.

2.3.3.2 Leaves and petioles. Due to the generally prostrate growth habit of white clover, at any given time, there will be leaves at varying elevations from ground level. Thus unless defoliation is extremely close (e.g. less than around 5 mm height), there will always be some RLA remaining after defoliation of clover, to provide current photosynthate for regrowth. For example, Hay *et al.* (1993) found that following rotational grazing of perennial ryegrass/white clover pasture by cattle, 21% more leaves of young branching stolons (less than 4 nodes/branch) remained undefoliated compared to leaves of parent stolons. This result tended to decrease as stocking rate (and hence defoliation intensity) increased from 2.5 to 5 to 7.5 bulls/ha. It was reported that this avoidance of defoliation was due to the short petiole length of leaves on younger stolons. This may also explain the finding that small-leafed varieties of white clover perform better under more frequent defoliation than medium- to large-leafed varieties (Wilman and Asiegbu 1982a; Brock and Hay 1996), as a greater proportion of their leaves escape defoliation (Wilman and Asiegbu 1982b; Kang and Brink 1995).

A number of studies, in the glasshouse and in the field, have shown white clover adapts to an increase in defoliation severity with a decrease in leaf DM (Wilman and Asiegbu 1982b; Wilman and Acunap 1993; Lawson *et al.* 1995; Singh and Sale 1997b), leaf area (Wilman and Acunap 1993; Lawson *et al.* 1995; Hay and Newton 1996; Singh and Sale 1997b) and petiole length (King *et al.* 1978; Wilman and Asiegbu 1982a, b; Wilman and Acunap 1993; Hay and Newton 1996), resulting in a smaller, more prostrate plant.

To illustrate this point, it has been reported that DM yield and content of white clover in pasture may be increased by close defoliation, provided that the interval between

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defoliations allows the plant adequate time to recover (Reid 1959; Reid 1962; Appadurai and Holmes 1964; Frame 1966a, b; Boyd and Frame 1983; Frame and Boyd 1987; Brink 1995). For example, Brink (1995) defoliated 3 white clover cultivars to stubble heights of 25 or 100 mm every 7, 28 or 49 days from spring to autumn over a 2 year period, and found that regardless of interval, increasing closeness of defoliation increased DM yield, however a combination of both close and frequent defoliation was eventually detrimental to plant survival.

Boyd and Frame (1983) reported that the observed increase in white clover DM with close defoliation in a mixed perennial ryegrass/clover pasture was due partly to greater utilisation of herbage (i.e. more was harvested), and partly to a reduction in shading by the taller ryegrass. Similarly, Wilman and Acunap (1993) in a 3 year study of perennial ryegrass/white clover swards defoliated monthly to varying heights (20 mm intervals from 20 to 100 mm), found that lax defoliation (80 and 100 mm) resulted in a decline in clover content attributable to shading by ryegrass. Thus in a mixed grass/clover pasture, the response of clover to defoliation appears as closely linked to the effect of defoliation on reducing the competitiveness of the grass component, as it is to the effect of defoliation upon clover *per se*.

To maximise white clover DM production, there appear to be 2 contrasting defoliation regimes, based on leaf size. The productivity of both small- and large-leafed varieties will be increased by close (20 to 50 mm) defoliation, but large-leafed varieties require longer intervals between defoliations for the plant to recover, whereas smaller leafed varieties can more easily adapt to frequent defoliation. Conversely, larger leafed varieties can tolerate longer intervals and more lax defoliation heights better than small-leafed varieties.

Fulkerson and Slack (1996a) defoliated white clover-based pasture in the subtropics at an interval which was based on the lower leaves of the plant (i.e. younger leaves) beginning to senesce. Thus growth was allowed to proceed without shading becoming detrimental to clover survival. From field observations (W.J. Fulkerson, personal communication), this approach coincides with the 3-leaf stage of regrowth of the companion ryegrass plants. Therefore, in a mixed perennial ryegrass/white clover pasture, a defoliation interval of

around 3 leaves/tiller for the ryegrass plant would seem to be near-optimal for both components of the pasture.

2.3.3.3 Stolons. An increase in defoliation severity decreases stolon DM (Hay *et al.* 1989b; Brink 1995; Singh and Sale 1997b) due to a reduction in stolon length (King *et al.* 1978; Wilman and Asiegbu 1982b; Wilman and Acunap 1993; Lawson *et al.* 1995; Hay and Newton 1996) and diameter (Wilman and Asiegbu 1982b; Wilman and Acunap 1993; Hay and Newton 1996). It is also possible that, as defoliation decreases stolon reserve levels, the observed decline in DM could be partly attributable to a loss of reserves. However, close defoliation also causes an increase in stolon branching relative to lax or no defoliation (King *et al.* 1978; Wilman and Acunap 1993; Hay and Newton 1996), eventually leading to higher stolon densities under close, relative to lax, defoliation (King *et al.* 1978; Wilman and Acunap 1993). This is a direct effect of more light penetrating the pasture canopy, as even mild shading of stolons decreases branching through a delay in branch appearance (Robin *et al.* 1994a, b).

However, severe defoliation may be detrimental to the branching process. Hay and Newton (1996) subjected white clover plants to varying defoliation severities (related to progressive removal of leaves) over a 37 day period in the glasshouse, and found that relative to undefoliated plants, severe defoliation (removing either all unfolded leaves, or all except the most recently unfolded leaf) decreased branch formation from axillary buds. This was associated with both a decrease in bud viability (by 44% compared to undefoliated plants), and a decrease in bud outgrowth to form a branch (Newton and Hay 1996). Hay and Newton (1996) concluded that the loss of bud viability was associated with physiological changes induced by defoliation, rather than defoliation *per se*, as there was no loss of bud viability once plants had adjusted their phenotype to a smaller size. Defoliation usually removes only leaves and petioles, but can sometimes remove stolons. Evans *et al.* (1992), in a study with several white clover varieties in the United Kingdom, concluded that greater removal of stolons by grazing animals occurred in large-leafed genotypes, than in small-leafed genotypes.

Close defoliation may also be more detrimental to clover when temperatures are high (Brock and Caradus 1996). Under drought conditions in New Zealand, 75 to 90% of stolons died under rotational grazing which left a more open sward, compared to no stolon death under set stocking.

Piano and Annicchiarico (1995), in an assessment of 34 white clover varieties over 5 years, found that persistence was positively correlated to density and DM of stolons. Likewise, Norris (1981), in a study with 19 varieties of white clover, found that stolon DM was positively correlated to rapid growth and high leaf DM yields. Thus, a defoliation regime which is close enough to promote an increase in stolon density, but of long enough interval to allow stolon DM to recover, should maximise persistence and productivity of white clover.

2.3.3.4 Roots. As with ryegrass, defoliation of white clover results in a decrease in root growth and function, the decrease being more pronounced with increasing severity of defoliation. Singh and Sale (1997b) defoliated white clover plants in the glasshouse at either 9 or 18 day intervals for 36 days, and found that the more frequent defoliation decreased root DM by 86%, and root length by 58%. Not only is the established taproot structure decreased by defoliation, but the ongoing process of nodal rooting also declines. Under a comparison of set stocking and rotational grazing (at 24 to 60 day intervals throughout the year) of perennial ryegrass/white clover pastures by sheep in New Zealand, Newton and Hay (1994) found that more frequent defoliation decreased nodal rooting from 40% under rotational grazing to 29% under set stocking.

Evans (1973a) defoliated white and red clover, perennial ryegrass, timothy and cocksfoot plants every second day to heights of 25, 50 or 100 mm, and discovered that at 25 mm, root elongation ceased in all species except white clover, where it was reduced to approximately 5% of that of undefoliated plants. In grasses, root elongation was found to be related to RLA remaining after initial defoliation, but this relationship did not hold for the clovers. Thus, it appears that clovers either have a greater storage capacity for reserves, or divert a greater proportion of reserves into continued root production than do grasses. Hodgkinson and Baas Becking (1977) reported a similar lack of response to

complete defoliation by lucerne in terms of root extension, longevity and branching, compared to wallaby grass and saltbush species, and concluded that this was due to the ready availability of carbohydrates from the taproot.

Butler *et al.* (1959) found that white clover adapts well to recurrent defoliation (defoliation to approximately 10 mm height at 11 day intervals), with death of roots and nodules being more than counterbalanced by regeneration of new roots and nodules. Only after very severe defoliation (80 to 85% of shoot removed) was deterioration of nodules evident from microscopic examination, and even then, damage to nodules was repaired as regrowth progressed and there was no overall loss of nodules (Gordon *et al.* 1986).

As well as retarding root growth, defoliation also causes a decrease in root function, in relation to the severity of defoliation. For example, Hartwig *et al.* (1994) reported that removal of less than 50% of leaf area in white clover had no effect on the activity of nitrogenase (the enzyme responsible for N fixation), however removal of more than 50% of leaf area led to a decrease in nitrogenase activity in relation to leaf area loss. Moustafa *et al.* (1969) in a study with established white clover plants, found that defoliation to 12 mm stubble height decreased the rate of N fixation in roots within 24 hours, which remained at low levels for 6 days and then increased, reaching levels similar to undefoliated plants within 21 days. Gordon *et al.* (1990) and Kang and Brink (1995) have also reported a disruption to the N fixation process following defoliation of white clover.

2.3.4 Limitations to growth and persistence

As with ryegrass, (section 2.4) the climatic conditions prevailing during the cooler months in the subtropics are optimal for clover growth and function, however conditions during the warmer months may become stressful.

2.3.4.1 High temperature.
The optimum mean temperatures for overall growth (24°C) (Mitchell 1956) and photosynthesis (25°C) (Murata and Iyama 1963) of white clover are higher than those for perennial ryegrass (approximately 18 and 15°C, respectively), suggesting white clover should cope better under a subtropical summer.

Although Brougham *et al.* (1978) stated that some growth of white clover still occurred at temperatures up to 40°C, in a field study in New Zealand over 2 years, Watson *et al.* (1996) defined the critical temperature of surface soil (to 10 mm depth) at which stolon death occurred as 30°C, however this was only in conjunction with low soil moisture levels (below 30%). In temperate Australia, Archer and Robinson (1989) also found that white clover plant density was adversely affected when mean weekly maximum temperatures exceeded 20°C, and soil moisture levels were low. A reduction or cessation of white clover growth and function under conditions of low soil moisture has also been noted in a temperate environment by Stevenson and Laidlaw (1985), and in the subtropics by Ostrowski (1972) and Jones (1980).

Thus, as periods of high temperature are usually associated with low soil moisture (due to greater evaporation), for white clover, soil moisture may be more important than high temperatures, up to a point, in reducing plant growth and ultimately persistence. However, prolonged exposure to temperatures above the optimal range for white clover will eventually cause a decrease in growth and function, and the processes by which this occurs are the same as for perennial ryegrass (see section 2.4.1).

2.3.4.2 Competition from grasses. White clover has a distinct disadvantage in competition with most grass species, as prostrate plants are often shaded by the more erect grass species, and since even moderate shading is detrimental to white clover (Brougham 1958b; Ostrowski 1972; Brougham *et al.* 1978), productivity and persistence may be reduced in a mixed pasture.

Also, glasshouse studies by Evans (1977) with 3 legume and 5 grass species, and Caradus (1980) with 11 legume and 10 grass species, found that the roots of grasses were longer, thinner and more finely branched, with more root hairs, than roots of legumes. Thus grasses have a more extensive root system than legumes, and are able to explore a greater volume of soil, giving grasses in a grass/clover pasture mix a strong competitive advantage over clover in uptake of water and nutrients, especially relatively immobile nutrients such as phosphorus (Evans 1977, 1978; Caradus 1980).

Thus in Europe, Murphy (1982) reported a 14% reduction in white clover stolon DM when clover was grown with perennial ryegrass, in comparison to when clover was grown in monoculture. In perennial ryegrass/white clover pasture in the United Kingdom, Wilman and Asiegbu (1982a) found when grass growth was slow, white clover may benefit from infrequent defoliation, but under conditions of faster grass growth, more frequent defoliation favoured white clover. From a number of other studies (Brereton et al. 1985; Brock et al. 1988; Hay et al. 1989b; Wilman and Acunap 1993; Barthram and Grant 1995; Hayes and Williams 1995), it is apparent that in a mixed grass/clover sward, a defoliation regime which favours an increase in grass density will be at the expense of clover density. For example in New Zealand, studies comparing set stocking of perennial ryegrass/white clover with rotational grazing (24 to 60 day intervals) by sheep (Brock et al. 1988; Hay et al. 1989b) found that set stocking (i.e. more frequent defoliation) not only decreased clover plant size, but also reduced the space available for clover growth by increasing ryegrass tiller density. After one year, the density of clover growing points was 60% less (1,880 vs. 3,260/m^2), and the density of ryegrass tillers over twice as great (10,950 vs. 4,630/m^2), under set stocking compared to rotational grazing (Hay et al. 1989b).

Wilman and Asiegbu (1982a) showed that the addition of N fertiliser to pasture increased perennial ryegrass plant size (through an increase in tillering and plant height) compared to white clover, and this partly explains the adverse effect of N fertiliser application on white clover growth, also observed by Frame (1966a), Boyd and Frame (1983), Frame and Boyd (1987) and Yarrow and Penning (1994). The effect of N fertiliser on white clover will be discussed further in section 3.4.3.

As well as competition from the sown grass component of mixed pasture, white clover must also compete with vigorous tropical grasses during the warmer months. In a field trial conducted over 7 years in New Zealand, Lambert et al. (1979) grew perennial ryegrass/white clover either alone, or with kikuyu or paspalum, and found that the more erect tropical grass (paspalum) had a negligible effect on white clover content in pasture, but the more prostrate kikuyu decreased white clover DM yields by up to 37%. The proportion of kikuyu rose from 21% of pasture composition in the first year to 52% after 7 years. In the subtropics, where climate favours more vigorous growth of tropical grasses

than in temperate areas, the competition to white clover by tropical grasses may be expected to be more severe.

2.3.4.3 N fertiliser application. The process of atmospheric N fixation by white clover is not obligatory, as white clover will use mineral N first if it is available, then use N fixation if there is still a requirement for N in growth (Hoglund and Brock 1987). Thus N fertiliser applications reduce the amount of N fixation in white clover (Moustafa et al. 1969), and remove the competitive advantage of legumes over grasses in an environment with medium to low soil N levels. N fertiliser also enhances growth of grass relative to clover, and the resultant competition for light, water and nutrients may be more detrimental to white clover than the effects of N on clover alone (Frame and Newbould 1986).

2.3.4.4 Effect of plant pathogens. Colman (1964), in an investigation of plant-parasitic nematodes in subtropical pasture, found that after 182 days, white clover grown in fumigated soil (free of nematodes) produced 3 times more DM than plants grown in unfumigated soil, and the main cause of this was the presence of root-knot nematode (*Meloidogyne hapla*) in unfumigated soil. Similarly, Fulkerson and Slack (1996a, b) found that after 3 years, root-knot nematode numbers had risen to levels which severely affected white clover performance, and concluded that this may partly explain the lack of persistence of white clover in the subtropics beyond 3 or 4 years. In New Zealand, invasion by both root-knot and clover cyst nematodes (*Heterodera trifolii*) into roots of white clover was reported to severely reduce growth (Sarathchandra et al. 1995). Nematodes have long been implicated in reducing persistence of white clover in New Zealand (Watson et al. 1993, 1996) and in the United States (Carlson et al. 1985).

In subtropical Australia, Irwin and Jones (1977) noted that nematodes and plant pathogenic fungi combined to decrease the persistence of white clover, with *Pythium middletonii* isolated from stolons in the field. Other fungi, such as *Fusarium* and *Rhizoctonia*, are commonly isolated from white clover stolons and roots in the United States (Hollowell 1966; Carlson et al. 1985), New Zealand (Sarathchandra et al. 1995) and subtropical Australia (Irwin and Jones 1977), however it is not clear whether they are pathogenic, as their presence has little if any effect on the clover plant (Hollowell 1966;

Irwin and Jones 1977; Sarathchandra *et al.* 1995).

2.3.4.5 Stolon fragmentation. Following stolon breakup, the small stolon fragments must compete with larger, more established grasses for water, nutrients and light (Hollowell 1966), and it is expected that these stolon fragments will be vulnerable to environmental and managerial stresses at this time (Brock *et al.* 1988).

In New Zealand, Brock *et al.* (1988) noted that few of the nodal roots produced by white clover stolons just after breakup actually established, due to dry soil conditions and infestation with root-knot nematodes. In the subtropics, where stolon breakup could occur in summer (see section 3.2.2), it is expected that a combination of high temperature, vigorous tropical grass growth, and the possibility of waterlogging due to heavy rainfall at this time will hinder recovery of white clover.

2.3.4.6 Edaphic limitations and the influence of grazing animals. In a mixed perennial ryegrass/white clover pasture, white clover is often selectively grazed (i.e. defoliated more often and more severely) (Frame 1966a, b; Evans and Williams 1987), due to a higher digestibility (Fulkerson *et al* 1993a), and possibly palatability, than ryegrass.

In addition to the effects of selective grazing, white clover is also more adversely affected than grasses by soil compaction (Edmond 1964; Frame 1966b). In a study involving 10 different pasture species in New Zealand, Edmond (1964) found white clover yield and stolon survival were reduced more by soil compaction through treading by sheep, than were yield and survival of perennial ryegrass.

White clover is more sensitive than most grasses to low pH (Frame and Newbould 1986), and the process of root nodulation in particular is detrimentally affected by low pH (Ostrowski 1972). Carlson *et al.* (1985) and Frame and Newbould (1986) suggested a pH of at least 5.5 to 6.0 as optimal for white clover growth and function.

The loss of major nutrients from soil (see section 2.4.5) is particularly important, as white clover is also more sensitive than grasses to a reduction in soil phosphorus status (Caradus

1980).

2.4 Possible solutions to poor persistence of perennial ryegrass and white clover pasture in the subtropics

In attempting to improve the persistence of perennial ryegrass and white clover pasture in the subtropics, there are 2 general approaches which may be taken. Firstly, plant breeding objectives can focus on traits which are thought to be important for ryegrass and clover adaptation to, and survival under, subtropical conditions. Secondly, pasture management techniques can be fine-tuned, to optimise performance of current commercial plant cultivars.

As persistence of perennial ryegrass in the subtropics is closely related to plant survival through summer (Fulkerson *et al.* 1993b), many of the breeding objectives and management strategies are aimed at improving plant survival during this period.

2.4.1 Breeding objectives

In order for temperate pasture species to successfully adapt to subtropical conditions, Fulkerson *et al.* (1993a) outlined the following plant breeding objectives.

2.4.1.1 Primary breeding objectives. Primary breeding objectives include rust resistance, tolerance of high temperatures, summer dormancy and tolerance/resistance to plant nematodes.

a. Resistance to rust. Resistance to leaf rust fungus was seen as a primary breeding objective, as rust is the most damaging disease of ryegrass species (see section 2.4.4) and oats, and also infects fescue, cocksfoot and persian clover. Lowe and Bowdler (1995) found that all of the major commercial cultivars of perennial ryegrass currently available were highly susceptible to rust, as were many fescue and cocksfoot cultivars. No rust was observed on phalaris or brome cultivars over 2 years of experimental studies.

Annual ryegrass genotypes appear to have a higher degree of resistance to rust than perennial genotypes, although the variation in both is large, and rust resistance can successfully be selected for in breeding programs (Fulkerson et al. 1993a).

b. Tolerance to high temperatures. Another primary breeding objective for both perennial ryegrass and white clover is improved summer growth, and the benefits from this are 2-fold. Firstly, greater growth during the warmer months should increase the competitiveness of the temperate species against tropical grasses (Lowe and Bowdler 1984), and secondly, the greater growth would justify more frequent irrigation. At present, many farmers are reluctant to apply the frequent irrigation necessary to maintain persistence of perennial ryegrass over summer, as temperate pasture contributes little to milk production at this time (Fulkerson et al. 1993a).

In subtropical Australia, Lowe and Bowdler (1995) found a considerable range in summer growth activity and persistence among cultivars of perennial ryegrass, which suggested the ability to select varieties of ryegrass more adapted to subtropical conditions than current cultivars. In particular, the variety 'Dobson' yielded more DM over the summer months than other cultivars, and also increased tiller density over the 2 year experimental period. Also in the subtropics, Fulkerson et al. (1993c) found the summer-active white clover variety 'Osceola' produced more DM throughout the entire year, especially during the warmer months, and was better able to resist invasion of tropical grasses, than the more commonly sown variety Haifa.

c. Summer dormancy. An alternative to breeding perennial ryegrass for increased summer growth activity, is to select varieties which exhibit summer dormancy (see section 2.2.2). Silsbury (1961) found that summer-dormant cultivars of perennial ryegrass grown in temperate Australia exhibited greater persistence than existing commercial cultivars, due to better summer survival. Summer dormancy appears to be initiated and maintained by a combination of temperature and moisture stress (Hoen 1968; McWilliam 1978). However, if the addition of water causes dormancy to break in perennial ryegrass (Silsbury 1961; Biddiscombe et al. 1977; Arcioni et al. 1985c), then this would not be a useful trait as the subtropics are characterised by summer rainfall.

d. Tolerance/resistance to plant nematodes. Recently, the effect of plant-parasitic nematodes on persistence of white clover has become a concern (see section 3.4.4), as nematode infestation, particularly of root-knot nematode, appears to be far greater in the subtropics than in temperate regions (Fulkerson and Slack 1996a). In New Zealand, Van den Bosch and Mercer (1996a) tested 117 varieties of white clover, and found significant variation existed between varieties in resistance to root-knot nematode, although no plants were fully resistant, and concluded that breeding for resistance to root-knot nematodes was possible, but could be a slow process.

2.4.1.2 Secondary breeding objectives. Secondary breeding objectives also encompassed improved rooting depth of ryegrass plants, and an investigation of the effect of ryegrass endophyte (*Acremonium lolii*), which improves plant vigour by deterring pathogens (e.g. Argentine stem weevil, Prestige *et al.* 1992) and also possibly by an allelopathic effect on other pasture species (Fulkerson *et al.* 1993a).

If successful, some of the breeding objectives could be combined to create a more persistent perennial pasture. For example, a summer-dormant perennial ryegrass would better complement a summer-active white clover, than sowing perennial ryegrass and white clover cultivars which both have summer activity, and hence result in greater competition between the sown species during the major stress period (summer).

Yet another approach to attaining a perennial temperature pasture in the subtropics is to investigate the management necessary for biennial ryegrass genotypes to perennate. Biennial genotypes have the advantages of being more vigorous under appropriate defoliation regimes, have significantly larger tillers, greater root DM with depth, and higher WSC levels than perennial genotypes (Fulkerson *et al.* 1994). Biennial genotypes can persist for longer than one year under subtropical conditions (D.C. Goodenough, personal communication), but little is known regarding the conditions under which they will perennate.

2.4.2 Management options

2.4.2.1 Seedbed preparation. The key in preparing the seedbed is to reduce competition from tropical grasses by hindering their germination and establishment. This may be achieved by cultivation, herbicide application, growing a summer fodder crop (e.g. maize (*Zea mays* L.), or combinations of any of these.

Fulkerson and Slack (1994a) found the highest survival rate of perennial ryegrass plants, the lowest incursion of tropical grasses, and the highest ryegrass and white clover DM yields, were achieved through multiple cultivations (up to 6 disc harrowings) followed by a maize crop. However, this may not be possible on all farms, due to soil erosion concerns or lack of machinery, so in this case, herbicide would be a better option. Where none of the above management options are desired or possible, or if the seedbed is particularly weedy, the sowing date can be put back to later in the autumn, when disturbance of the soil in the then cooler temperatures may cause less tropical grass seeds to germinate.

Using lime to increase the naturally low soil pH in the subtropics, increased the DM of ryegrass and white clover, and led to less incursion of tropical grasses, than not liming the soil (Fulkerson *et al.* 1993b). To achieve this, 5 t of lime/ha was applied 3 weeks prior to sowing, which increased pH (in $CaCl_2$) from 4.6 to 5.8. In the United States, Carlson *et al.* (1985) recommend liming soil to a pH around 6 to 6.5 to obtain optimum productivity of white clover. Thus it appears that productivity of ryegrass and white clover are optimised more at pH around 6, than at lower pH.

Studies in which seeding rate was altered (Fulkerson *et al.*1993b; Fulkerson and Yeoman 1994) show that the best balanced pasture was obtained from 15 to 20 kg perennial ryegrass seed/ha, along with 4 to 5 kg white clover seed/ha. Higher seeding rates of ryegrass resulted in a pasture too dense for clover to readily establish, and requiring more N than the clover could immediately provide. Lower seeding rates of ryegrass resulted in a less dense pasture, and provided little competition toward tropical grass incursion.

To deal with the often copious summer rainfall, the soil surface needs to be able to shed water rapidly, to prevent pasture damage through prolonged waterlogging, or through pugging by stock. The type of drainage will depend on soil type, and on the existing slope of the land. Having the ability to shed water relatively quickly would also give farmers more confidence in applying the frequent summer irrigation necessary to maintain persistence (Fulkerson *et al.* 1993a, see also section 4.2.3).

2.4.2.2 Grazing. Grazing is the most flexible, and least expensive, of the management options. Grazing management encompasses interval (when to graze), intensity (how hard to graze) and duration (how long to graze) of grazing.

Grazing at set time intervals, or when the pasture sward reaches a given height, are 2 popular means of setting defoliation interval, however neither takes into account plant development or regrowth stage. For example, as plant size is influenced by mineral nutrition and soil moisture status (Turner and Begg 1978; Langer 1979), it is possible that under favourable conditions, plants may be quite large, but still in the early stages of regrowth. Likewise, grazing at set time intervals does not account for different rates of growth due to changes in climate.

From section 2.3, it appears that following defoliation, allowing ryegrass plants to regrow at least 2 new leaves/tiller before again defoliating, ensures that root growth has resumed and stubble WSC levels have begun to be replenished. Defoliating more frequently than at the 2- or 3-leaf stage of regrowth may jeopardise plant survival, and this may be associated with a decrease in WSC reserve levels, and a retarded root system, whereas defoliating any later than around 3 leaves/tiller will result in wastage of pasture through leaf senescence. Thus the optimum grazing interval to maximise the utilisation, productivity and persistence of *Lolium* species appears to be around 3 fully expanded leaves/tiller, however, so far, this has only been evaluated under cutting.

From section 2.3, it also appears that the optimum height of defoliation for ryegrass is around 50 or 60 mm stubble height. Defoliating any lower than this will deplete WSC through physical removal of part of the major WSC storage organ (stubble), and may

expose soil, which during the warmer months, can lead to high soil temperatures, detrimental to survival of ryegrass and white clover. Leaving greater than 50 to 60 mm stubble height after grazing may also be detrimental to the survival of ryegrass and clover, through shading of young tillers and growing points. Although Watson *et al.* (1996) found that maintenance of a dense pasture canopy during summer in New Zealand prevented soil temperatures from rising to levels at which white clover stolons were killed, in a subtropical environment, lax grazing practice could allow rust to proliferate, thus reducing productivity and survival of ryegrass plants.

The duration of grazing should be limited to no more than 2 days in any one area, as a longer duration allows stock to regraze plants in the early stages of regrowth, when they are particularly vulnerable to defoliation (Fulkerson 1994).

2.4.2.3 Irrigation. In the subtropics, the shallow rooting of perennial ryegrass, the variability of rainfall events and the often high temperatures in the warmer months, necessitates the application of relatively frequent irrigation to maximise productivity and persistence of this species. By contrast, kikuyu, with a deeper root system and more efficient water use, requires less frequent irrigation. Fulkerson *et al.* (1993a) developed an irrigation schedule for both ryegrass and kikuyu on the north coast of New South Wales, based on mean evapotranspiration figures for the region, a rooting zone of 300 mm for ryegrass and 600 mm for kikuyu, and a soil water-holding capacity of 25 and 50 mm available water, respectively. This is shown in Figure 2.3.
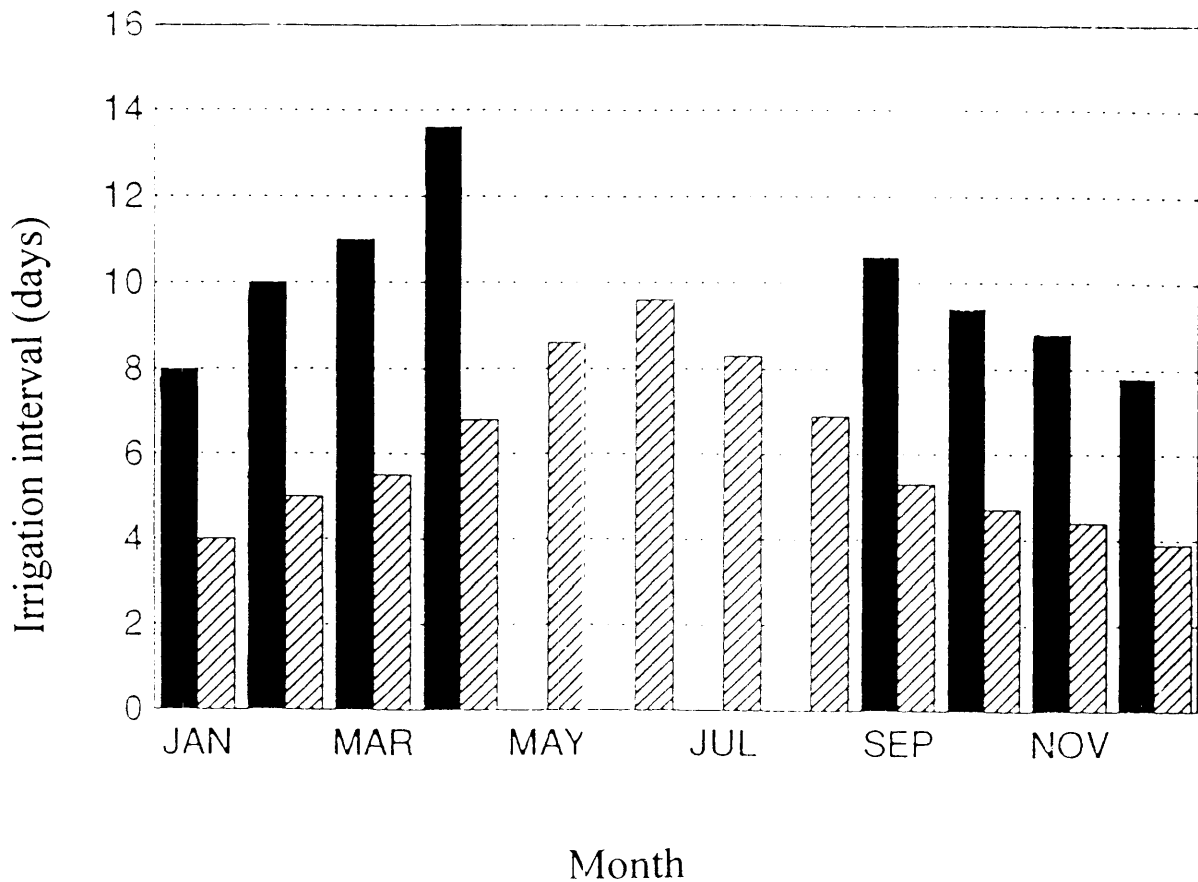
Figure 2.3. Appropriate irrigation interval (days) for ryegrass (▨) and kikuyu (■) on the clay flood plains of northern New South Wales (source: Fulkerson *et al.* 1993a).

In the absence of 'substantial' rainfall (defined as more than about 10 mm), to maximise persistence of perennial ryegrass, 25 to 30 nm of irrigation water needs to be applied each 2 weeks in winter, each week in autumn and spring, and each 4 to 5 days in summer. Applying any more than the recommended 25 to 30 mm of water at a time will result in waterlogging of pasture, while Figure 2.3 indicates that irrigating less frequently than recommended will only benefit the deeper-rooted tropical grasses and weeds.

Through a relatively dry subtropical summer, Fulkerson and Slack (1994a) found that irrigation sufficient only to keep plants alive (irrigating each 2 weeks in the absence of sufficient rainfall), increased the DM yield of perennial ryegrass and white clover by 95% in summer, and by 68% in the following autumn and winter, and increased survival of ryegrass plants by 34%, compared to not irrigating. However, the maximum DM

production possible from well-irrigated perennial ryegrass/white clover pasture in the subtropics still needs to be quantified.

2.4.2.4 Nutrient replacement. Section 2.4.5 highlights the need to at least replace major nutrients removed through grazing of pasture. An appropriate fertiliser application regime should be based on analysis of soil (Fulkerson *et al.* 1993a). On the north coast of New South Wales, it is recommended to maintain Colwell phosphorus levels at 100 ppm or higher, and potassium levels at 0.4 meq/100 g soil or higher. N is generally applied at 40 to 50 kg N/ha, once or twice in late winter of the establishment year only, and thereafter, the white clover component is relied on to supply pasture N requirements (W.J. Fulkerson, personal communication).