White clover dynamics in New Zealand pastures

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Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
dr ir L. Speelman
in het openbaar te verdedigen
op woensdag 8 november 2000
des namiddags om half twee in de Aula.

ISBN 90-5808-299-7

J.B. (Ina) Pinxterhuis, 2000
White clover dynamics in New Zealand pastures
Ph.D. thesis, Wageningen University,
with references and summaries in English and Dutch.
Photo's: background cover - PR Lelystad, author with Friesian bull -
Elisabeth Watson, others - author/AgResearch Grasslands.

Stellingen

1. De aanwezigheid van een wortel op dezelfde ouderlijke knoop is niet noodzakelijk voor de ontwikkeling van een zijscheut bij witte klaverplanten, of omgekeerd.
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2. Een zijscheut en een wortel op dezelfde ouderlijke knoop hebben voordeel van elkaar.
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3. In het voorjaar verloopt het afsterven van oud stolon materiaal sneller dan de productie van nieuw stolon materiaal.
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5. Betreding en/of depositie van excreta dragen voortdurend bij aan het opbreken van witte klaverplanten in grasland.
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6. De mogelijkheden om de structuur en morfologie van witte klaverplanten te beïnvloeden door het aanpassen van beweidingsbeheer, om de persistentie van klaver te vergroten als stikstofkunstmest wordt gebruikt, zijn beperkt.
   Dit proefschrift.

7. "It has been found that girls become the best assistants on this (herbage) dissection work..."
   P.D. Sears (1951), The technique of pasture measurement. New Zealand Journal of Agricultural Science and Technology 33: 1-29.
   In 1989 the same "girls" taught me the art of herbage dissection!

8. "The benign environmental image of grass/clover is not a feature of the clover itself, but of the two species growing together."
   D.F. Chapman, A.J. Parsons and S. Schwinning (1996), Management of clover in grazed pastures: expectations, limitations and opportunities. Agronomy Society of New Zealand 11/ Grassland Research Practice Series 6: 55-64.

9. Er is een significant verschil tussen statistische en praktische significantie.

10. "De kracht van het Nederlands kennissysteem is de binding tussen onderzoek en praktijk. Dit was de drijvende kracht achter de vernieuwing. Concentratie van het onderzoek in Wageningen, en daarmee terugtrekking van het onderzoek (dat wordt uitgevoerd) op de regionale proefstations, is funest voor deze binding."
A.J. Vijverberg, 63-jarige promovendus van J.D. van der Ploeg, Rurale Sociologie, Wagenings Universiteits Blad 97-1, 9 januari 1997.

11. "Het blijkt zo moeilijk voor elkaar te krijgen dat mannen hun deel van de verantwoordelijkheid nemen voor huishouden en kinderen, dat die wens vaak verschoven wordt naar de eis van betere kinderopvang."
Anja Meulenbelt, Casablanca, of de onmogelijkheden van de heteroseksuele liefde, 1990.

12. Indien de maatregelen van de Nederlandse boeren om nutriëntenverliezen te reduceren niet samen gaan met gelijke inspanningen van de burgers, zal de toestand van het milieu in Nederland niet verbeteren.

13. Als er geld is, is er tijd.

Stellingen behorende bij het proefschrift "White clover dynamics in New Zealand pastures", J.B. Pinxterhuis. Wageningen, 8 november 2000.

ACKNOWLEDGEMENTS

Too many people have supported me during the ten years in which I have worked on this thesis to mention them all. The thesis has been "nearly finished" for about six years, and the people who showed interest in its progress have certainly helped me to accomplish the task. My brother read the summary and said: "And thát needed ten years to be sorted out?" Ah well...

I very much appreciated the company of the Herbage Ladies, Yvonne Gray, Christine van Meer and Margaret Greig. These ladies from the Herbage Dissection Lab of AgResearch Grasslands (Palmerston North, New Zealand) were amazing with their enormous speed of herbage dissection, counting tillers and growing points, and even being able to recognise grass species in freeze dried samples. In the mean time they managed to keep up a nice atmosphere and much talk in their lab. I enjoyed working with them, and they taught me a lot.

John Hay I thank for his supervision of my MSc project, and for his efforts to get me a PhD study allowance from the DSIR. Jocelyn Tilbrook I owe many thanks for her always cheerful help with whatever horrible job. Her "I hear an earthquake coming" I'll never forget, because she was right! I am grateful to Mike Hay for the discussions and comments on my manuscripts, and to John Brock for discussions and helping me to put things in perspective again. The late Siri Wewela from the Applied Mathematics group of DSIR/AgResearch I want to mention, because she managed to get me interested in statistics. After she sadly passed away, Gary Thomas and Bob Fletcher took over helping me out. I want to thank them for their time and patience. The people from Aorangi Research Station I thank for the good company and the help. Especially Craig and Trevor for the help with harvesting and taking tiller plugs, and Bill for looking after my transport. It is not easy to remember to take someone to the Research Station every ten or eleven days!

Special thanks go to my co-promotor and supervisor at Grasslands, Dave Chapman, for his great support. The E-mails and the days he spent in Lelystad to get me going again certainly helped a lot!

Then, the people of Wageningen University. First of all my promotor professor Leendert 't Mannetje. His comments on my manuscripts and his support are very much appreciated. Anjo Elgersma I thank for her care when I returned to Wageningen from New Zealand to work on my thesis. I only had a short but enjoyable time at the Department of Agronomy. Anjo was right when she warned me that accepting a job with the Research Station for Cattle, Sheep and Horse Husbandry (PR) would make it difficult to finish my thesis, but I would not have liked to miss this opportunity to work with the Grasslands Group.

My boss, Willem Luten, from the Grasslands Group, I want to thank for his flexibility and patience. Even though the thesis was not related to my job, being able to adapt my working times has helped greatly to keep up work on the thesis. Geert André, statistician of the PR, I thank for his comments on the methodology.

I'm indebted to family and friends, all over the world, for their support and never fading sympathy. Even though it took so long, I've never felt that anyone lost faith in the party to come. I'm sorry my dad did not live to see the thesis finished. He always stimulated his children to do what they enjoy and to see a lot of the world. His and my mums enthusiasm made the physical distance feel a lot shorter.

Last, but not least, Peng. He deserves a special place. Always patient and always taking good care of me. He's been wondering what kind of partner he would have once the thesis was finished, with suddenly more spare time on hand. Others say it just gets filled up with more work, but since May last year we've got a wonderful daughter, Xoë. So I think I'll still spend a lot of time at home, but less on the computer!

InaP

ABSTRACT

J.B. (Ina) Pinxterhuis (2000), *White clover dynamics in New Zealand pastures.*
Ph.D. thesis Wageningen University, Wageningen, The Netherlands.

The study presented in this thesis is based on the overall target of increased herbage production in cooler times of the year, through the use of fertiliser N, while maintaining the benefits of white clover (*Trifolium repens* L.) in New Zealand pastures. To help identify management strategies and plant breeding goals to attain this target, seasonal dynamics in cattle-grazed pastures of clover population and plant structure and stolon dynamics were studied, including the effects of fertiliser N applications, grazing management and their interactions. The grazing treatments applied were continuous stocking with 4.9 Friesian bulls/ha and rotational grazing with either 4.9 or 7.4 bulls/ha. Fertiliser N (urea) was applied in mid autumn and late winter, at 50 kg N/ha per dressing.

Average total herbage dry matter accumulation was about 16 tonnes/ha/year. The average efficiency of the applied fertiliser N was 17.5 kg DM/kg N in 1991 and 12.9 kg DM/kg N in 1992. The grazing treatments applied did not result in great differences in herbage accumulation or composition, and clover was maintained under all grazing treatments. Fertiliser N tended to decrease clover DM accumulation by 15% and clover content by 3.6%.

Stolon growth dynamics and structures of populations and plants showed great seasonal variation. Growth was related positively to average soil temperature at 10 cm depth, which explained the greatest part of the variation for most growth parameters. Rooting was not related to temperature, rainfall, distribution of rainfall nor radiation.

Continuous stocking during the cooler periods of the year, shifting to rotational grazing when temperatures rise, may help to maintain clover in the sward. Moderate fertiliser N applications in the cooler times of the year increase herbage accumulation and do not compromise clover permanently. However, it remains to be confirmed that the inhibited root production in spring, when N is applied, makes clover plants or branches in grazed swards more susceptible to local or temporal stress, such as drought. Plant breeding should be directed to improved rooting, and maintenance or improvement of N fixation.

Key words: biomass allocation; branching; cattle grazing; climate; continuous stocking; fertiliser nitrogen; grassland; population and plant structure; radiation; rainfall; rainfall distribution; rooting; rotational grazing; stolon growth dynamics; temperature; *Trifolium repens* L.; white clover.

CONTENTS

Chapter 1

INTRODUCTION

1. INTRODUCTION

1.1 White clover and nitrogen in New Zealand pastures

White clover (*Trifolium repens* L.) is an important species in New Zealand grassland farming due to its high quality as an animal feed and, especially, its ability to fix atmospheric nitrogen (N) in symbiosis with *Rhizobium trifolii*. Rogers and Little (1982) estimated the total amount of N fixed per annum by pasture legumes in New Zealand to be about one million tonnes N. A more recent estimate is 1.57 million tonnes N annually (Caradus *et al.*, 1996). White clover utilises the fixed N for its growth. When clover plant parts die, this N becomes available for other plants in the sward, especially the grasses. The other important pathway of N transfer in grassland farming is through the grazing animal.

White clover is successfully used in New Zealand to supply N in grass/clover swards, and grass/clover swards can attain herbage dry matter yields equivalent to pure grass swards fertilised with 300-600 kg N/ha/year (Ball and Field, 1985). An average annual N fixation rate of 185 kg N/ha was found on grazed lowland pastures for nine sites throughout New Zealand (Hoglund *et al.*, 1979). However, N deficiency is widespread in New Zealand pastures (Field and Ball, 1978; Ball and Field, 1982), and for many grass/clover swards, fertiliser N application results in increased herbage dry matter yield (Harris and Hoglund, 1977; Ball *et al.*, 1978; Crush *et al.*, 1982; Feyter *et al.*, 1985).

The seasonal pattern of growth of a grass/clover sward imposes limits on animal production for livestock systems relying on pasture as the sole, or dominant, feed source such as in New Zealand. Clover growth is reduced, but grass can still be productive in the cooler times of the year in New Zealand (Hoglund *et al.*, 1979). From late autumn to early spring, temperatures can be limiting for mineralisation, resulting in less N available for the grass. The first significant rains in autumn can also result in a net immobilisation of soil inorganic N, due to a sharp increase in microbial activity (Ball and Field, 1982). So N deficiency will be more pronounced at these times and production responses to fertiliser N can be expected (e.g. Ball *et al.*, 1978; Field and Ball, 1978; Luscombe, 1980; Thomson and Roberts, 1982; Feyter *et* *al.*, 1985). Using pasture cores under simulated winter/spring temperatures, Ledgard *et al.* (1989) showed that plant uptake of applied N in a cold winter (6 °C day/1 °C night) was as rapid as in a mild winter (11 °C day/7 °C night). However, an N application before a cold winter was leading to a greater immobilisation of N by the microbial biomass as compared to a mild winter. Later in the season when temperatures increase and mineralisation increases, the immobilised N subsequently will become available for plant growth.

In late winter and early spring, feed deficits are particularly important in New Zealand since this is the usual time of lambing and calving and, hence, feed demand from lactating animals is high. Highest fertiliser N responses can be expected when temperatures increase in spring (Frame and Boyd, 1987; Roberts and Thomson, 1989), provided soil moisture levels are non-limiting, but at this time of the year production is already high and most farmers have a surplus of fodder for their animals (Buxton, 1982). Beside this, spring applications may result in decreased summer production, because the clover population can decline to such an extent that in summer clover growth and hence N fixation is less than in non-fertilised swards (Ledgard and Saunders, 1982; Ball and Field, 1985). To compensate for this, farmers may need to apply further fertiliser N, thus changing the pasture production system from one reliant on N fixation into one more reliant on fertiliser N.

In the early 1990's in some areas of New Zealand this change of production system started already. Some dairy farmers have moved to a similar system to Dutch farmers, with fertiliser N application rates of up to 400 kg N/ha/year. These farmers perceive the management of N fertilised pasture as being easier than clover-based pasture, because of greater predictability of pasture production and less year to year variation (Barr, 1996). These high rates of fertiliser N application increase dry matter production per hectare, but can only increase profitability if animal production also increases. Hence higher stocking rates are required, and harvesting pasture surpluses for silage is necessary. Profitability depends on the prices of fertiliser N and animal products. With recent price levels for milk, the gross margin for dairy farming is estimated to be highest with only 100 kg fertiliser N/ha/year (Clark and Harris, 1996), but, at this level of fertiliser input, gross margins are only NZ$ 50 per hectare higher than without fertiliser N.

In general, the change in system from clover-based to fertiliser N based pastures is seen as undesirable because of its environmental effects. The international view of a "clean green" New Zealand is exploited in the marketing of New Zealand's agricultural products and the low costs of the production system keeps the New Zealand farmer competitive in world markets. New Zealand farmers do not receive any subsidies and sell their produce at prices determined solely by what international markets are prepared to pay for it. Maintaining the green image may well be crucial in future when the environment becomes a more important selection factor for consumers. Hence in the 1990's comparisons of environmental aspects of grass/clover versus grass plus fertiliser-N were performed.

The grass/clover based system is estimated to be 20 times more efficient in the use of non-renewable resources such as fossil fuel, than pastures reliant on fertiliser N (Walker, 1996). A grass/clover based system is less likely to show direct N losses, but it is generally accepted that at similar production levels, indirect N losses to the environment may be similar to those from a fertiliser N based system. With fertiliser N higher levels of dry matter production per hectare can be obtained. This is only profitable when utilised by the grazing animals, but increases N losses, mainly nitrate leaching, nitrous oxide emission and ammonia volatilisation from urine patches. Parsons *et al.* (1991c) showed substantially higher N losses in a grass sward fertilised with 420 kg N/ha/year compared to a grass/clover sward. Leaching losses from a grass sward fertilised with 210 kg N/ha/year were much lower than that of the grass sward fertilised with 420 kg N/ha/year, and closer to that of the grass/clover sward. Ledgard *et al.* (1996) showed direct nitrate leaching loss of urea fertiliser N in a 400 kg N/ha/year treatment was negligible in a Waikato trial grazed by dairy cows, but Carran and Clough (1996) cite Ruz Jerez (1991) who calculated 0.6 g N leached per grazing day in a grass/clover system, compared to 3.1 g N in a grass plus 400 kg N/ha/year urea fertiliser in a Manawatu trial grazed by sheep. Carran and Clough (1996) argue that beyond a threshold value of fertiliser N rate both direct and indirect losses increase dramatically. Variation, however, is large due to the influence of soil type and weather conditions.

With its climate very suitable for clover growth, New Zealand is in a good position to maintain a low cost, low input pasture production system, relying mainly on N fixation. Thus the incentive to maintain a good clover component in the pasture is strong, and the interest in increasing pasture production, without reducing the input of clover, is great. For improved cold season dry matter yield and maximising clover N input, a balance of fertiliser N in winter and N fixation in summer should be sought. The rate of fertiliser application and its timing, and the optimal grazing management (stocking rate, grazing method, etc.) are among the factors to be determined. Furthermore, the mechanisms causing the decline in clover when soil N is increased need to be defined, to identify plant breeding strategies leading to varieties which have the desired characteristics for maintaining an optimum clover population in the sward. In this respect, knowledge of the processes in the mixed sward involved in the competition between grass and clover for N is important. Main points of the current knowledge on this topic are summarised in the next section.

1.2 Competition for N in mixed pastures

When grass and clover are grown together in a mixture, they compete with each other for available N (Ledgard and Saunders, 1982; Davidson and Robson, 1985b). Grasses are generally considered to be more effective competitors for N than clover. However, clover, with its associated N fixation capacity, can switch between uptake of inorganic N and N fixation and so maintain a relatively constant N supply rate. At low levels of fertiliser N, clover can benefit from the inorganic N without a measurable reduction in N fixation (Eltilib and Ledgard, 1988). In monocultures inorganic N also increases dry matter yield from clover but, especially at higher temperatures, a considerable part of N fixation is replaced by inorganic N uptake (Davidson and Robson, 1986). Clover can then utilise more of the energy derived from photosynthesis for new growth, since N fixation is an energy-demanding process (Ryle *et al.*, 1979a, b, 1981). N fixation in the light is primarily fuelled by current photosynthesis (Ryle *et al.*, 1985, 1986), and consumes approximately 12% of the carbon fixed (Ryle *et al.*, 1979b, 1988, 1989). During the dark period, another proportion of the assimilates, reserves stored in the nodules, is used to sustain N fixation (Ryle *et al*, 1985, 1988). Sheehy *et al.* (1991) estimated that, for a lucerne crop in the field, 20% of daily photosynthesis is required to support maximum N fixation rates.

In grass/clover swards at higher N application rates, N fixation rate decreases (Moustafa *et al.*, 1969; Ledgard and Saunders, 1982; Murphy *et al.*, 1986; Boller and Nösberger, 1987; Nesheim *et al.*, 1990) as clover utilises more inorganic N (Hoglund and Brock, 1982; Davidson and Robson, 1985b). However, N application will benefit ryegrass more than white clover with ryegrass showing a faster and greater N accumulation than white clover following N application (Davidson and Robson, 1985b; Murphy and Ball, 1985; Murphy *et al.*, 1986; Boller and Nösberger, 1988). This is probably due to a faster growing root system (Evans, 1977) and higher density of roots (number of root tips per gram of herbage) of ryegrass compared to white clover (Jackman and Mouat, 1972). Another reason mentioned for greater N uptake by grasses compared to clover is the faster water uptake by ryegrass relative to white clover (Mitchell and Kerr, 1966) which increases water tension and decreases availability of nutrients. The greater competitive ability of ryegrass to attain inorganic N results in a greater increase of shoot yield of ryegrass relative to white clover, increasing the shoot competition (Martin and Field, 1984) and the competition for other nutrients (Mouat and Walker, 1959). The greater shoot competitive ability of ryegrass at higher N levels is more pronounced at lower temperatures (Davidson and Robson, 1986). There is some indication of white clover cultivar differences in competitive ability for N uptake (Goodman and Collison, 1986).

At continued high levels of N application and constant favourable environmental conditions clover is able to take up N as effectively as ryegrass (as nitrate uptake per unit root dry weight; Davidson and Robson, 1985b), and can indeed increase its dry matter yields in a grass/clover sward (Davidson and Robson, 1985a). However, in the field, when N application is continued, soil N levels increase and generally the clover content in the sward decreases ('t Hart, 1954; De Vries and Kruijne, 1960; Aldrich, 1970; Hoen, 1970; Ball *et al.*, 1978; Denehy and Morrison, 1979; Connolly, 1981; Luscombe *et al.*, 1981; Rhodes, 1981; Wilman and Asiegbu, 1982; Boyd and Frame, 1983; Morrison *et al.*, 1983; Hall and Scott, 1985; Frame and Boyd, 1986; Hoglund and Brock, 1987; Mouat *et al.*, 1987).

1.3 Mechanisms behind clover reduction

The mechanisms behind the decrease of clover in swards that have been fertilised with N are not fully understood. It was initially assumed that increased grass growth leads to greater sward height, which in turn increases the shading of clover plants (Stern and Donald, 1962). Shading could reduce the photosynthesis of clover plants to such an extent that they eventually die through energy deficit (Chestnutt and Lowe, 1970; Rhodes and Stern, 1978). This effect would be strongest in spring, when grass growth is already vigorous; hence the greatest suppressing effect of fertiliser N is seen when it is applied in spring. However, more recent research has shown that white clover is capable of maintaining light interception, and hence photosynthesis, in grass-dominant pastures by increasing petiole length, thus maintaining leaf area in the well-illuminated layers of the sward near the top of the canopy (Dennis and Woledge, 1982, 1985; Woledge, 1988). These authors concluded that these mechanisms made clover even more efficient in retaining its net photosynthesis per leaf area unit/hour than *Lolium perenne* as sward height increases. So apparently other mechanisms are involved in the decrease in clover content and production in N fertilised grass/clover swards. Parsons *et al.* (1991a) argue that because growing leaves of clover remain folded longer during their development than grass leaves, so that the specific leaf area of developing clover leaves is lower than of developing grass leaves, photosynthesis of clover lags behind that of grass following defoliation. As sward height increases, clover invests proportionally less in lamina tissue than in non-lamina tissue, resulting in a lower rate of acceleration of photosynthesis of clover compared to grass (Parsons *et al.*, 1991b). These two attributes could counteract the positive effect on growth capacity of the greater specific leaf area of fully expanded clover leaves and the ability to maintain a greater net photosynthesis per leaf area unit/hour of clover compared to grass.

Woledge (1988) showed that fertiliser N did not influence the clover content in the sward in the course of one growing season, but that, over the same time period, the clover content in a sward which was not fertilised increased. She suggested that stress factors like defoliation, low temperatures, drought, pests or diseases might result in the decline of white clover content found in the long term. Dennis and Woledge (1987) hypothesised that inhibition of clover branching due to shading at ground level could be a factor in the decline of clover following fertiliser N use. In a growth room experiment, lower light intensity decreased branching (Beinhart, 1963). Davies and Evans (1990) showed that shading inhibited branch outgrowth in glasshouse experiments when stolons were defoliated.

1.4 Breeding for clover persistence in the presence of fertiliser nitrogen

In the past many breeding programs have been carried out to produce clover varieties that would show increased persistence in N fertilised pastures. Aldrich (1970) found differences between cultivars in reduction of yield following N applications: large leafed cultivars were less affected than small leafed cultivars. Large-leafed cultivars would be better competitors with grass in the presence of fertiliser N. At the Welsh Plant Breeding Station, a programme began in 1949 to develop larger leafed clover varieties, resulting in the varieties Sabeda, Olwen, Kerry and Katrina (Williams, 1987). In a checklist of clover varieties, Caradus (1986) mentions better yielding capacity with fertiliser N for the following varieties, referring to various publications: Aberystwyth S.100, Aran, Blanca, Crau, Katrina, Kersey, Menna, Merwi, Olwen, Ross, Sabeda and Siwan. However, variable results are reported for some varieties. Generally, varieties with high yields at lower temperatures show less depression in growth when N is applied (Connolly, 1970; Brock and Hoglund, 1974; Hoglund and Brock, 1974; Hoglund and Williams, 1984). These varieties commonly have some Mediterranean parentage. In evaluating clover varieties, however, care must be taken in choosing the experimental environment because of interactions between variety and defoliation frequency (Rhodes and Harris, 1979; Hoglund and Brock, 1983) and temperature (Brock and Hoglund, 1974).

In a 3-year field experiment, Caradus *et al.* (1993) evaluated 15 cultivars for tolerance to fertiliser N in a perennial ryegrass/clover sward grazed by sheep. In this experiment, significant differences between varieties were found for all clover characteristics measured, but no interaction between clover variety and N treatment was found. In general the performance of the varieties with 225 kg N/ha/year was related to their performance without N application, similar to results of Laidlaw (1984). N application decreased growing point density, stolon weight and stolon length/m^2, perhaps due to increased shading of the stolons (Dennis and Woledge, 1987; Caradus and

Chapman, 1991), thus it was suggested to evaluate the variation of branching capacity of white clover stolons between different varieties.

In the earlier work the breeding target was improved harvestable yield (larger leaves) of clover, which would make the clover more competitive for light, especially under infrequent defoliation. Rhodes and Harris (1979) suggested that this breeding target may have resulted in a change in the harvest index, rather than increased total aboveground yield. The associated sacrifice of stolon density may have increased the sensitivity of clover to other stresses such as severe defoliation or drought, due to fewer sites being available for regeneration of branches and new plants, and less capacity for storage of energy and protein reserves in stolon material.

The ratio of kg N fixed/kg clover dry matter harvested is reduced by fertiliser N application, but with greater grass yield total N fixation increases (Harris and Hoglund, 1977), presumably because increased grass growth reduces inorganic N available to clover. Selection for a clover - *Rhizobium* association which is relatively insensitive to inorganic N would be another approach towards increased production from a grass/clover sward (Steele, 1982). The concept is that if such associations could be established, higher soil inorganic N levels due to N fixation and fertiliser N application would then be available for grass growth only. To achieve this it has been suggested that clover plants be screened for nodulation ability in the presence of high inorganic N levels which will not limit growth, since phenotypic variation in nodulation or N fixation are strongly related to the time of inorganic N depletion (Rys and Mytton, 1985). Within one variety (Aberystwyth S.100) genetic differences were shown for nodulation and N fixation with applied N (Mytton and Rys, 1985). However, because the agricultural significance of the differences found was very small, greater genetic improvements are required.

1.5 Morphology and physiological organisation of white clover plants

In this section a short description of white clover morphology and the importance of branching are given. The description is confined to plants growing in the vegetative state. Seed production and seedling establishment is not considered here, because establishment of clover plants through seed is infrequent in grazed, temperate pastures (Turkington *et al.*, 1979; Chapman, 1983, 1987). In these environments clover propagation occurs mainly through vegetative (clonal) growth.

An extensive review of white clover has been published by Baker and Williams (1987) covering many aspects of the biology and management of the species, e.g. morphology, reproduction, physiology, mineral nutrition, N fixation, pests and diseases. Figure 1.1 shows a white clover plant (after Thomas, 1987a, b). The clover plant usually consists of a main or parent stolon with nodes that are separated by internode stolon tissue. The internodes undergo elongation in the region immediately basal to the apex. At the apical meristem itself, new nodes with a leaf primordium are continuously developed. A young node bears a leaf, two root primordia and an axillary bud. Usually, only one of the root primordia (the lower most) develops into a nodal root when it is in contact with a moist medium. From the nodal roots lateral roots develop, and infection of *Rhizobia* is usually most abundant on these lateral roots. In some types of clover the nodal root can develop in a taproot. The axillary bud can grow out into an inflorescence or a branch (lateral) stolon, but not both. Outgrowth of an axillary bud typically starts at older nodes; at younger nodes it is inhibited by apical dominance. A branch stolon possesses all the same structures as the parent stolon, and when it has established roots it is capable of surviving the death of the parent stolon. This is the basis of clonal growth in the species.

White clover shows strong phenotypic plasticity in the size of different plant organs, and partitioning of dry matter to these organs. This phenotypic plasticity enables clover to survive periods of stress and exploit better conditions and could well be an important characteristic to select for, since it is under genetic control (Forde *et al.*, 1989). The clover population in sheep-grazed grass/clover pastures consists of many small plants and a few large individuals, irrespective of mean plant shoot (stolon plus leaf) dry weight (Hay *et al.*, 1990). Hay *et al.* (1989b) showed that, in pastures continuously stocked with sheep, clover plant density was less than in rotationally grazed pastures, while for total pasture biomass and ryegrass tiller density the opposite was true. It was shown that the dry weight per clover plant was less under continuous stocking, compared to rotational grazing, while plant structure remained similar (Brock *et al.*, 1988; Hay *et al.*, 1988, 1989a). Plants of similar branching structure showed minor differences in number of nodes

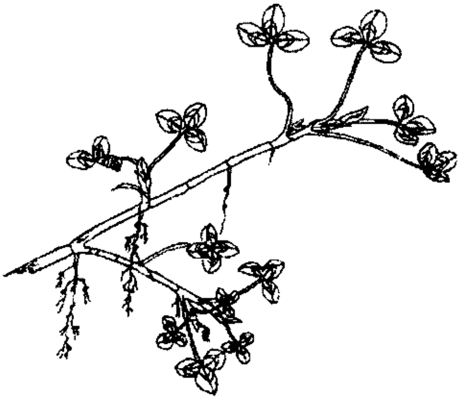

Figure 1-1 A white clover (*Trifolium repens* L.) plant.

per plant between differing grazing management or season (Hay *et al.*, 1991). Brock *et al.* (1988) and Hay *et al.* (1988) showed seasonal variation in clover plant structure in sheep-grazed pastures, due to differing balances between growth and death of plant parts. In spring plant break up occurred, mainly due to death of older buried stolon material (Hay *et al.*, 1983), resulting in the release of many new, smaller plants with simpler branching structure. In summer stolon growth was greater than death, resulting in increasing percentage of plants with a more complex branching structure.

Within a plant, a high level of integration between main stolon and branch stolons exist, with reciprocal exchange of carbohydrates between branches and parent stolon, and between branches (Chapman *et al.*, 1992b). Turkington and Klein (1991) showed that the effects of different neighbouring grass species on branches were evened-out when branch stolons were still interconnected through the parent stolon. When stolons are defoliated, changes in carbon translocation occur. Carbon is exported to defoliated branches and the apex of the parent stolon, at the expense of root and stolon tissue (Chapman and Robson, 1988, 1992; Chapman *et al.*, 1992a, b). After prolonged defoliation, however, exported carbon is mainly used to maintain existing stolon and root tissue (Chapman and Robson, 1988). The strong integration within a white clover plant should provide it with a buffer against stress factors like defoliation and soil heterogeneity (Chapman *et al.*, 1992b). The buffer would be greater when plants are more profusely branched.

1.6 Objectives of this study

The target for pasture management and clover breeding is increased animal production per hectare without increasing N losses from the system nor trading N input by clover for N input by fertiliser. This is achieved by utilising total herbage production increases while maintaining an optimal balance between N fixing clover and N utilising grass. An optimum clover content is assumed to be somewhere between 20 to 50% of total pasture yield (Clark and Harris, 1996). Higher clover contents increase N losses, and do not significantly increase animal production. A significant proportion of grass is needed to utilise the inorganic N becoming available when clover plant parts die or recycle through the grazing animal, before this N is out of reach of plant roots.

To bring total herbage production to a higher level, grass growth should be less limited by N supply. Fertiliser N can be applied in periods with limited contribution of clover N or mineralisation, i.e. in the period late autumn - early spring. In the warmer seasons the N becoming available by increased mineralisation should be mainly utilised by grass, while clover maintains a high N input in the cycle through N fixation.

The proposition upon which this study is based is that knowledge of the effects of added mineral N on clover growth processes and plant and population structure in grazed N fertilised grass/clover pastures can be used to help identify grazing management or plant breeding strategies that improve the growth and persistence of clover populations in the presence of moderate-high fertiliser N application, and thus help increase overall production without losing the benefits of white clover. This target is particularly relevant to cattle-grazed pastures, since intensification of production and the use of fertiliser N in New Zealand are mostly occurring on dairy farms. Also, in other parts of the world, for example western Europe, intensive grasslands are mainly used for cattle production, especially dairying.

The in-depth knowledge of clover in grazed pastures that is currently available is mainly based on work with sheep-grazed pastures. It is important to extend this knowledge also to cattle-based systems, especially because it has been shown that sheep and cattle differ in their grazing patterns and the sward structures that typically develop under them (Briseño de la Hoz and Wilman, 1981). Thus, differences in clover growth processes and resulting plant structure between sheep- and cattle-grazed pastures could also be expected. In the present study, no direct comparison with sheep-grazed pastures is made, but work by Hay and Brock (Brock *et al.*, 1988; Hay *et al.*, 1988; see Chapter 4) gives extensive information on population and plant structure in sheep-grazed pastures in a similar environment from which comparisons can be drawn.

In the present study the interactions between cattle-grazing management, N application and seasonal dynamics of clover growth processes and plant and population structure are studied. Similar comparisons have not been made up to date. For sustained contribution of clover in intensive cattle systems, it is important to establish the size and direction of these interactions, since the farmer can manipulate management and fertiliser use. If interactions are found at times of the year that are critical for clover plant survival, then it may be possible to adjust management accordingly to improve clover persistence when fertiliser N is used. In aid of this, relationships between climatic variables and clover growth processes in cattle-grazed pastures are established. Some of these relationships have been quantified earlier (see references in Chapter 5), but again only in sheep-grazed pastures, or under controlled-environment conditions.

The approach of this study is novel, in that it comprises a relatively long term (28 months) field study in cattle-grazed pastures, combining information on clover growth processes from marked stolons (Chapter 3) with information on plant and population structure from turves (Chapter 4). Some measurements at the whole sward level were taken as well, to establish the broad-scale environment for clover growth and the productive potential of the pastures (as indicated by herbage production and sward structure, Chapter 2). This provides the background for the detailed measurements. The simultaneous use of differing methods provides a means to compare the results and establish the advantages and applications of each method. The results are also valuable for future modelling of clover growth in grazed pastures.

Chapter 2

THE GRAZED SWARD

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SETTING THE SCENE

2. THE GRAZED SWARD - SETTING THE SCENE

2.1 Introduction

When dealing with clover plants in a sward, the sward characteristics, such as tiller density, amount of biomass produced and species composition are of great importance for determining the plant's environment. Treatments imposed on the sward can affect the clover plants for example by changing the light environment or competition for nutrients.

This chapter sets the scene for the following chapters. It describes the environment of the clover plants: the experimental site, and the climatic data. The treatments imposed and their effects on some important variables of the sward are described.

2.2 Aorangi - the experimental site

The experiment in which this study was conducted was located at Aorangi field research station, AgResearch Grasslands' experimental area at Kairanga near Palmerston North, New Zealand, at 40°20' S and 175°28' E. The experiment was established in 1987 to investigate the economics of beef production under different grazing managements as there was little information available on the effects of stocking rate or grazing system on profitability. The pastures were about 40 years old when the grazing trial commenced, and were probably based on an original sowing of New Zealand Certified White, a precursor to the widely used cultivar 'Grasslands Huia'. Williams and Cornegé (1979) screened white clover populations at Aorangi in 1975 and found that approximately 50% of the plants were Huia type (medium leaf size, about 75% cyanogenic), while the remainder was smaller leafed and less cyanogenic.

The soils of the site are classified as Kairanga fine sandy loam and Kairanga silt loam. Soil characteristics are summarised in Table 2-1. The site has been drained with subsoil tiles, but drainage is still imperfect in winter. The soils have high extractable phosphorus and low phosphorus retention (20-30% in 0-8 cm). They are low in well-decomposed organic matter. In the Kairanga silt loam, iron oxides often occur at 1 m depth, almost forming an iron pan (Rijkse and Daly, 1972). More recent soil analyses on some of the farmlets (Crush *et al.*, 1982) showed 10% organic matter, 6000 kg total N/ha and similar C%, N%, C:N ratio and pH to those shown in Table 2-1.

An annual maintenance dressing of 250 kg/ha/year of superphosphate was applied.

Table 2-1 Characteristics of the top 30 cm of fine sandy loam and silt loam soils in Kairanga, Manawatu, New Zealand (Source: Rijkse and Daly, 1972).

	Soil depth	Fine sandy loam	Silt loam
pH(H_2O)	0-15	5.7	5.1
	15-30	6.3	5.4
Organic matter			
C%	0-15	3.8	3.8
	15-30	1.9	2.3
N%	0-15	0.39	0.35
	15-30	0.20	0.25
P extracted by normal sulphuric acid (mg%)	0-15	30	33
	15-30	29	34

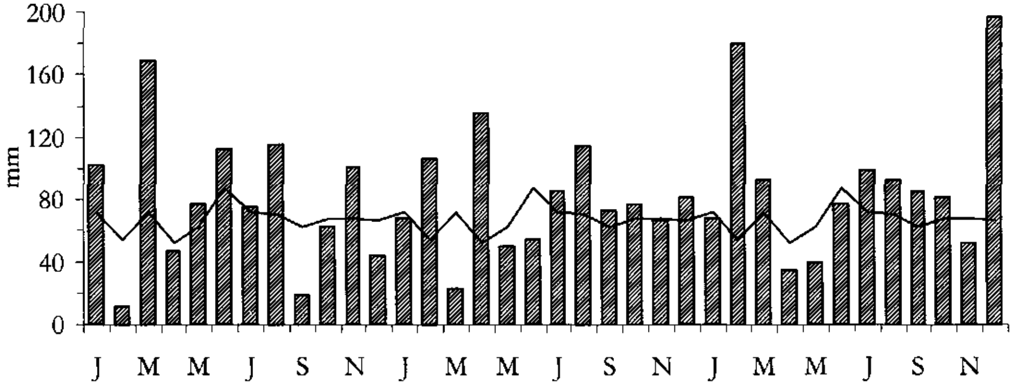
2.3 Climate

Weather data for the duration of the study were collected from a meteorological station situated 650 m from the trial area. All air temperature readings and relative humidity are measured in a double louvered "Stephenson Screen" set at 1.2 m above ground level. Grass minimum temperature was measured at 2.5 cm above closely mown lawn grass. Soil temperature measurements are made under bare soil at 10 cm depth. The 5" (12.5 cm) rain gauge rim was positioned 12" (30 cm) above the ground.

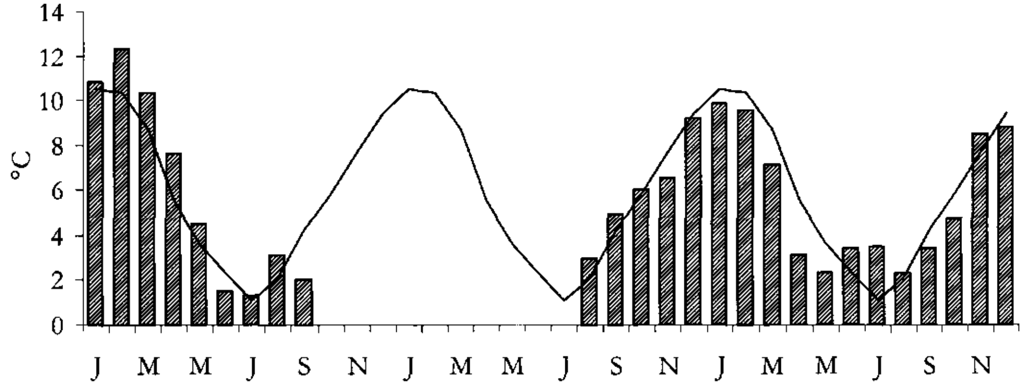
The results, including ten-year means (1980 - 1990) are presented in Fig. 2-1. On average, the rainfall is distributed evenly throughout the year. However, during the experimental period, below and above average rainfall was experienced in several months. The high rainfall in winter 1991 caused partial flooding of the experimental area for several days at a time on several occasions.

The mean daily air temperatures did not deviate much from the long-term average values between October 1990 and June 1991. Therefore, the average daily grass minimum and soil temperatures are assumed to have been

a) Average monthly rainfall



b) Average daily grass minimum temperatures



c) Average daily soil temperatures at 10 cm depth

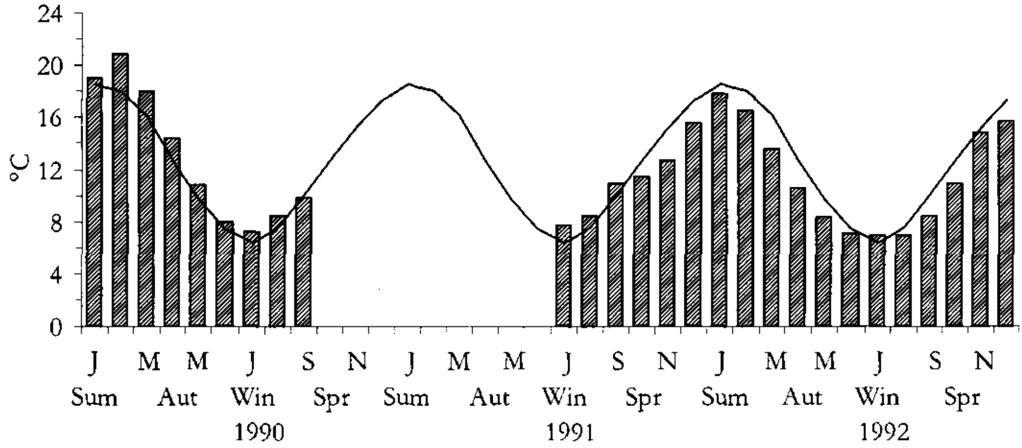
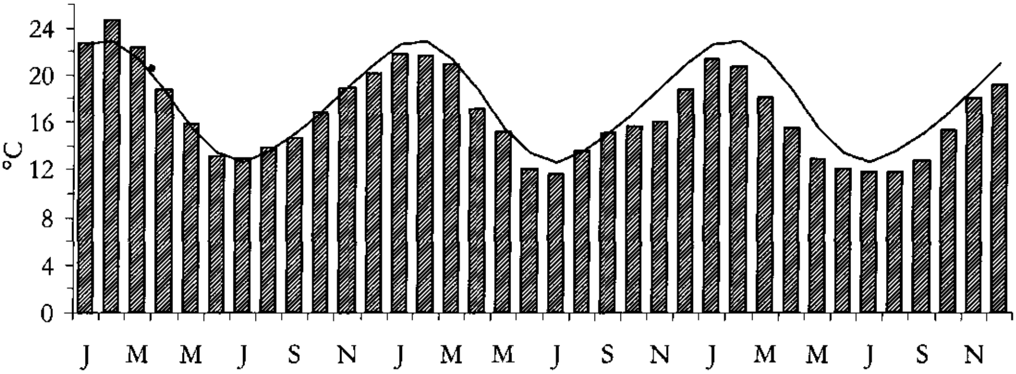


Figure 2-1 Weather data for the Aorangi experimental farm. Long term means (1980-1990; line) as well as monthly data from January 1990 until December 1992 (bars) are given. Grass minimum and soil temperatures were not recorded between October 1990 and June and July 1991, respectively.

d) Average daily air maximum temperatures



e) Average daily air minimum temperatures

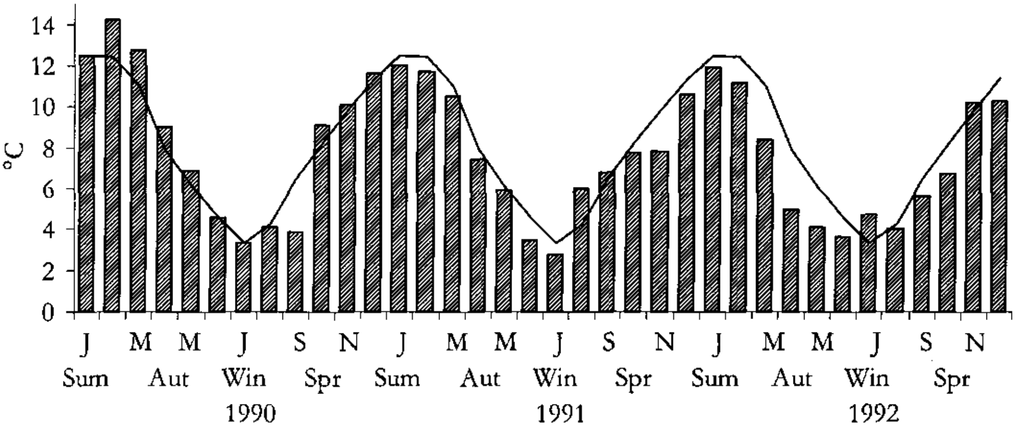


Figure 2-1 Continued

close to the long-term average in the period when these were not recorded. Below average temperatures were experienced from late spring 1991 to autumn 1992 and in spring 1992.

2.4 Treatments

2.4.1 Grazing treatments

The three grazing treatments compared in the present experiment were strict rotational grazing with either 4.9 or 7.4 Friesian bulls per hectare (RG4.9 and RG7.4, respectively), and continuous stocking with 4.9 bulls per hectare (CS4.9). Treatments were imposed on self-contained farmlets each of 1.62 ha, subdivided into 8 paddocks for the rotational grazing treatments.

Rotation length (i.e. the time taken for animals to return to any area of pasture after the previous grazing) varied from around 14 days in spring/summer to 60 days in winter. Stock within the CS4.9 treatment had access to the whole farmlet at all times. There were two replicates of each treatment.

Each November or December appropriate numbers of calves (three months old) were placed on the treatments, grazing ahead of the older bulls. The older bulls stayed on the farmlets until February/March, depending on feed availability and live weight. The older bulls were then removed from the trial altogether. Fig. 2-2 shows the stocking rates on each treatment expressed on a metabolic liveweight basis.

The grazing management treatments had been in place for 2 years and 7 months when measurements for this study started in September 1990. The measurements continued till December 1992.

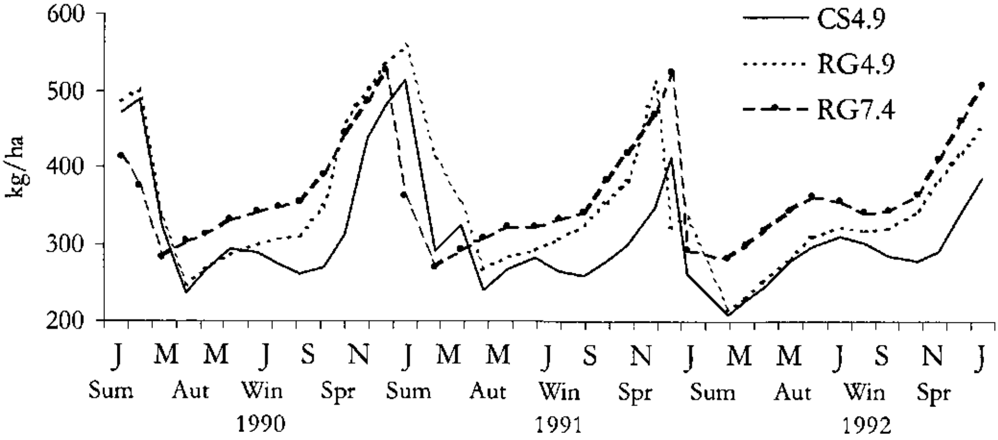


Figure 2-2 Stocking rates of the three grazing treatments used in the present experiment (metabolic liveweight, kg/ha).

2.4.2 Nitrogen treatments

Nitrogen (N) treatments were first applied in April 1991. N fertiliser was applied by hand to 5 m strips running the length of each paddock. Thus approximately 10% of the area of each replicate received N fertiliser. This provided an N treatment area of adequate size for destructive sampling at regular intervals while minimising disturbance to the larger trial within which this study was conducted. This area will be referred to as plus-N. The remaining area in each paddock, which did not receive N fertiliser, will be referred to as no-N.

N fertiliser was applied on 22 April 1991, 31 July 1991, 24 April 1992 and 7 August 1992 (mid - autumn and late winter). In each dressing, 50 kg N/ha was applied, in the form of urea, $CO(NH_2)_2$. Urea is the main N fertiliser used by New Zealand farmers (Rogers and Little, 1982), being usually the cheapest per unit N (During, 1984). Also, urea is the type of N fertiliser produced in New Zealand itself at the Taranaki urea factory, which was constructed in the mid-eighties. With this timing of N fertiliser application, good responses can be expected (see section 1.2), alleviating the feed deficit at this time of the year. The rate of 100 kg N/ha/year was considered high at the time of the experiment, in terms of economic benefits (Buxton, 1982). This rate however, was shown to increase total herbage production by 8-12 kg DM/kg N applied in the Manawatu (Ball *et al.*, 1978) or in the Waikato (Ledgard and Saunders, 1982; Feyter *et al.*, 1985), while decreasing the herbage production of clover, the effect this present experiment aims to study.

2.5 Methods

2.5.1 Herbage production

A movable exclosure cage technique where herbage is cut to a fixed height before the cage is placed (Brown, 1954) was used to measure net herbage production. In each treatment, three randomly chosen areas of 1 x 0.5 m were cut to a stubble length of approximately 1 cm using electric shearing clippers, and covered by cages to prevent grazing. After regrowth periods, herbage under the cages was harvested by cutting to the same stubble length as at the start of the regrowth period. After harvest, cages were moved to newly cut areas at a new location. This method should have minimised errors associated with the use of cages when estimating herbage production under RG (Frame, 1981) but would probably have overestimated herbage accumulation under the CS4.9 treatment because of changes in sward structure and differences in average leaf area index under cages compared to under continuous stocking (Collett *et al.*, 1981; Parsons *et al.*, 1984). The error is likely to be greatest in spring because ryegrass reproductive stem growth can accumulate under cages but is often removed by animals in the early stages of stem elongation under continuous stocking (Lambert *et al.*, 1983). However, the errors associated with frequent cutting to simulate continuous stocking is likely to lead to even larger sampling errors due to incomplete recovery of short herbage (Grant, 1981).

After cutting, herbage was placed in plastic bags and taken to a laboratory. The fresh weight was determined on the same day to avoid losses of moisture ('t Mannetje, 1978). A subsample of 200 g was dried for 24 hours in a forced-draft oven at 85 °C and weighed to determine dry weight percentage. An additional subsample was taken for botanical dissection to determine sward composition (Sears, 1951). Categories separated were *Lolium* species, other grasses, *Trifolium repens*, other species and dead matter.

2.5.2 Sward density

The density of grass tillers, clover growing points, and plants of other species (number/m^2), as well as clover stolon length or weight per m^2 were assessed using the following destructive sward sampling technique.

With a soil corer of 50 mm diameter, fifty turf plugs were removed from both the plus-N and no-N treatments, in each of two paddocks per farmlet. While walking along straight lines at set distances across the paddocks, one plug was taken at a set number of paces. Thus the whole treatment area was evenly sampled, to take the heterogeneity of the swards into account (Jewiss, 1981). Number of tillers of *Lolium* species and other grass species, white clover growing points and number of plants of other species were counted per plug. Clover stolon length and dry weight were measured in a random subsample of ten plugs per treatment within each sampled paddock.

2.5.3 Statistical analysis

For analysis, two different data sets were created. The first, from here on referred to as NN, included all data from the no-N treatment from September 1990 to December 1992 and was used to determine seasonal and grazing treatment effects. The second set, referred to as PN, consisted of all data from April 1991 to December 1992 from both the no-N and plus-N treatments and was used to determine the main effect of N, and interactions between N and date of sampling or grazing treatment. Data were tested for homogeneity of variances, transformed where needed and then analysed by analysis of variance using the General Linear Models (GLM) procedure of SAS/STAT (Anonymous, 1990). A split plot model was used, with the main plot being grazing treatment, sub-plot N treatment and sub-sub-plot sampling date. The correlation between the (destructive) samplings at different dates was considered small enough to include sampling date in the analysis in this way.

Least squares means of the full model and their related standard errors were calculated and used to test differences between treatments when the effect was significant (the PDIFF option of GLM). Where transformed data were used in the tests, least squares means were back-transformed and resulting values are presented; where non-transformed data were used, least squares means are presented.

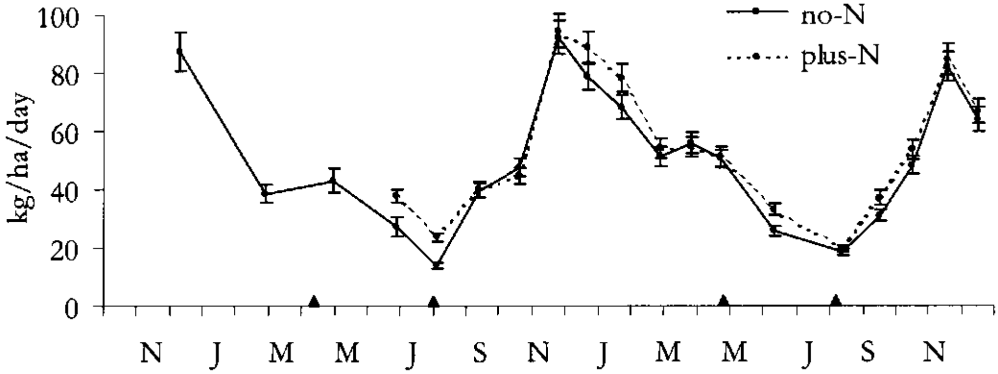
2.6 Results

2.6.1 Herbage production

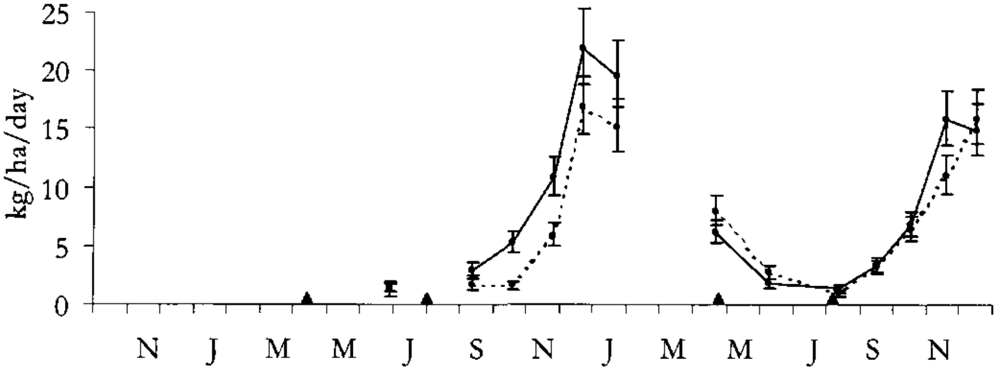
Total pasture herbage accumulation rate is presented in Fig. 2-3a. Total annual accumulation was 16.6 tonnes DM/ha in 1991 and 16.0 tonnes in 1992. The seasonal fluctuations in botanical composition were highly significant for all five components ($P<0.001$). This is shown in Fig. 2-4. Clover content ranged between 30% of total DM in summer and 10% in winter. The content of *Lolium* species fluctuated between 40% of total herbage DM in summer to 65% in winter. Other grasses (mainly *Poa* species, couch (*Elymus repens*) and browntop (*Agrostis capillaris*)) comprised between 15% (late summer/autumn) and 30% (late winter/early spring). Other species contributed little, reaching a maximum of 3% in summer. Dead matter content was highest in mid-summer (13% of total DM), after which it decreased to around 2% in late winter/early spring.

Grazing management had no effect on herbage DM accumulation or composition. N application tended to increase the total herbage DM accumulation ($P=0.07$). The average efficiency of N use was 17.5 kg DM/kg N applied in 1991 and 12.9 kg DM/kg N applied in 1992. The greatest

a) Total herbage accumulation rate



b) Accumulation rate of white clover



c) Accumulation rate of grasses other than *Lolium*

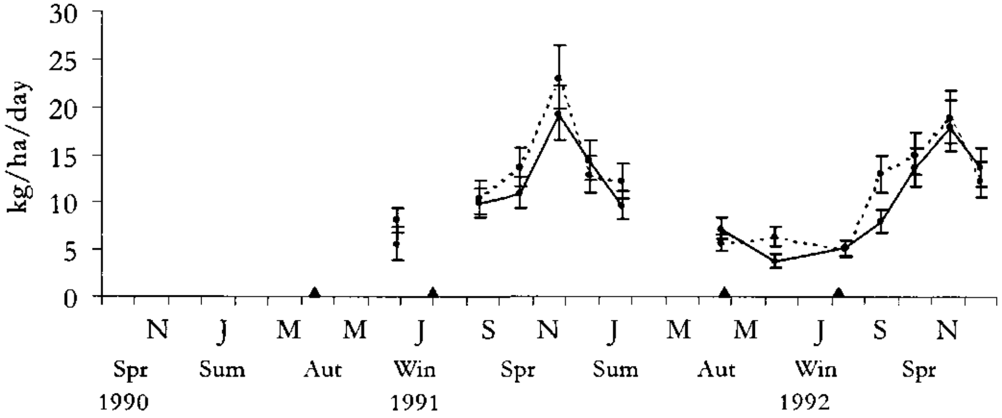
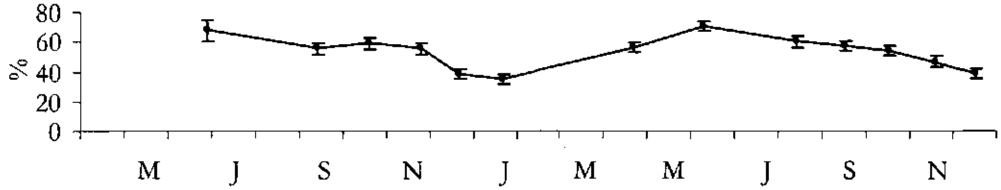
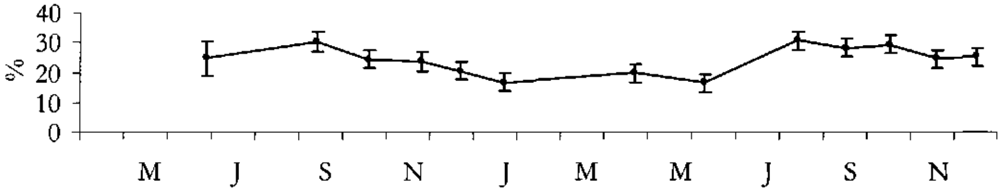


Figure 2-3 Seasonal variation and effect of N application on DM accumulation rate of a) total herbage, b) white clover, and c) grasses other than *Lolium*. Mean of grazing treatments. Triangles on the x-axis show the timing of N applications.

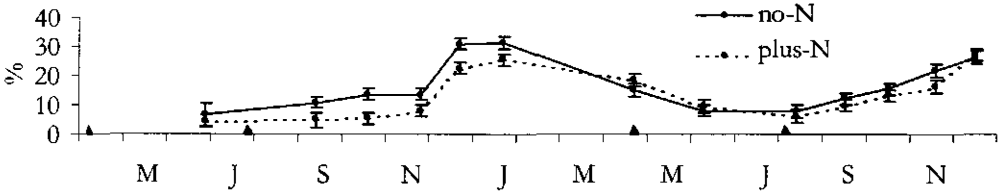
a) Percentage of *Lolium*



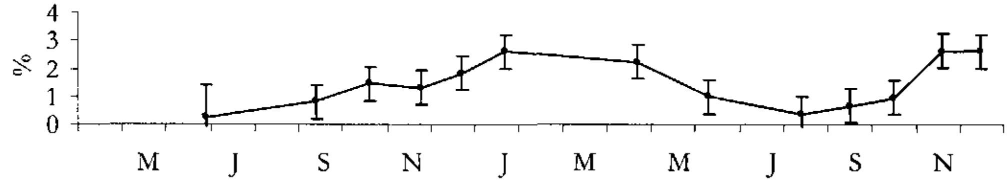
b) Percentage of other grasses



c) Percentage of white clover; triangles denote timing of N application



d) Percentage of other species



e) Percentage of dead matter

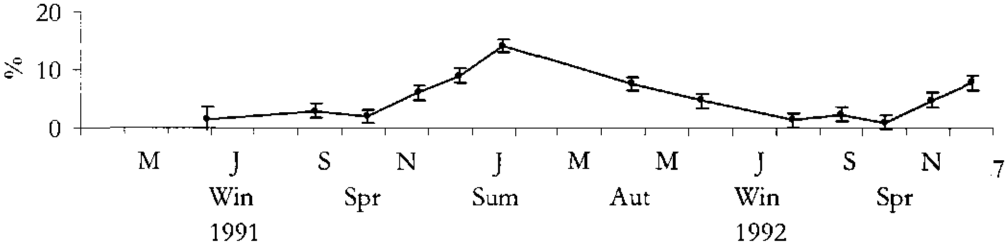


Figure 2-4 Seasonal variation in herbage composition and effects of N application on percentage of white clover. Mean of grazing treatments.

increase occurred after the early autumn N applications in April (significance of interaction between N treatment and time $P<0.05$; Fig. 2-3a). Immediate responses to the late winter N application were smaller.

N application tended to reduce clover DM accumulation by 15% ($P=0.07$; Fig. 2-3b) and decreased the clover content of the swards by 0.036% /kg N applied ($P=0.05$; Fig. 2-4b). There was a significant interaction between N treatment and sampling date for clover DM accumulation ($P<0.01$) due to a strong suppression by N in spring 1991 (Fig. 2-3b). N did not significantly increase the DM accumulation of *Lolium*, other species, or of dead matter, nor did it affect the percentage of total herbage comprised by these components. N increased the DM accumulation of other grasses than *Lolium* at some sampling dates (Fig. 2-3c).

2.6.2 Sward density

Grass tiller, clover growing point and stolon density results are presented in Table 2-2. Density of *Lolium* species (mainly *Lolium perenne*) averaged 7200 tillers/m^2, while other grasses collectively averaged 6600 tillers/m^2. Clover growing point density was highest in the late summer/autumn/early winter period, especially in the second year with the density in February 1992 being significantly higher than that recorded at the other samplings. Clover stolon content (g or m/m^2) showed similar seasonal fluctuations to growing point density. The stolon weight per unit length dropped significantly in spring.

While within RG stocking rate did not show an effect, CS significantly increased the average tiller density of *Lolium* species (8800 tillers/m^2 under CS4.9 versus 6420 under RG; $P<0.05$). Significant interactions between grazing treatment and sampling date were recorded for *Lolium* tiller density and clover growing point density (both $P<0.001$), and stolon length/m^2 ($P<0.01$). The interaction for *Lolium* tiller density resulted from the lack of a significant effect of the grazing treatments in the February and September 1992 samplings, while at other times the tendency was that CS4.9 showed higher densities compared to RG. For clover growing point density and stolon length/m^2 the interaction resulted from the higher density measured under CS4.9 in February 1992, whereas no significant differences were measured in the other samplings.

Table 2-2 Seasonal variation in grass tiller density, clover growing point density and stolon content. Different capital letters denote significant differences between sampling dates ($P<0.05$). For variables with significant interaction between grazing treatment and sampling date, differences between grazing treatments within sampling date are indicated by different small letters, level of significance is given (~ $P<0.10$ and * $P<0.05$). Values are means of NN data (and grazing treatments where interactions were not significant).

Density of:	Grazing Treatment	AUG 1990 winter	MAR 1991 autumn	JUL 1991 winter	OCT 1991 spring	FEB 1992 summer	JUN 1992 winter	SEP 1992 spring
Lolium species	CS4.9	13140*	8760 a	13230 a	8400 a	6570	10300 a	6410
(tillers/m^2)	RG4.9	7040*	5330 b |~	8390 b |~	5360 ab |~	5570	6790 b |*	5750
	RG7.4	10700*	7920 a	6670 b	4940 b	6520	8120 ab	6210
*(*Lolium* + other grass spp)			BC	A	C	C	AB	C
Other grass species (tillers/m^2)		–	3870 C	5240 C	6190 B	7340 BC	10390 A	7700 AB
Clover growing	CS4.9	3720	4810	3700	2610	8780 a	4980	3070
points	RG4.9	2160	3990	3450	2690	6430 ab |*	5110	2580
(no./m^2)	RG7.4	4040	3290	2070	2190	5310 b	5190	3730
		CD	C	D	D	A	B	D
Clover stolon length	CS4.9	118	104	100	62	245 a	148	102
(m/m^2)	RG4.9	75	111	117	84	131 ab |*	138	100
	RG7.4	87	74	67	70	125 b	115	113
		CD	CD	CD	D	A	B	C
Clover stolon weight (g/m^2)		54.8 CD	51.8 CD	55.8 C	34.8 D	106.7 A	82.2 B	53.6 CD
Clover stolon weight/length ratio (g/m)		0.6 AB	0.53 CD	0.58 BC	0.48 D	0.64 A	0.61 AB	0.51 D

N application decreased the density of tillers of grass species other than *Lolium*: 7270 tillers/m^2 for no-N, compared to 6320 tillers/m^2 for plus-N (P=0.01). No other significant effects of N treatment or significant interactions between N treatment and sampling date were detected.

2.7 Discussion

Total annual herbage accumulation rate and sward composition were similar to those recorded earlier on the same site (Brougham *et al.*, 1975; Clark *et al.*, 1979; Crush *et al.*, 1982; Hay *et al.*, 1985; Cosgrove and Brougham, 1988). The response of herbage accumulation rate to N applications was higher than reported for similar N application levels on Manawatu grass/clover swards by Ball *et al.* (1978), and similar or higher than responses recorded in lowland pastures in Taranaki (Thomson and Roberts, 1982) or in the Waikato (Ledgard and Saunders, 1982; Feyter *et al.*, 1985). It was comparable to responses to similar application levels in Europe (Denehy and Morrison, 1979; Laidlaw, 1984; Wilman and Hollington, 1985; Reid, 1986; Frame and Boyd, 1987).

The well-documented suppressing effect of N application on clover growth in mixed grass-clover swards (see section 1.1) was again apparent in this experiment. The average reduction in clover DM accumulation rate of 15% was equal to that reported by Ball *et al.* (1978), but the reduction in clover content (0.036% per kg N applied) was less than the 0.07 - 0.12% per kg N reported by Frame and Boyd (1987). However, in the latter study the average clover content at the no-N treatment was higher than in the present study.

No significant differences in herbage accumulation rates were detected between grazing treatments in this study. Sward structure differed slightly, with the RG swards having lower population densities than those under CS, in accordance with earlier published results (Briseño de la Hoz and Wilman, 1981; Bircham and Korte, 1984; Frame and Newbould, 1986). The difference in population density between the grazing treatments was relatively small compared to differences seen in sheep-grazed pastures. This suggests smaller relative differences in defoliation patterns between CS and RG under cattle compared to sheep (Briseño de la Hoz and Wilman, 1981). Indeed, results presented later in Chapter 3 indicate that defoliation patterns of individual stolons resulted in similar defoliation rates for the grazing treatments.

In general, increasing defoliation frequency reduces production of a grass/clover sward (for example Chestnutt and Lowe, 1970; Curll and Wilkins, 1983; Frame and Newbould, 1984; Reid, 1986; Brock *et al.*, 1988) due to reduced leaf area and hence decreased photosynthesis per tiller. However, within a wide range of standing biomass or LAI a balance between population density and production per individual growth unit will establish (Hodgson and Wade, 1978; Bircham and Hodgson, 1983; Grant *et al.*, 1983), resulting in similar herbage DM accumulation in the long run. The results from the present experiment suggest that this state was reached in the swards, implying that the swards studied were in equilibrium and adjusted to the grazing treatments.

Tiller density of grasses other than *Lolium* species decreased with the application of N. DM accumulation increased slightly for this component. A shift in species composition was likely to contribute to this effect. *Poa annua* will be suppressed by the taller species such as *Lolium* and *Elymus repens* when N is applied. Additionally, production per tiller generally increases following N application (Bircham and Hodgson, 1984; Woledge, 1988).

The clover content was below desirable levels of 20-50% (Clark and Harris, 1996) during a large part of the year during the period of the present study, but similar to those often found on New Zealand farms. Caradus *et al.* (1996) mention an average of 20% clover in total pasture yield for lowland regions. Even though a higher clover content could buffer the adverse effects of fertiliser N better (Frame and Paterson, 1987), in the present experiment clover was capable of maintaining stolon density and recovering to the same level of herbage accumulation as the no-N treatment in the summer of the second year.

In conclusion, the response to fertiliser N of the grass/clover swards used in the present experiment was generally consistent with previously published results, under climatic conditions reasonably close to the long-term average for the location. Therefore, the effects of N application on clover plant characteristics described in the following chapters can be considered representative for cattle-grazed permanent pastures in similar environments to that of the present experiment.

Chapter 3

GROWTH DYNAMICS OF STOLONS

3. GROWTH DYNAMICS OF STOLONS

3.1 Introduction

Information on the effects of fertiliser nitrogen (N) on white clover growth dynamics in grazed swards may provide insights into the reasons for the often observed decline of clover when fertiliser N is applied (see Chapter 1). Since fertiliser N is mainly used in intensive production systems such as dairying, this information is particularly important for cattle-grazed swards. Research comparing clover growth dynamics under different managements in cattle grazing systems is lacking. Therefore, a detailed study was undertaken of the influence of cattle grazing and fertiliser N on clover growth dynamics. Important parameters for clover growth dynamics in temperate swards are development of nodes, branches and roots. Leaf production and utilisation are important parameters for the yield of harvestable matter and the influence it has on photosynthesis, energy supply and plant growth.

In temperate pastures white clover persists mainly through vegetative growth. The development and establishment of branches at nodes are important processes for continued persistence of this species (Turkington *et al.*, 1979; Chapman, 1983, 1987; Sheath and Hodgson, 1989). Branching is also important for production of harvestable dry matter (DM), by increasing the number of growing points. Each growing point continually develops nodes, each bearing a leaf. Each node is a potential site for the development of another branch since an axillary meristem is also present. The rate of node development depends on genotype (Knight, 1953; Beinhart *et al.*, 1963) and environment, particularly moisture availability and temperature (Brougham, 1962; Beinhart, 1963; Chapman *et al.*, 1983; Sackville Hamilton and Harper, 1989). Light intensity has less impact on node (and hence leaf) appearance rates (Beinhart, 1963).

Grazing also influences the growth dynamics of white clover. The clearest effects of grazing are seen in the dynamics of leaf growth. Initially after defoliation, new leaf growth is supported by increased partitioning of C from residual sources (remaining leaf tissue or energy reserves) to the apex at the expense of other organs such as stolon tissue and roots (Chapman and Robson, 1988, 1992; Chapman *et al.*, 1992a, b). Continued leaf removal leads to progressive reductions in lamina size and petiole length. From the results presented in Chapter 4 it can be derived that also in the present experiment the leaves were smaller under continuous stocking compared to rotational grazing, and under the higher stocking rate under rotational grazing in the present experiment (as dry weight per leaf).

Development of branches is regulated by assimilate supply and correlative factors within the plant which influence the allocation patterns of assimilates (Harvey, 1970; Newton *et al.*, 1992). Leaf removal reduces branching rates (Jones and Davies, 1988; Davies and Evans, 1990) and considerably lowers the viability of young axillary buds (Newton *et al.*, 1992), the loss of viability being greater under continuous stocking than rotational grazing in sheep-grazed swards. Particularly young branches are vulnerable to removal of parent stolon leaves, because these branches quantitatively import more carbohydrates from the parent stolon leaves than they fix themselves, while at the same time, they export more carbohydrates to the rest of the plant than they import from the parent stolon leaves (Chapman *et al.*, 1989; Chapman *et al.*, 1992b). Clearly, the carbohydrate source capacity of young branches is still small.

Shading increases the allocation of assimilates to stolon and petiole extension, at the expense of branching (Solangaarachchi and Harper, 1987; Thompson and Harper, 1988; Thompson, 1995). Davies and Evans (1990) found shading inhibited the rate of initiation of axillary buds and development of branches only when leaflets and petioles were removed from the parental node, while shading did not affect branch development when leaves were retained. Thus, a rest period after leaf removal is beneficial for branching, by allowing more leaves per growing point. However, a prolonged rest period results in a high herbage mass, with older leaves senescing and thus an increasing number of nodes which are mature enough to branch, but which are both shaded and leafless (Davies and Evans, 1990). Exposure of these nodes to light can subsequently induce branching.

Other factors that result in less branching are higher temperatures (Beinhart, 1963; Hoglund and Williams, 1984) and an increase in inorganic nitrogen (N) supply (Hoglund and Williams, 1984). Both factors increase the apical dominance of the parent stolon, and result in higher rates of leaf appearance. With increasing temperatures, the decrease in branching induced by application of mineral N is less (Hoglund and Williams, 1984).

In addition to knowledge of rates of leaf appearance and branching, information on rates of leaf development, senescence and leaf removal is useful for evaluation of leaf utilisation and the contribution of leaf senescence to the litter pool. Such information is available for sheep-grazed pastures in some environments (Chapman *et al.*, 1984; Clark *et al.*, 1984). These studies have shown the similarity in leaf utilisation of rotational and continuous sheep stocking systems, due to a balance between defoliation severity and frequency. The term "severity" has been used for the number of leaves defoliated per stolon per defoliation event (Clark *et al.*, 1984); "frequency" is used for the number of leaf removal events per time unit (Hodgson, 1979).

Chapman (1983) showed strong seasonal fluctuations and little effect of grazing management on branch and root development of clover populations in grazed hill country swards. Development of adventitious roots from stolons is essential for water and nutrient supply for the whole plant. Much less is known about the patterns of distribution of nutrients and water from source roots to sink shoots than is known about C distribution from source leaves to sinks throughout the plant. However, the information that is available confirms the importance of the stolon apex as a sink for these resources, and the existence of specific, intra-plant source-sink connections such as those between the root and branch growing at the same node (Chapman, 1983; Chapman and Hay, 1993; Kemball and Marshall, 1994).

The present study describes seasonal variation in clover growth dynamics, characterised by rates of leaf appearance, removal and senescence, stolon elongation, branching and rooting of nodes, and branch and root senescence, in lowland pastures grazed by cattle. The effects of application of fertiliser N in autumn and winter and differing grazing treatments are established. Thus, this study explores interactions between management practices and environment in a field setting as an important step in defining possible improvements in grass/clover pasture management in relatively intensive cattle systems where fertiliser N is used.

3.2 Materials and methods

3.2.1 Treatments

The swards used and treatments applied are described in detail in Chapter 2. Briefly, three grazing treatments were compared: continuous stocking with 4.9 bulls/ha (CS4.9) and rotational grazing with either 4.9 bulls/ha (RG4.9) or 7.4 bulls/ha (RG7.4). Each treatment was imposed on two replicate self contained farmlets. Two N treatments were imposed: no fertiliser N applications (no-N) and 100 kg N/ha/year (plus-N) applied to 5 m strips running the length of each paddock. N treatments started in April 1991, with N in the form of urea being applied twice per year (in autumn and winter) at 50 kg N/ha per dressing.

3.2.2 Marking stolons

In September 1990, four quadrats of 100x15 cm were randomly positioned within each replicate of each treatment (two in each of two paddocks). A metal frame was used to divide the quadrat into ten equal blocks. Clover stolons were marked in the inner eight blocks. These blocks were used to provide the location co-ordinates of the stolons. Five stolons per quadrat were selected and an inverted U-shaped metal marker was placed over the stolon and pushed into the soil immediately behind the node bearing the oldest leaf present on the stolon. Only main stolons (after Thomas, 1987a) with at least eight nodes and bearing roots were marked. Contact with the marked stolon was avoided as much as possible to minimise disturbance. Herbage and soil, if this was moved to relocate the marker, was replaced immediately after measurements were taken.

Measurements were collected weekly starting in mid September 1990. Following the initiation of the N treatments in April 1991, the number of marked stolons was doubled with a complete replicate set of marked stolons in the plus-N treatment. Subsequently, measurements were collected every ten or eleven days. In late autumn of the second year, longer intervals (14 to 21 days) between measurements were imposed since growth rates were much slower. Intervals of ten or eleven days were reimposed the following spring as growth rates increased. Measurements continued until mid December 1992. The scoring intervals which were used in the second year fell within the recommended length of time of between one and one-and-a-half leaf appearance intervals (Davies, 1981). During the first year and the last spring, the scoring intervals were slightly shorter than the average leaf appearance interval during those periods.

3.2.3 Measurements on stolons

At each measurement the presence of a leaf (petiole plus leaflets), petiole (not carrying leaflets), root, axillary bud, branch or inflorescence was recorded for each node from the marker up to the apex. The length of the stolon from the marker to the tip of the apex was also recorded. Fig. 3-1 shows the major morphological features and events that were noted, and also gives a schematic representation of the recorded data and the rates and age-specific data that were derived from these.

A node was considered to have developed a root when the root was long enough to penetrate the soil surface. An axillary bud was defined as a vegetative shoot in a leaf axil which had developed less than one fully unfolded leaf (after Thomas, 1987a). As soon as one fully unfolded leaf was present, the bud was considered a branch and reclassified. When rates of branch production and development were high (e.g. in the spring months), the axillary bud stage was sometimes missed between observations, and therefore not all branches observed were recorded also as axillary buds. The stage of development of young leaves was scored using the Carlson scale (Carlson, 1966b). The number of nodes on the marked stolon (from marker to tip) was determined. The nodes bearing young leaves (<1 of the Carlson scale) were not counted as one, but as the value of the stage of development according to the Carlson scale (Fig. 3-1). This permits calculation of more accurate rates of leaf appearance, especially in winter when growth is slow (Davies, 1981).

The state of every leaf was recorded at each measurement. Leaves were classified as either intact (all leaflets undamaged by grazing and completely green), partially grazed (one or more leaflets partially or totally removed by grazing or by invertebrate herbivory), or senescent (one or more leaflets showing signs of chlorosis). The onset of senescence of petioles, axillary buds, branches and roots (i.e. the main root, which is visible between the node and the soil surface, senesced) was also recorded. When a leaf passed into the senescent category it could not subsequently be reclassified as having been removed by grazing since it would have been difficult to determine whether a senescent leaf had disappeared between two scoring dates through leaf removal or decay.

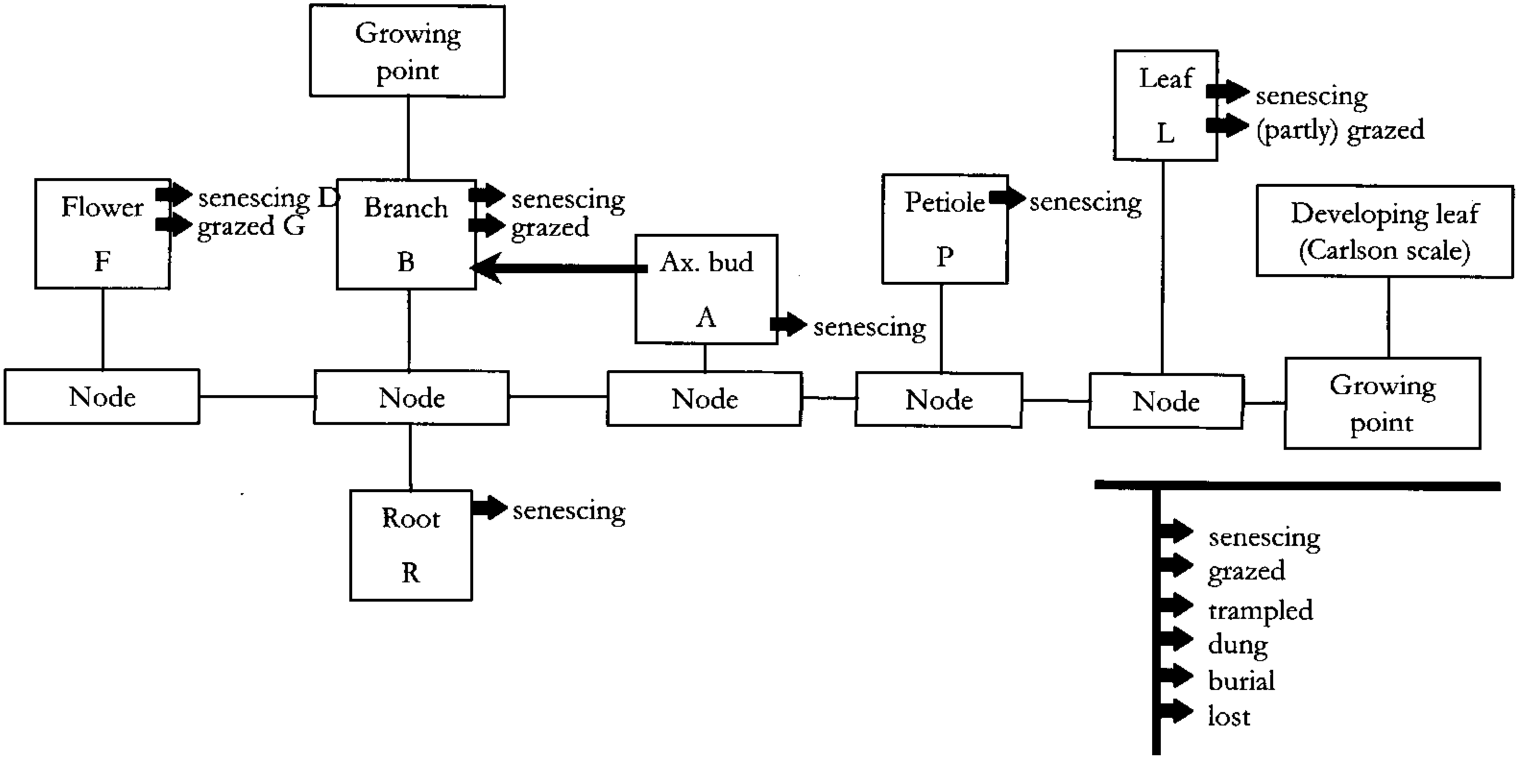
Leaf removal was defined as the disappearance of all leaflets between two successive scoring dates for either intact or partially grazed leaves. Thus, no differentiation between leaf removal by cattle and by for example slugs, insects or rabbits, could be made. Under RG it would be possible to identify removal due to other causes than cattle grazing during the resting period of the sward. Under CS this was not possible. It was assumed that the leaf removal patterns due to agents other than cattle were similar under both CS and RG, and no further differentiation was attempted.

When a marked stolon was lost from the sample population, the cause of loss was recorded in the following categories: grazing (i.e. entire section of marked stolon removed), senescence of whole stolon or the stolon apex, trampling damage, burial under cattle dung, burial due to other causes (e.g. by the hoof of an animal), or simply inability to relocate the marker. A replacement stolon was selected for the sample population, and marked in the same way as described above for the original sample population.

3.2.4 Data handling

Raw data collected from the field were entered in spreadsheets using Quattro Pro software, as shown in Fig. 3-1. Variables such as rates of stolon elongation, leaf appearance, branching, rooting and flowering, frequency and severity of leaf removal, and longevity of plant parts, were derived from these for each rotation period using Quattro Pro macros specifically created for this purpose. At the time of appearance of an axillary bud, branch, root or inflorescence (i.e. the first time it was noticed), the relative position of the node where this occurred was determined. Node position 1 was always assigned to the youngest node on the main stolon, node 2 the next, etc. (Fig. 3-1). The age of plant parts when removal or onset of senescence occurred was estimated by the number of days between the date when the plant part was first present and the date when it had disappeared or started to die.

a) Diagram of white clover main stolon and possible events.



b) Example of spreadsheet with measurements taken of main stolon. The node with position 1 is the youngest on the stolon (marked [illegible]). Rot = rotation period. Stolon length in cm.

Date	Rot	Stolon length	Nodes									
12-aug	1	1.1	L	0.8	0.1							
22-aug	1	1.1	L	P	L	0.2						
2-sep	1	1.2	LDA	PR	L	L	0.8					
12-sep	2	1.4	LDB	PR	LDR	PF	LG	L	0.1			
23-sep	2	1.8	BR	PDR	R	PDF	LG	P	P	0.6		
3-okt	2	2.1	BR	PDR	AR	FD	LDA	P	A	L	0.6	
14-okt	3	2.4	BR	0	BR	0	0	B	A	LG	LG	0.8

c) Example of derived data from spreadsheet by means of macro's. SER = stolon elongation rate (cm/week); Leaves = number of leaves/stolon; RLA = rate of leaf appearance (all rates as number per stolon/week); Petioles = number of petioles/stolon, without leaflets; LR events = leaf removal events (number per stolon/week); LR severity = leaf removal severity (number of removed leaves per event); L Age = age of removed leaves (days); AAR = axillary bud appearance rate; RR = rooting rate; Node pos. = node position where axillary bud or root appeared; RSR = root senescence rate; R Age = age of root at the onset of senescence.

Rot	SER	Leaves	RLA	Petioles	LR events	LR severity	L Age
1	0.03	2.3	0.97	0.7	0.30	1	
2	0.20	1.8	0.85	1.7	0.45	1.5	14

Rot	AAR	Node pos.	RR	Node pos.	RSR	R Age
1	0.33	5	0.33	4	0	
2	0.68	5	0.45	6.5	0.23	42

Figure 3-1 a) Diagram of white clover main stolon, b) example of measurements taken which are entered in a spreadsheet, and c) example of derived data from the spreadsheet by means of macro's.

Table 3-1 Division of scoring period (25 September 1990 to 14 December 1992) in rotation periods (as applied to RG treatments) and seasons. Boundaries between seasons were determined by rotation periods.

Season	Date	Number of rotations	Rotation lengths (days)
Spr90	25 Sep 90 - 04 Dec 90	3	35, 14, 21
Sum9091	04 Dec 90 - 05 Mar 91	5	20, 16, 20, 21, 14
Aut91*	05 Mar 91 - 30 May 91	4	21, 21, 23, 21
Win91	30 May 91 - 12 Sep 91	2	53, 52
Spr91	12 Sep 91 - 02 Dec 91	3	21, 28, 32
Sum9192	02 Dec 91 - 26 Feb 92	3	35, 21, 30
Aut92	26 Feb 92 - 20 May 92	3	26, 28, 30
Win92	20 May 92 - 24 Aug 92	2	40, 56
Spr92	24 Aug 92 - 14 Dec 92	3	60, 20, 32

* The N treatment started in the middle of the Aut91 period, hence for the PN data set, the last two rotations only were used: 17 April 91 to 30 May 91. From here on seasons are the same for the NN and PN data sets.

The entire measurement period was divided into 29 rotation periods (a rotation period started on the first day the animals went into the first paddock and ended when they returned to this paddock) and nine seasons as shown in Table 3-1. Means were calculated of each quadrat in each season for statistical analysis.

3.2.5 Statistical analysis

As described in Chapter 2, NN (no-N from September 1990 to December 1992) data were used to determine seasonal and grazing treatment effects and possible interaction between season and grazing treatment. PN (no-N and plus-N from April 1991 to December 1992) data were used to determine N effects and interactions between N and season or grazing treatment. Thus, where seasonal fluctuations or grazing treatment effects are discussed, data from NN only were used in the analysis and these are the data presented in the results sections. Data were transformed where necessary for homogeneity of variances. Presented values are least-squares means, back-transformed where applicable. SAS/STAT statistical software was used for the analyses (Anonymous, 1990).

Since the same stolons were scored at different dates (where stolons were not lost), the data resulting from these measurements are not independent. Covariances were expected to differ between seasons, since consecutive seasons are likely to be more closely related than seasons that are further apart in time. Thus, multivariate analysis was required, using season as multivariate variable (Cole and Grizzle, 1966; Snedecor and Cochran, 1980; Steel and Torrie, 1980). The number of observations of axillary buds and roots senescing were too small to use repeated measures analysis on the age of axillary buds or roots at senescence. Hence univariate analysis was used for these variables using a split plot for time (Snedecor and Cochran, 1980; Steel and Torrie, 1980). The node positions where an axillary bud, branch, root or inflorescence appeared were averaged over seasons and quadrats. The frequency distributions of these means were analysed with chi-square tests.

Senescence of axillary buds, branches or inflorescences on rooted nodes was initially distinguished from senescence on non-rooted nodes. To test if rooting affected the longevity of these plant parts, a chi-square test was used on the frequency distribution of the age of the plant part at the onset of senescence. The number of observations on rooted nodes was too low to analyse for seasonal or treatment effects. Hence the total number of occurrences on rooted and non-rooted nodes, and average age, were used in these analyses.

3.3 Results

3.3.1 Loss of marked stolons

In Table 3-2 an account is given of the frequency with which stolons were lost and new stolons were marked. Highest rates of loss occurred in summer, when more stolons died (either totally, or just the stolon apex), more stolon apices were removed by grazing, and a higher number were lost under a dung patch than during the rest of the year. More stolons were lost due to burial and trampling damage in the wet autumn and winter of 1991 and winter and spring of 1992 (see Chapter 2 for weather data). The wet conditions in autumn and winter 1991 resulted in a higher number of stolons that could not be relocated.

Table 3-2 Percentage of stolons which were replaced per week and concurrent causes. Different capital letters denote significant difference between seasons (P<0.05). Mean of NN data.

Season	Total	Lost	Stolon dead	Growing point dead	Grazed	Dung	Trampled	Buried	Other[1]
Spr90	2.5 F	0.8 CDE	0.8 E	0.1 F	0.8 BC	0.0 B	0.1 B	0.0 C	0.3 BC
Sum9091	10.6 A	0.7 CD	4.4 A	0.8 B	3.9 A	0.4 AB	0.1 B	0.0 C	0.5 B
Aut91	8.3 B	1.6 B	3.2 AB	0.3 CDEF	0.9 BC	0.2 AB	0.1 B	0.2 BC	1.8 A
Win91	5.8 CD	2.7 A	0.9 E	0.2 EF	0.4 C	0.1 B	0.5 A	0.7 AB	0.3 BC
Spr91	4.0 DE	0.9 BC	1.0 E	0.3 DEF	0.5 C	0.2 AB	0.3 AB	0.0 C	0.9 AB
Sum9192	8.1 B	0.3 DE	3.1 B	1.9 AC	1.8 B	0.5 A	0.1 B	0.0 C	0.4 B
Aut92	5.1 CDE	0.3 DE	1.8 CD	1.0 AB	1.7 BC	0.1 AB	0.1 B	0.0 C	0.2 BC
Win92	3.7 EF	0.2 E	1.0 DE	0.5 BCDE	1.1 BC	0.1 AB	0.5 A	0.1 C	0.2 BC
Spr92	5.8 C	0.4 CDE	2.2 BC	0.9 BCD	0.6 C	0.1 AB	0.5 A	1.0 A	0.1 C

[1] New stolons were marked, either due to replacing the whole quadrat or the reason for losing the marked stolon was not recorded. The value is especially high in Aut91, because several existing quadrats ended up in the plus-N treatment when this was applied, so that new quadrats for the no-N treatment had to be placed.

3.3.2 Leaf and petiole complement per stolon

The number of intact, partially grazed, and senescent leaves, and the number of petioles (not carrying leaflets), per stolon are shown in Fig. 3-2. The average over grazing treatments is presented, since no interactions of season and grazing treatments occurred. Seasonal differences were significant for all three variables ($P<0.001$). The number of green leaves per marked stolon decreased gradually from summer to the next spring in both years. The number of senescing leaves per stolon was lowest in winter for both years and was higher in the second year. The number of petioles per stolon decreased from spring to autumn in both years.

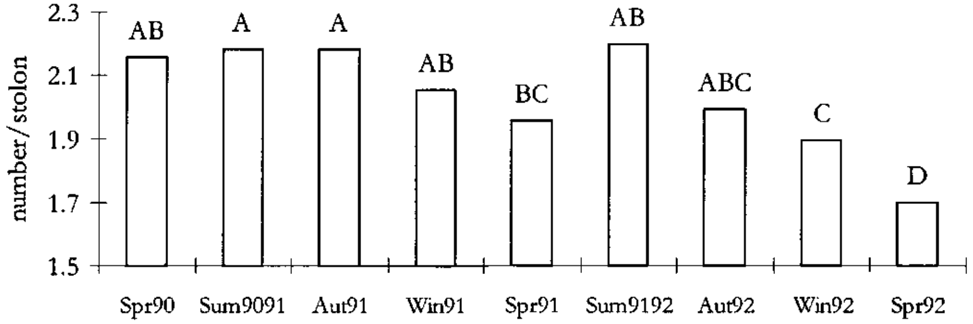
The average number of intact leaves per stolon was higher under CS4.9 (2.2 leaves/stolon) than under RG4.9 and RG7.4 (2.0; $P<0.05$). Grazing treatments did not significantly affect the number of senescent leaves or the number of petioles per stolon. Significant effects of N were found for number of senescent leaves ($P<0.001$), with average values being 0.25 per stolon for no-N and 0.40 per stolon for plus-N. The interaction between N and season was significant ($P<0.001$). This was due to a similar number of senescing leaves in Aut91, but a consistently higher number for plus-N from then on (Fig. 3-2b).

3.3.3 Rates of stolon elongation and leaf appearance

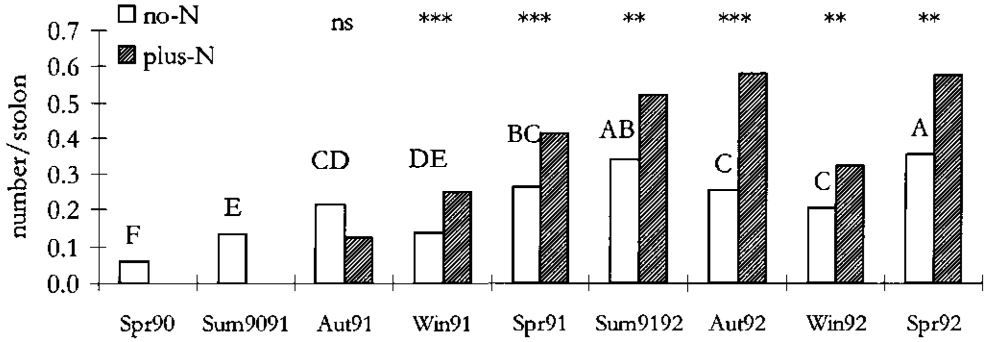
Stolon elongation rate showed strong seasonal variation, with lowest rates in winter and highest in summer and Spr90. Rate of leaf appearance showed a very similar pattern (Table 3-3).

Neither variable was significantly affected by grazing treatment. Overall, N increased stolon elongation rate from on average 0.15 to 0.19 cm/week ($P<0.05$). Interactions between N treatment and season were significant for stolon elongation ($P<0.01$) and for rate of leaf appearance ($P<0.001$). Both effects were due to a stronger response to N in Aut91. In this season stolon elongation rate averaged 0.14 cm/week in no-N compared to 0.22 cm/week in plus-N, while rate of leaf appearance averaged 0.58 and 0.66 leaves per stolon/week in the respective treatments. Note that these figures are for the PN data set, hence the different rate of leaf appearance value for no-N compared to the figure for Aut91 in Table 3-3, which presents NN data.

a) Number of green leaves per stolon, mean of NN data



b) Number of senescing leaves per stolon, mean of grazing treatments



c) Number of petioles (without leaflets) per stolon, mean of NN data

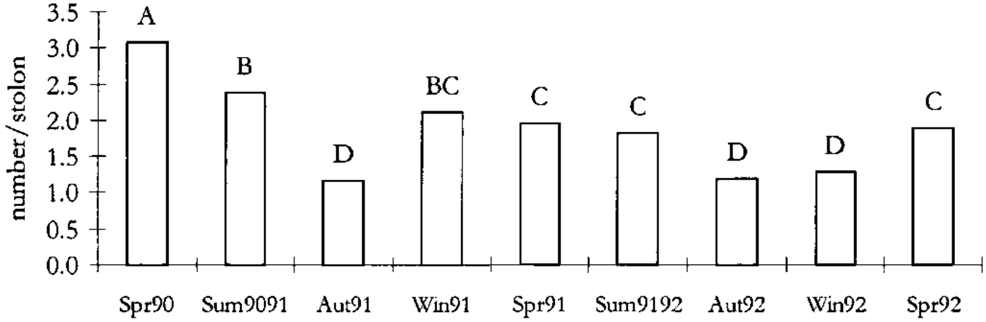


Figure 3-2 Average number of a) green leaves, b) senescing leaves and c) petioles (without leaflets) per stolon. Different capital letters denote significant difference between seasons ($P<0.05$). Levels of N effects within season are given by ** ($P<0.01$) and *** ($P<0.001$).

Table 3-3 Average leaf appearance (leaves per stolon/week) and stolon elongation rates (cm/week) and effect of N on stolon elongation rate ($P<0.05$). Different capital letters denote significant difference between seasons ($P<0.05$). Significance of N effect within season is given by * ($P<0.05$). Mean of grazing treatments.

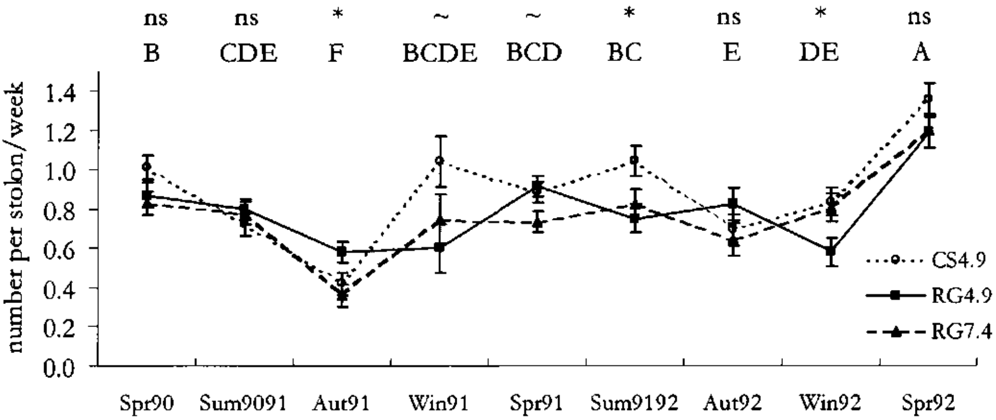
Season	N treatment	Leaf appearance rate	Stolon elongation rate
Spr90	no-N	0.89 A	0.48 A
Sum9091	no-N	0.94 A	0.29 B
Aut91	no-N	0.64 C	(0.15)[1] 0.14 C
	plus-N		0.22 *
Win91	no-N	0.44 D	0.08 D
	plus-N		0.07
Spr91	no-N	0.69 B	0.18 C
	plus-N		0.21
Sum9192	no-N	0.92 A	0.3 B
	plus-N		0.45
Aut92	no-N	0.73 B	0.19 C
	plus-N		0.23
Win92	no-N	0.39 E	0.02 E
	plus-N		0.03
Spr92	no-N	0.64 C	0.17 C
	plus-N		0.18

[1] See footnote Table 3-2.

3.3.4 Leaf removal patterns

Leaf removal patterns are presented in Fig. 3-3. Seasonal differences were significant ($P<0.001$) and a significant interaction between season and grazing treatment occurred for both leaf removal frequency and severity ($P<0.01$). Under CS4.9 leaf removal frequency was higher than under RG4.9 in the summer and winter of the second year. CS4.9 also had a higher frequency than RG7.4 in summer of the second year. The higher stocking rate treatment under RG (7.4 bulls/ha as opposed to 4.9 bulls/ha) did not result in increased leaf removal frequency: indeed in Aut91 and Spr91 the leaf removal frequency was lower at the higher stocking rate. The main effect of grazing treatment was significant for severity of leaf removal ($P<0.001$), with on average fewer leaves being removed per leaf removal event under CS in

a) Leaf removal frequency, mean of NN data



b) Leaf removal severity, mean of NN data

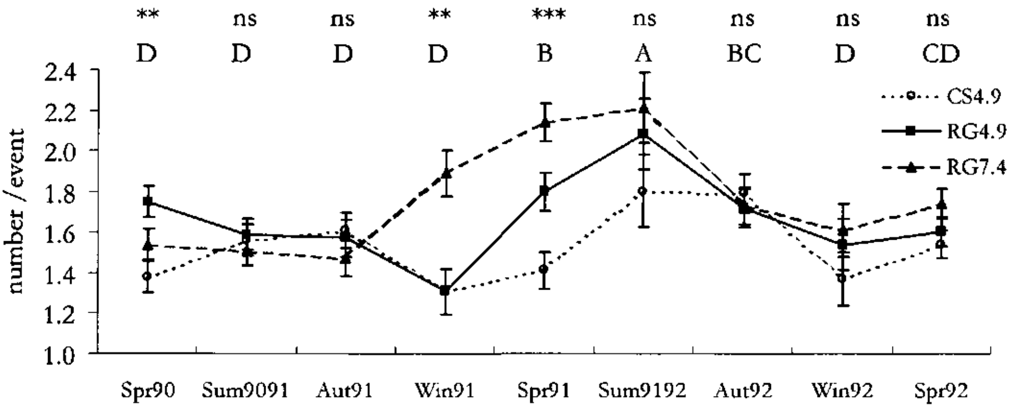


Figure 3-3 Seasonal and grazing treatment effects on a) leaf removal frequency and b) severity and c) N effects on frequency and severity. Different capital letters denote significant difference between seasons ($P<0.05$). Levels of grazing treatment effects are given by ~ ($P<0.10$), * ($P<0.05$), ** ($P<0.01$) or *** ($P<0.001$).

comparison to RG. In Win91 and Spr91 more leaves were removed per event under RG7.4 than under RG4.9.

N application resulted in lower leaf removal frequency in Aut91 and Spr92 (interaction $P<0.05$). In Aut91 there was an average of 0.38 leaf removal events per stolon per rotation for no-N compared to 0.18 for plus-N while in Spr92 the respective frequencies were 1.24 and 1.08. Severity of leaf removal also showed a significant interaction between season and N treatment (P<0.05), in Aut91 1.56 leaves were removed per event for no-N and 1.06 for plus-N.

3.3.5 Leaf removal rate and leaf and petiole senescence rates

When the frequency and severity of leaf removal are combined, an overall rate of leaf removal for individual stolons can be calculated. This information is presented in Table 3-4 along with data for the mean age of leaves at removal. The corresponding data for the rate of leaf and petiole senescence combined with the age of the leaf or petiole at senescence are also presented in Table 3-4. Seasonal fluctuations were significant for all of these variables (P<0.001). The rate of leaf removal was lowest in autumn and winter and highest in summer. Leaf and petiole senescence rates were lowest in winter.

As may be expected, leaf age at removal or onset of senescence was generally lower when leaf removal or senescence rates were higher and *vice versa.* Leaves were on average 23 days old when defoliated and 31 days old at the onset of senescence. After removal of leaflets, the petiole remained green for another 15 days before the onset of senescence.

Table 3-4 Average rates of leaf removal and leaf and petiole senescence (number per stolon/week), with corresponding age (days; for petiole the age is the number of days after removal of the leaflets) when removal or senescence occurred. Different capital letters denote significant difference between seasons (P<0.05). Mean of NN data.

Season	Leaf removal		Leaf senescence		Petiole senescence	
	rate	age	rate	age	rate	age
Spr90	0.43 AB	17.0 G	0.05 E	24.4 DE	0.29 B	12.3 F
Sum9091	0.48 A	17.0 FG	0.13 C	25.1 DE	0.36 A	13.6 E
Aut91	0.23 E	18.9 DE	0.19 AB	30.0 C	0.20 C	13.9 DE
Win91	0.16 F	36.2 A	0.10 D	45.0 A	0.11 D	18.4 AB
Spr91	0.38 BC	21.2 C	0.17 BC	31.4 C	0.21 B	15.9 BC
Sum9192	0.42 ABC	18.3 EF	0.19 AB	24.0 E	0.27 B	14.2 DE
Aut92	0.31 D	20.1 CD	0.13 C	26.0 D	0.20 C	15.1 CD
Win92	0.16 F	32.8 A	0.08 DE	42.0 B	0.10 D	18.9 A
Spr92	0.37 C	24.7 B	0.21 A	32.0 CD	0.21 C	17.2 AB

Grazing treatment did not affect removal and senescence rates or concurrent ages. Interaction with season was significant for age of leaf when defoliated (P<0.01). In Spr90, Aut91 and Sum9192 leaves were on average 3 days older when defoliated under CS4.9 than under RG7.4. In Spr91 leaves were on average 3 days older, and in Spr92 3 days younger under CG4.9 than under RG4.9. RG7.4 was intermediate.

Fertiliser N did not significantly influence removal or senescence rates, or age at removal or senescence. Interaction with season occurred for age of leaf at the onset of senescence (P<0.05), due to a lower leaf age in Aut91 with N. However, this was an artefact of the experimental set-up, because new stolon tips were tagged for plus-N: older leaves were not in this sample. A significant interaction between N and season was found for petiole senescence rate (P<0.01). It was lower with N in Aut91 (0.03 and 0.12 petioles per stolon/week for plus-N and no-N, respectively) and higher with N in Win91 (0.14 for plus-N and 0.11 petioles per stolon/week for no-N).

The loss of marked stolons contributed considerably to loss of leaves. Combining the number of leaves per stolon (Fig. 3-2) and the chance of losing a stolon (Table 3-2), between 6 and 28% of developed leaves were lost this way. These leaves were not included in either the removed or senescent categories. Leaf senescence and loss of leaves with the senescence or loss of the marked stolon (excluding loss of stolon due to grazing) combined, add up to an average of 0.22 leaves per stolon/week. The loss of marked stolons due to grazing accounted for removal of 0.024 leaves per stolon/week.

3.3.6 Node demography

On average 17% of the nodes which appeared during the course of the experiment developed axillary buds, 17% developed branches, 14% developed roots and 2% developed inflorescences, but seasonal variation was significant for all variables (Table 3-5; P<0.001). A lower percentage of the nodes that appeared in winter developed axillary buds and branches compared to nodes appearing in spring. More roots developed on nodes that appeared in autumn and winter than on nodes that appeared in summer. Root development was also high from nodes that appeared in the first spring. Flowering occurred mainly on nodes that had appeared in spring and summer.

Table 3-5 Average percentage of nodes, formed within each season, which developed axillary buds, branches, roots or inflorescences. Different capital letters denote significant differences between seasons (P<0.05). Mean of NN data.

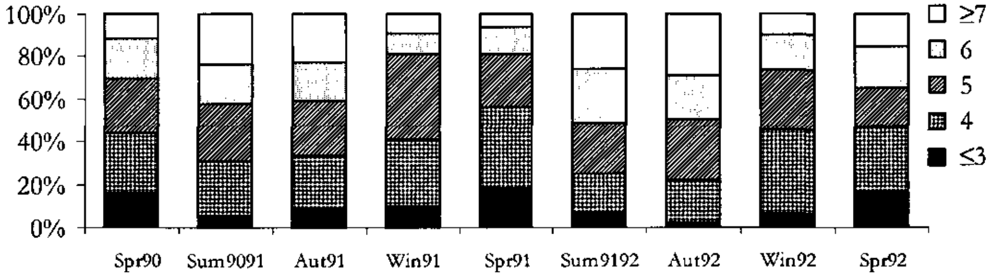
Season	Axillary bud	Branch	Root	Inflorescence
Spr90	31 A	33 A	19 A	7.1 A
Sum9091	17 BC	15 C	8 D	2.7 B
Aut91	18 BC	10 D	18 AB	0.1 D
Win91	14 C	15 C	13 BC	0 D
Spr91	22 B	29 AB	10 CD	2.7 B
Sum9192	16 C	22 B	8 D	3.4 B
Aut92	16 BC	13 CD	19 AB	0.8 C
Win92	7 D	6 E	15 ABC	0 D
Spr92	13 C	13 CD	12 CD	2.3 B

Note: In seasons when rates of branch production were high, the axillary bud stage was sometimes missed. Therefore, branch % values may sometimes be > axillary bud % values.

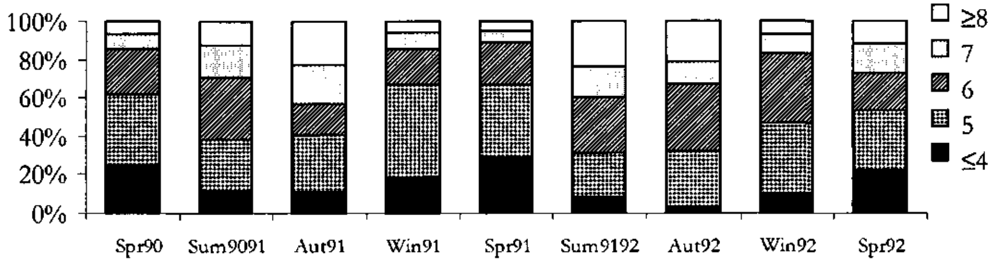
The average percentage of nodes developing roots was significantly higher under RG4.9 (17%) than under RG7.4 (13%). The percentage under CS4.9 (11%) was significantly lower than under the other two treatments (main effect of grazing treatment P<0.05). An interaction between season and grazing treatment was found only for flowering (P<0.05). Significantly fewer nodes which had appeared in Spr90 developed an inflorescence under RG7.4 (3.6%) than under RG4.9 and CS4.9 (8.8 and 9.0%, respectively). No significant effects of N were detected, and there were no significant interactions between N and season.

The distribution of the node position where a root, axillary bud, branch or inflorescence first appeared is presented in Fig. 3-4. The majority of axillary buds and roots appeared on nodes 4 to 6 (72 and 67%, respectively). Branches developed mainly on nodes 5 to 7 (71%). Thus, branches appeared on average on nodes of higher position than roots, i.e. nodes were older when branching occurred in comparison to rooting.

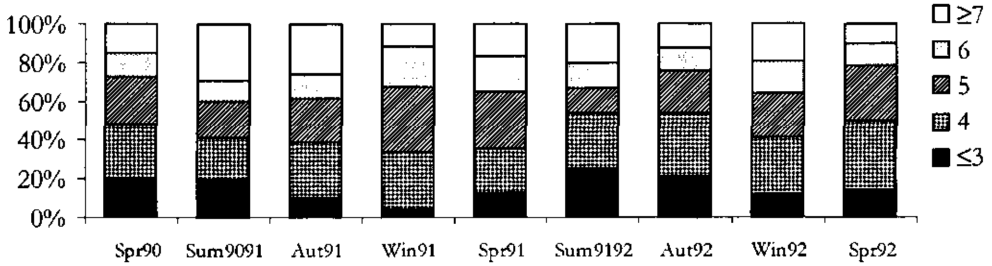
a) Axillary bud



b) Branch



c) Rooting on node



d) Inflorescence

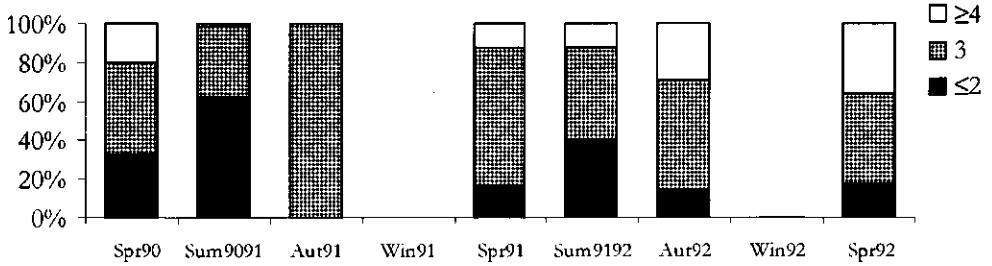


Figure 3-4 Frequency distribution of node position where a) an axillary bud or b) a branch appeared, c) a node rooted or d) an inflorescence appeared. Node position 1 is the youngest node on the marked stolon. Mean of NN data.

Seasonal variation was significant for these four distributions of node positions ($P<0.001$; Fig. 3-4). The position of the nodes was higher when axillary buds and branches developed in summer and autumn. In the first year, the nodes were of a higher position when they formed roots in summer and autumn than in the second year. In the first winter a lower percentage of nodes 3 and younger rooted, while rooting occurred more on node 5 and 6. Inflorescences appeared mainly on nodes of position 3. However, in Sum9091 the majority of the inflorescences appeared on nodes of position 2 or 1. The percentage of inflorescences appearing on the node of position 3 was higher under RG7.4 than under the other grazing treatments (65% and 42% under CS4.9 and RG4.9; $P<0.01$), while less appeared on older nodes under this grazing treatment.

Main effects of N treatments were not significant for node position at which organs first appeared. Appearance of axillary buds on nodes 7 and higher was increased by N applications in Spr91: 15% with N and 6% without N (interaction N treatment and season $P<0.05$).

3.3.7 Axillary buds

The average rates with which axillary buds appeared, developed into branches or died, and their age at which further development or senescence occurred, are given in Table 3-6. The highest rates were recorded in the first season (Spr90), declining to minimum rates in the subsequent winter. In the second year, highest rates were recorded in summer, after which they declined again to low levels in winter ($P<0.001$). The rates and seasonal fluctuations did not differ between grazing or N treatments.

3.3.8 Branches

The average appearance and senescence rates of branches and concurrent age at senescence are given in Table 3-7. Seasonal fluctuations were similar to those described for axillary buds with minimum rates recorded in winter. When senescence rates were lower, concurrent ages were higher. An exception was Spr92, where branches of a comparatively high age died.

Grazing and N treatments did not affect the branch appearance or senescence rates or age at senescence, and interactions with season were not significant. When branching rates were higher, a lower proportion appeared on rooted nodes. The presence of a root at the node of origin on the parent stolon significantly affected the longevity of a branch by increasing the mean age at senescence from 32 to 50 days ($P<0.05$).

Table 3-6 Average observed appearance rates of axillary buds, development rates of axillary buds into branches, and senescence rate of axillary buds (number per stolon/week) and corresponding age (days) when development or senescence occurred. Different capital letters denote significant difference between seasons ($P<0.05$). Mean of NN data.

Season	Appearance	Development into branch		Senescence	
	rate	rate	age	rate	age
Spr90	0.36 A	0.23 A	8.8 DE	0.06 A	10.7 E
Sum9091	0.20 B	0.12 B	8.7 E	0.04 AB	11.4 DE
Aut91	0.16 BCD	0.09 BC	10.1 CD	0.03 BC	13.5 CD
Win91	0.08 E	0.05 D	15.9 AB	0.01 E	14.1 AB
Spr91	0.15 CD	0.10 BC	11.0 C	0.01 DE	13.5 BC
Sum9192	0.17 BC	0.11 B	11.0 C	0.02 CDE	15.1 BC
Aut92	0.16 BCD	0.07 C	12.9 B	0.02 CD	15.5 BC
Win92	0.06 E	0.04 D	16.9 A	0.01 DE	22.7 A
Spr92	0.12 D	0.06 CD	15.2 A	0.03 C	16.9 B

Table 3-7 Average branch appearance and senescence rates (number per stolon/week), and average age (days) when senescence occurred. Different capital letters denote significant difference between seasons ($P<0.05$). Mean of NN data.

Season	Appearance	Senescence	
	rate	rate	age
Spr90	0.39 A	0.06 AB	15.8 D
Sum9091	0.23 B	0.08 A	27.3 C
Aut91	0.10 E	0.03 DE	30.9 BC
Win91	0.06 F	0.01 EF	51.3 AB
Spr91	0.18 BC	0.03 D	27.9 C
Sum9192	0.31 A	0.06 AB	30.2 BC
Aut92	0.16 CD	0.03 CD	29.3 BC
Win92	0.06 F	0.01 F	40.7 ABC
Spr92	0.13 DE	0.05 BC	60.2 A

3.3.9 Roots

Seasonality and the effect of N application on the rates of root development and senescence are presented in Table 3-8. Root development rates were highest in spring and autumn. Highest root senescence rates were found in spring. N significantly suppressed root development and root senescence rates in Aut91 and in spring.

The appearance and senescence of roots on nodes with or without a branch was distinguished. Between 13 and 34% of the roots developing did so on nodes which were already bearing a branch. This proportion was higher when branch appearance rate was higher, but it was not related to root appearance rate.

The presence of a branch on a node significantly increased the lifespan of roots: roots were on average 16 days old at the onset of senescence on non-branched nodes, whereas the average age of senescence of roots was 44 days at branched nodes ($P<0.01$). Significant interactions between the presence of a branch and season on the appearance and senescence of roots were recorded. The seasonal variation in rates of appearance or senescence of roots was much less pronounced for branched nodes than for non-branched nodes. The appearance rate of roots showed a significant interaction between N treatment and presence of a branch ($P<0.001$). N reduced root development rate of branch-bearing nodes (0.091 branched nodes developing a root per stolon/week for no-N compared to 0.065 for plus-N) but there was no significant difference between N treatments for non-branched nodes (0.023 and 0.021 non-branched nodes developing a root per stolon/week for no-N and plus-N, respectively).

The main effect of grazing treatment was significant for root senescence rate ($P<0.05$). Senescence rate was lower under CS4.9 compared to RG4.9 (0.027 and 0.036 roots senescing per stolon/week, respectively), with RG7.4 intermediate (0.031 roots senescing per stolon/week). There were no significant interactions between grazing treatment and season.

3.3.10 Inflorescences

Table 3-9 presents the average rates of inflorescence appearance, removal and senescence. Seasonal differences were significant ($P<0.001$). Spring and summer were the main seasons of flowering, and only a few inflorescences appeared in autumn. Rates of removal and senescence followed the same pattern. Grazing treatment and season interacted significantly ($P<0.05$). In Spr90 inflorescence appearance rate was lower under RG7.9 (0.029 inflorescences appearing per stolon/week) than under CS4.9 and RG4.9

(0.072 and 0.071, respectively). No grazing treatment effects were detected in other seasons. N application significantly decreased the average age of an inflorescence when senescence occurred (from 18.0 days in no-N to 14.3 days in plus-N, $P<0.05$), but did not affect inflorescence appearance or senescence rates.

Table 3-8 Average rooting rate and root senescence rate (number per stolon/ week), and average age (days) when senescence occurred. Different capital letters denote significant difference between seasons ($P<0.05$). Significance of N effects is given by * ($P<0.05$), ** ($P<0.01$) or *** ($P<0.001$). Mean of grazing treatments.

Season	N treatment	Rooting	Senescence	
		rate	rate	age
Spr90	no-N	0.20 A	0.10 A	10.5 D
Sum9091	no-N	0.08 CD	0.06 BC	13.0 CD
Aut91	no-N	(0.11)1) 0.14 BC	(0.02)1) 0.01 E	14.5 CD
	plus-N	0.05 ***	0 **	
Win91	no-N	0.07 D	0.03 DE	18.4 AB
	plus-N	0.08	0.02	
Spr91	no-N	0.11 B	0.05 CD	16.5 BCD
	plus-N	0.08	0.03 *	
Sum9192	no-N	0.07 D	0.03 E	21.4 ABC
	plus-N	0.07	0.03	
Aut92	no-N	0.15 AB	0.04 CDE	21.6 ABC
	plus-N	0.12	0.03	
Win92	no-N	0.05 D	0.03 E	34.0 A
	plus-N	0.06	0.02	
Spr92	no-N	0.16 A	0.08 AB	32.6 A
	plus-N	0.11 *	0.05 *	

1) See footnote Table 3-2.

Table 3-9 Rates of inflorescence appearance, removal and senescence (number per stolon/week). Different capital letters denote significant difference between seasons (P<0.05). Mean of NN data.

Season	Appearance	Removal		Senescence	
	rate	rate	age	rate	age
Spr90	0.058 A	0.029 A	12.7 B	0.004 A	9.0 C
Sum9091	0.025 BC	0.021 B	16.2 A	0.010 A	20.6 AB
Aut91	0.001 DE	0.001 DE	-	0 B	-
Win91	0 E	0 E	-	0 B	-
Spr91	0.014 C	0.003 CD	-	0.004 AB	12.5 BC
Sum9192	0.032 B	0.020 B	16.3 AB	0.006 A	24.4 A
Aut92	0.005 D	0.004 CD	-	0 B	-
Win92	0 E	0 E	-	0 B	-
Spr92	0.020 BC	0.008 C	16.0 AB	0.004 AB	-

3.4 Discussion

In this chapter a study of the dynamics of clover growth in lowland swards is presented, concentrating on seasonal changes and the effects of cattle grazing management and fertiliser N application in autumn and winter. The aim was to establish reasons for the often observed negative effects of fertiliser N use on clover persistence, and to determine possible interactions with grazing management. Interactions between grazing management and N treatments would indicate possible management options to improve clover persistence in cattle-grazed, N fertilised swards. However, significant interactions did not occur in the present experiment. Several differences between grazing or N treatments were found and these are discussed below.

The clover population and plant characteristics generally showed great variation at any point in time, perhaps reducing the occurrence of statistically significant effects of treatments. However, this variation also demonstrates the great phenotypic variability typically found in pastures, which is likely to benefit the persistence of clover in the spatially and temporally patchy environment of a grazed sward.

3.4.1 Leaf demography

In the present experiment rates of leaf appearance were similar to those presented by Chapman (1983). Grazing treatment did not influence rate of leaf appearance in either study. Only in the first autumn of this experiment did N application have a significant effect on rate of leaf appearance. Apparently rate of leaf appearance of clover is a characteristic which is not likely to be influenced by grazing management or soil N, but more by environmental factors as temperature. Rate of leaf appearance is important for harvestable clover production and, since it implies development of new nodes, which are potential sites for branch development, for persistence.

The ratio of leaves removed to leaves produced is a measure of utilisation of clover leaf growth. Values found during this experiment were between 36 and 58% (average 46%), being lower in autumn and winter than summer and spring. The lower utilisation in autumn and winter has been reported before (Chapman *et al.*, 1984; Chapman, 1986) and can be attributed to shorter petioles in winter (Brougham, 1962, 1966) and therefore the higher incidence of leaf surfaces being positioned below grazing height compared to other seasons. The average utilisation was lower in the present experiment than that recorded in earlier experiments (40-66% in Chapman *et al.*, 1984; 61% in Chapman, 1986). This is likely to be due to a higher surplus pasture growth in at least part of the year in the present experiment, in comparison to the earlier experiments. While in the experiment of Chapman a set number of breeding cows was used (approximately 2.5 cows/ha; Chapman, 1986), in the present experiment the one year old bulls were taken from the trial in summer. Thus, only calves were left, so that the stocking rate was less than 300 kg metabolic liveweight/ha during a large part of summer and autumn (Fig. 2-2). Also, the annual herbage DM accumulation was higher in the present experiment (16-17 tonnes/ha/year, in comparison to 11-12 tonnes/ha/year for the earlier trials; Lambert *et al.*, 1983). Further, removal of leaves after the onset of senescence was not determined in the present experiment, so utilisation may be underestimated. The average leaf age when defoliated was 23 days in the present experiment (from Table 3-4). Inclusion of removal of senescing leaves would likely have increased this average age. This could explain the lower average age in the present experiment compared to the average age of 27 days recorded by Chapman (1986).

Under CS, leaf removal severity was on average lower and the average number of leaves per stolon was higher than under RG. This was also found under sheep grazing (Chapman, 1986). Under RG, a higher stocking rate resulted in a similar or sometimes more severe leaf removal per event and a similar or sometimes lower frequency of leaf removal. It is possible that the removal of more leaves per event results in lower frequency of leaf removal, because the first new leaves to appear after defoliation have shorter petioles than those appearing subsequently (Brougham, 1966; Culvenor *et al.*, 1989a). Petioles elongate until the leaf is in the top of the canopy (Thompson, 1995). Just after a grazing event, the canopy is obviously shorter than at the time of the next grazing. Thus, the first leaves to appear in a regrowth period are likely to be below the grazing height of the cattle during the next grazing. In a continuous stocking system, with more frequent defoliation, the canopy height is more stable. Thus, under continuous stocking, defoliation frequency and severity are unlikely to trade off in the same way as under intermittent defoliation. The effect of increasing grazing pressure under continuous grazing (by increasing stocking density) could therefore be to increase *both* the frequency and severity of defoliation as shown by Curll and Wilkins (1982), leading to different equilibrium states of leaf size and turnover. In the present experiment only one stocking rate was used in the continuous stocking system, so no such comparison can be made for cattle.

Even though differences in leaf removal frequency and severity occurred, the leaf removal rate measured as number of leaves per stolon/week removed did not differ between the grazing treatments. Since leaf appearance and senescence rates did not differ either, utilisation was also similar for the grazing treatments. With the similar growing point densities (Chapter 2), leaf appearance and senescence rates, the lack of effect of grazing treatment on clover DM accumulation, as measured with exclosure cages, can be explained (Chapter 2). This is an example of the great phenotypic plasticity of clover (e.g. Brougham *et al.*, 1978; Chapman and Lemaire, 1993) leading to relatively similar levels of primary production under apparently quite different management regimes.

When comparing the age of plant parts when senescence or removal occurred, it is important to remember that the interval between measurements differed between seasons. This will have had an effect on the age, since the length of the interval determines the lowest value possible. The influence of this would have been greater when senescence or removal rates were higher. Thus, the lower ages found in the first spring and summer (with weekly measurements) in comparison with spring and summer of the second year (with intervals of 10 or 11 days) could be partly due to this. However, the general seasonal trend can be assumed to be genuine. The average age of the leaves at the onset of senescence was below the average rotation length in Win91 and from Sum9192 onwards. This implies that under RG the first leaves that appeared after a grazing died before a chance of removal during the following grazing had occurred. If redistribution of nutrients was efficient, the senescence of leaves, petioles and stolons could have benefited the overall nutrient economy of clover plants. For example, in lucerne around 80% of the N in shaded and senescing leaves is recovered by the rest of the plant (Lemaire and Culleton, 1989; Durand *et al.*, 1991; Lemaire *et al.*, 1991); in perennial ryegrass this figure is 30-50% (Hunt, 1983). The remaining nutrients in decaying material will have contributed to the soil litter pool and nutrient cycling. The life span of leaves also has implications for the herbage accumulation measurements. At nearly all harvests for herbage measurements (Chapter 2), the regrowth period was longer than the average age of clover leaves at the onset of senescence. Thus, gross clover growth was probably underestimated. The same would apply for grasses if their leaf life spans were similar to white clover. However, Chapman *et al.* (1984) measured a longer life span for clover leaves than grass leaves in winter, but a shorter life span in spring/early summer. Thus, the level of underestimation is likely to differ between clover and grass.

An interesting finding was the long life span of petioles after removal of leaflets. On average, 1.8 petioles were present per stolon for 15 days before the onset of senescence. To establish the importance of photosynthesis of the petioles for the whole plant, the photosynthesis/respiration ratio of petioles should be measured along with laminae. The contribution of petioles to the carbon economy of clover populations is often overlooked, but may be substantial; for example, Korte (1984) recorded that petioles contributed 16% of the total photosynthesis of clover in hard-grazed swards.

3.4.2 Stolon elongation

Stolon elongation rates were comparable to those recorded under rotational cattle grazing in hill country (Chapman, 1983). However, greater stolon elongation rates recorded by Chapman (1983) under rotational compared to continuous stocking with sheep were not observed in this study with cattle. Stolon elongation rate increased with fertiliser N application. Increased stolon elongation increases the spreading ability of clover and increases the ability to escape unfavourable conditions and explore the soil surface. Since N had generally no effect on rate of leaf appearance greater stolon elongation must have been primarily due to increased internode length. This is in accordance with results of Hoglund and Williams (1984) under controlled conditions. Shading at the node increases internode length (Thompson, 1993, 1995). Obscuring this photoperceptive site caused a redirection of resources from branching into stolon elongation and petiole elongation (Thompson, 1995). Robin *et al.* (1992) showed additional far-red light (thus lowering the red/far-red ratio, as with shading), promoted stolon and petiole elongation, and redirected photosynthates to petioles and leaves, at the expense of stolon (including branches) and root material. In the present experiment, fertiliser N application resulted in greater herbage mass (Chapter 2); hence shading at the nodes would have been greater, in comparison to no-N.

3.4.3 Node demography

In this study, the nodes 4 to 7 were the most important for development of axillary buds, branches and roots. Newton *et al.* (1992) recorded the maximum proportions of nodes with "incipient" branches (i.e. buds that had made visible growth at sampling, but had no visible stolon) on node 4 to 8. In their experiment 21.5% of the nodes had developed branches at sampling, a slightly higher percentage than the 17% of the current experiment. On average, branching and rooting frequency was considerably higher in the experiments of Beinhart (1963; 28-79%) and Chapman (1983; 10-43%) compared to the frequency measured here. Great seasonal fluctuations were observed in all experiments.

The important limiting climatic factors are temperature, light and soil moisture. Soil moisture was unlikely to have limited branching and rooting in winter during the present experiment; it is more likely that lower temperatures or light levels caused decreased branching and rooting of nodes in this season. In summer, a lower soil moisture status and a higher temperature appeared to inhibit rooting of nodes to a greater extent than branching of nodes. In the second summer, when below average temperatures were experienced, branching of nodes was less inhibited.

Branching and rooting rates were positively correlated, similar to earlier findings (Chapman, 1983), but the correlation coefficient was low. The presence of a root on a node was not likely to induce a higher branching probability, nor did the presence of a branch enhance root development at the parental node. The presence of a root is not a prerequisite for the development of a branch, or *vice versa.* A significant finding of the present experiment was the benefit to both branch and root survival when they occurred on the same parental node: the life span of a branch was increased when the parental node had rooted, and the life span of a root was increased when the parental node had branched. Thus not only did the branch benefit from the presence of the root, as was shown previously (Chapman, 1983; for example through supply of water and nutrients (Chapman and Hay, 1993; Kemball and Marshall, 1994)), but also the root was supported by the branch (through supply of photosynthates, as is now shown conclusively by Chapman *et al.*, 1992b, c). Obviously, the success rate of establishment of new plant units when the parental stolon dies depends on the combination of branching and rooting. With high rates for both variables, the chance that both occur at the same node increases. This would increase the chance for successful establishment of young branches, which, following severance of the parental stolon, are the new generation clover plants.

One aspect determining the number of sites where branches can develop is flowering. Once a node bears an inflorescence, this site is lost for branch development. In the present experiment only a small percentage of nodes developed inflorescences. These were often removed through grazing or senesced within 9 to 24 days of their appearance. Thomas (1987c) refers to an experiment where it took white clover plants with initiated inflorescences a minimum of 17 days to blossom after being transferred to a controlled environment. Within 12 days after pollination about 50% of the seed is viable (Thomas, 1987c). Hence, the results indicate that reproduction of clover through seed setting played a very minor role in the present experiment, similar to earlier findings in hill country (Chapman, 1983, 1987).

An important result of this experiment was the consistent and significant effects of grazing and N treatments on root development and longevity. With the same stocking rate, the percentage of nodes developing roots was lower under continuous stocking than under rotational grazing. Under rotational grazing the percentage of nodes developing roots was lower with a higher stocking rate. During regrowth periods, clover may have invested relatively more of its assimilates in the main and existing branch apices to maintain leaf area in the top of the canopy (Dennis and Woledge, 1982; Fisher and Wilman, 1995), thus reducing assimilate supply for initiation or maintenance of nodal roots. This reduction will be greater when stolons are defoliated more often or more severe. The same would apply to the clover plants in the swards receiving fertiliser N. Fertiliser N application suppressed rates of root development on nodes in spring, particularly on branch bearing nodes. In spring herbage accumulation rates were high (Chapter 2). Also in this situation clover may have invested more in leaf and petiole tissue on existing apices.

Since the relative senescence rate of roots was similar for no-N and plus-N, the number of rooted nodes per main stolon must have been lower in the N fertilised swards. This was confirmed by the population census data (Chapter 4). Decreased rooting will make clover more vulnerable to stresses such as drought, and lower its ability to compete for nutrients. In addition to the direct negative effect on N fixation of fertiliser N application, decreased rooting can lower N fixation per plant as well in the longer run through a decrease in number of potential sites for nodule establishment. Lower frequency of root development will also result in fewer branches that are attached to a root-bearing node. Since the presence of a root on the parental node increased the longevity of branches, N application and increased leaf removal severity or frequency could decrease longevity of a proportion of the branches. Since the proportion of branches that had a root on the parental node was low, this effect was not detectable in the present experiment. More research is required in which the effects of leaf removal and N application on frequency of root development on nodes and on branch development and survival are established. This work can probably be aimed at the spring and subsequent summer period. Spring is a crucial time in the establishment of clover, a period in which plant break up occurs due to high turnover rates of plant material, and the clover plant population consists of many small individuals (see Chapter 4). At this time, competition from fast-growing grasses for light and nutrients is strong. Extra stress could then result in a lower survival rate of clover plants. For example, a spring drought resulted in a collapse of clover in Canterbury (Vartha and Hoglund, 1983; Hoglund, 1985; Brock, 1988). This happened under rotational sheep grazing; in continuously stocked swards the clover recovered rapidly. The dense sward would have reduced direct solar radiation to the soil surface and hence heat stress to the plants (Brock, 1988). Subsequent work confirmed the importance of sward density for drought survival of white clover (Brock and Kim, 1994).

The environment of clover nodes is crucial for the persistence of the species in grazed swards. Many factors influence this environment, and management should aim to develop sufficiently dense swards to protect the nodes from heat or water stress, and to sustain the sward height within a range where sufficient leaves are maintained for photosynthate supply and where nodes receive sufficient light to enable branch development and establishment. These requirements are equally valid for a system with or without the use of fertiliser N. However, they are perhaps harder to meet where N is used since the growth potential of the pasture is increased with added N, and management decisions must be both timely and accurate in order to maintain sward height and density within desired ranges for the clover population.

Rooting was inhibited under CS4.9 and RG7.4 compared to RG4.9. Branching was not affected by grazing treatment. From the literature it is derived that defoliation decreases the allocation of photosynthates to stolon and root material. With the defoliation regimes of the present experiment, it appeared that root development was more affected than branch development. Indirectly this can still have its implications for branch development when conditions are adverse, since the presence of a root on the parental node increases the longevity of a branch, and hence increases the chance of a successful establishment of a branch.

Chapter 4

POPULATION AND PLANT STRUCTURE

4. POPULATION AND PLANT STRUCTURE

4.1 Introduction

White clover plays an important role in supplying nitrogen for the growth of New Zealand pastures. However, N deficiency is widespread in New Zealand (Field and Ball, 1978; Ball and Field, 1982) and in relatively intensive production systems fertiliser N is used to boost herbage production. With continued use of fertiliser N, clover content in the sward generally decreases (see Chapter 1). In this Chapter, the effects of fertiliser N on white clover population and plant structure are explored as a basis for increasing our understanding of the processes involved in the decrease of clover when fertiliser N is used on mixed grass/clover pastures. This information is particularly important for cattle-grazed pastures, since fertiliser N is mainly used in intensive productions systems, such as dairying.

Populations of higher plant species are usually comprised of many small plants and a fewer number of larger plants (Harper, 1977). The small plants within the population are often more vulnerable to adverse conditions for growth and survival since they do not have the same capacity for resource acquisition and storage that large plants possess, nor the same ability to integrate the effects of localised patchiness in resource availability by transferring resources within the plant (Harper, 1977; Turkington and Klein, 1991). Therefore, when exploring the possible mechanisms for clover reduction in N fertilised, mixed swards, it is important to consider the effects of treatments on the structural organisation of clover plants and populations, and how this may affect the sward composition.

In the 1980's some important research on the dynamics of clover stolons and plants in sheep-grazed swards was conducted (e.g. Brock *et al.*, 1988; Hay *et al.*, 1988). This work confirmed that the clover population in mixed swards consists of many small and a few large individuals. Furthermore, Brock *et al.* (1988) established that the branching structure of plants shows strong seasonal variation, with plant break-up occurring principally in spring. Thus the size distribution of individuals within the population is particularly skewed in spring, a time when the survival of small clover plants in the face of intense competition from associated grasses could be problematic.

Similar detailed information on the morphology of clover plants and populations under cattle grazing was not available at the onset of this experiment. Furthermore, there was virtually no information available on the effects of fertiliser N on clover plant and population structure in grazed swards. Previous work includes the observations that fertiliser N decreased the secondary stolon/primary stolon weight ratio in potted clover plants (Hoglund and Williams, 1984), and decreased growing point density and stolon length and weight per unit area in sheep-grazed grass/clover swards (Caradus *et al.*, 1993). However, there is no systematic basis for predicting the effects of fertiliser N on clover plant and population measures in realistic, grazed situations. In Chapter 3 it has been shown that fertiliser N applied in autumn and winter did not decrease branching, but decreased rooting of nodes in the following spring. Thus the establishment and survival of clover branches could be limited when fertiliser N is applied, and this could have important consequences for population survival in this clonally growing species.

This chapter describes a study of the effects of fertiliser N and cattle grazing management on plant morphology, and its dynamics, based on a census sampling approach using removal of intact turves from the sward to extract whole plants. Season and treatment effects are determined for whole plants and for individual stolon classes. The concept underlying this work is that knowledge of the size of individual ramets increases the understanding of their establishment and fate within the sward, and hence of the response of the whole population to management factors or environmental conditions. Preliminary results from this study were published by Pinxterhuis *et al.* (1993).

4.2 Material and methods

4.2.1 Treatments

Three grazing treatments were applied: continuous stocking with 4.9 bulls/ha (CS4.9), and rotational grazing with either 4.9 bulls/ha (RG4.9) or 7.4 bulls/ha (RG7.4). Two self-contained replicate farmlets were assigned to each grazing treatment. N treatments consisted of no N application (no-N) or application of 50 kg N/ha as urea in autumn and again in winter (plus-N). A detailed description of the treatments and swards is given in Chapter 2.

4.2.2 The quadrat size

A pilot study was undertaken in early September 1990 to determine the optimal quadrat size for sampling the populations in the current experiment. In selecting the quadrat size, the goal was to be able to extract sufficient 'uncut' plants (where no plant part has been severed by the cutting edge of the quadrat as it is pushed into the soil) from each turf to give unbiased estimates of plant size, while keeping the turf size manageable. In general, the average plant size of uncut plants in the quadrat initially increases with increasing quadrat size after which a plateau is reached. This represents the 'realistic' average plant size of the sward sampled.

Clover plants were recovered from the sward using a steel quadrat (5 cm deep) and a spade to remove the turves. These were washed and dissected into individual clover plants and other plant material (Brock *et al.*, 1988). A clover plant is defined as the unit of stolon (i.e. stem), leaf and root material which is physically attached to one another: a physically-distinct individual (Sackville-Hamilton, 1989). Dissection of the turf was done carefully, to avoid breaking stolons.

Four replicate turves each of the sizes 10x10, 15x15, 20x20, 30x30 and 40x40 cm, were randomly taken from pastures under RG4.9, which was expected to have the largest plants of all the treatments in the experiment. The number of cut (by the quadrat edge) and uncut plants was recorded, as well as total plant, leaf and stolon dry weights (DW), and the data subjected to analysis of variance. The leaf and total plant DW was not significantly different between the five quadrat sizes. The stolon DW per uncut plant was significantly lower in the 10x10 cm quadrat than in the 40x40 cm quadrat. The number of uncut plants per turf increased significantly with quadrat size: on average 2.5, 21.8, 38.0, 62.3 and 111.8 uncut plants were found per turf of the five quadrat sizes in increasing order, respectively. From earlier work (Brock *et al.*, 1988, Hay *et al.*, 1988) it was determined that a subsample of at least 20 plants was needed to obtain information on the 4th order plants, the most complex plants usually found in NZ swards and comprising 5-10% of the population in sheep-grazed swards. In the September 1990 pilot study, a quadrat size of 15x15 cm yielded enough uncut plants for this purpose. However, plants are smaller in spring than in other periods of the year (Brock *et al.*, 1988; Hay *et al.*, 1988). This means that the number of uncut plants per turf recorded in this pilot study was probably at a maximum. Thus to increase the probability of obtaining at least 20 uncut plants per quadrat in other seasons, a larger quadrat size was considered necessary. The 40x40 cm quadrat was difficult to handle without breaking turves and thus plants. This was not a problem with the 30x30 cm quadrat size. For these reasons, a quadrat size of 30x30 cm was selected for the census sampling used in this study.

4.2.3 The sampling of plants

Two turves per treatment were taken from swards just prior to grazing. Turf sampling sites were selected randomly, but bare areas and visible dung patches were excluded. Turves were taken every four weeks between September 1990 and April 1991 (before N treatments were applied). Thereafter turves were taken every 7 weeks in winter when growth rates were low, and every 5 weeks in spring, summer and autumn, until December 1992.

Clover plants were retrieved from the turves as described for the pilot study (section 4.2.2). The plants were divided into cut and uncut plants, and the number of plants in both groups was recorded. The cut clover plants (cut by the quadrat edge) were disregarded for further dissections. From the uncut plants, a random subsample of 20 (or all the uncut plants if the total number was equal to or less than 20) was taken for further dissection. The remaining uncut plants and the cut plants were dried in a forced-draft oven at 85 °C for at least 24 hours and the total dry weight (DW) of both plant groups was determined.

4.2.4 Measurements on subsampled white clover plants

The subsampled uncut plants from a single turf were grouped according to their degree of stolon branching (Brock *et al.*, 1988). First, second, third, fourth and fifth plant orders categories were separated. A higher order plant was found only a few times, and 34 fifth order plants were found over the entire experimental period. In all measurements were collected on 3111 first order plants, 3971 second order plants, 1529 third order plants and 270 fourth order plants during the study. For each of these plants, the number of stolons (i.e. number of branches) and their cumulative length, and the number of growing points, nodes, leaves, petioles, rooted nodes and inflorescences were recorded for each stolon order. Each plant was then dissected into stolon, leaf, root and inflorescence material. Within each subsample, stolon and leaf material from each stolon order was bulked for each plant order. All root and inflorescence material of the same plant order was bulked within each subsample. Thus for example, all second order plants of one subsample were dissected into primary stolon, primary leaf, secondary stolon, secondary leaf, root and inflorescence material. Primary leaves are produced by the growing point of the primary stolon, secondary leaves by the growing points of secondary stolons (Beinhart, 1963; Fig. 4-1). All plant material was dried in a forced draft oven at 85°C for at least 24 hours and weighed thereafter.

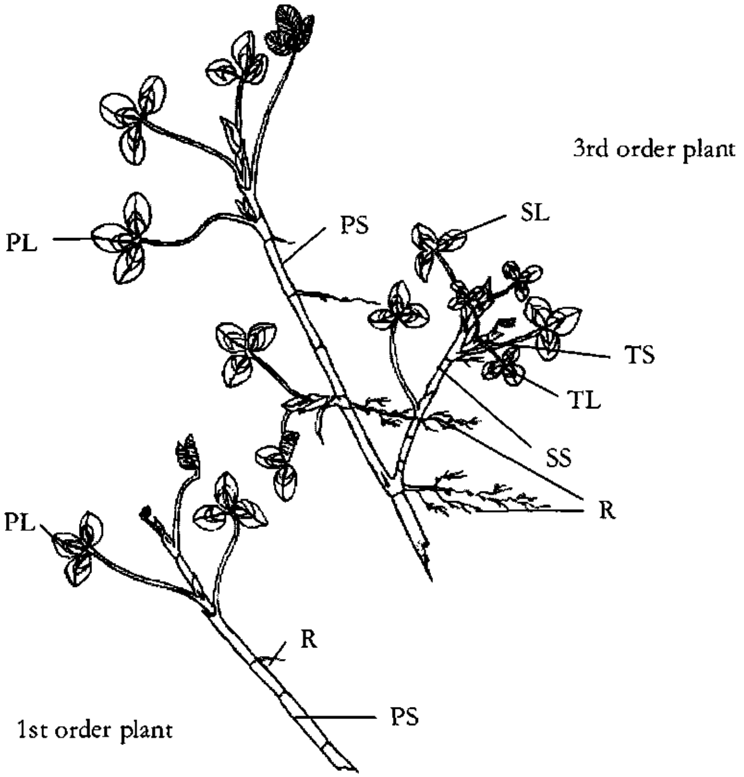


Figure 4-1 Distinguished parts of a white clover plant at dissection: PS = primary stolon, PL = leaf from primary stolon, SS = secondary stolon, SL = leaf from secondary stolon, TS = tertiary stolon, TL = leaf from tertiary stolon, R = root (not shown inflorescence material).

4.2.5 Separating the effects of defoliation, treading and excreta return

The process of grazing includes several dimensions: defoliation, treading, excreta return. The fragmentation of plants by the treading action of cattle hoofs can be expected, while the return of nutrients in excreta could also affect plant parameters. To separate the effects of defoliation from the effects of treading and return of excreta on the size of plants, frames of 2x1 m, allowing cattle to defoliate the enclosed area, but preventing treading and return of excreta were placed on the swards in August 1991. In both replicates of the grazing treatments, a frame was placed on both the plus-N and no-N areas in either of two paddocks. However, under RG4.9 the bulls moved the frames. Thus only results for CS4.9 and RG7.4, a total of 16 frames, could be obtained.

On 30 November 1992 one turf was sampled from each of the frames. At this date the areas underneath the frames had been free from treading and excreta return for 15 months. Plants were extracted from turves, subsampled, and measured using the same procedures described above.

4.2.6 Treatment of data

As the chance of a plant being cut by the quadrat edge is greater for larger plants, the sampling method may be biased against larger plants. Even though an optimal quadrat size was selected (section 4.2.2), all data were weighted using a program developed for this purpose (Brock *et al.*, 1988). The program calculates a weighting factor for a plant: $w = 1 / (1 - p)$, with p being the probability that a plant of that particular size had been cut and excluded from the sample while its centre fell within the quadrat. The p of a particular plant was calculated from the length and width of the plant. To estimate the length and the width from the data collected, four "stolon sizes" were calculated, PS (length of the primary stolon), SS (twice the average length of the secondary stolons or the length of the secondary stolon if only one secondary stolon was present), TS and QS (both calculated as for SS, but using the tertiary and quaternary stolons, respectively). The largest of these values was considered to be the plant length, the next largest the plant width.

All variables were analysed by plant order. The averages per plant were calculated per subsample and plant order for all characteristics. The sum of the weighting factors of the plants which made up this average was used as a weighting factor in the analysis, thus also taking into account the number of plants making up the mean.

4.2.7 Statistical analysis

Each plant order was separately subjected to analysis of variance, using a split plot in time (Snedecor and Cochran, 1980; Steel and Torrie, 1980). The GLM procedure (SAS/STAT, Anonymous, 1990) was used, using the appropriate error strata to test effects of treatments or sampling date. It was assumed correlation between sampling dates would be small enough to include sampling date as a main effect in the analysis. For analysis of the main effect of the grazing treatments, and the differences between year of

Table 4-1 Dates of turf sampling and division in years for the statistical analyses of the main effects of grazing treatments and differences between years of sampling. * = these samplings were not used in the analyses of the main effect of grazing treatments and year differences.

Sampling	Date	Year
01	17 Sep 1990	*
02	22 Oct 1990	*
03	19 Nov 1990	*
04	17 Dec 1990	*
05	14 Jan 1991	1
06	11 Feb 1991	1
07	11 Mar 1991	1
08	08 Apr 1991	1
09	27 May 1991	1
10	15 Jul 1991	1
11	28 Aug 1991	1
12	07 Oct 1991	1
13	13 Nov 1991	1
14	16 Dec 1991	1
15	20 Jan 1992	2
16	24 Feb 1992	2
17	09 Apr 1992	2
18	25 May 1992	2
19	15 Jul 1992	2
20	31 Aug 1992	2
21	05 Oct 1992	2
22	04 Nov 1992	2
23	07 Dec 1992	2

sampling, a model with year and grazing treatments as factors was used. This way, the data was balanced for seasonal effects. For this analysis, the year factor was assigned as shown in Table 4-1. For analysis of main effects of sampling dates, year and grazing treatments, and their interactions, NN-data (no-N data only, see Chapter 2) were used. PN-data were used to test main effects of N treatment and its interactions with grazing treatment or sampling date. For analysis of effects of treading and excreta return, the data from the turves taken underneath the frames and from the turves taken one week later in the corresponding treatments for the repeated samplings were used.

Data were transformed where necessary for homogeneity of variances. The presented values are the least squares means and their standard errors (LSMEANS option of GLM procedure) of the full model, after back transformation where applicable. Where differences were significant, the PDIFF option of the LSMEANS option was used when needed, to establish where the differences occurred.

4.3 Results

4.3.1 Distribution among plant orders

The distribution of clover plants among different plant orders is shown in Fig. 4-2. Second order plants dominated the population at most sampling dates and maintained on average 46% of the population. For this plant order, the main effect of sampling date was not significant. For 1st, 3rd and 4th plant orders, this effect was highly significant ($P<0.001$): a strong seasonal pattern in their distribution occurred. The percentage of 1st order plants peaked in spring, while the percentages of 3rd (especially) and 4th order plants were lowest in spring and peaked in early autumn.

Effects of grazing management or N treatments for the distribution of plants among plant orders, nor interactions between grazing or N treatments and sampling date were found.

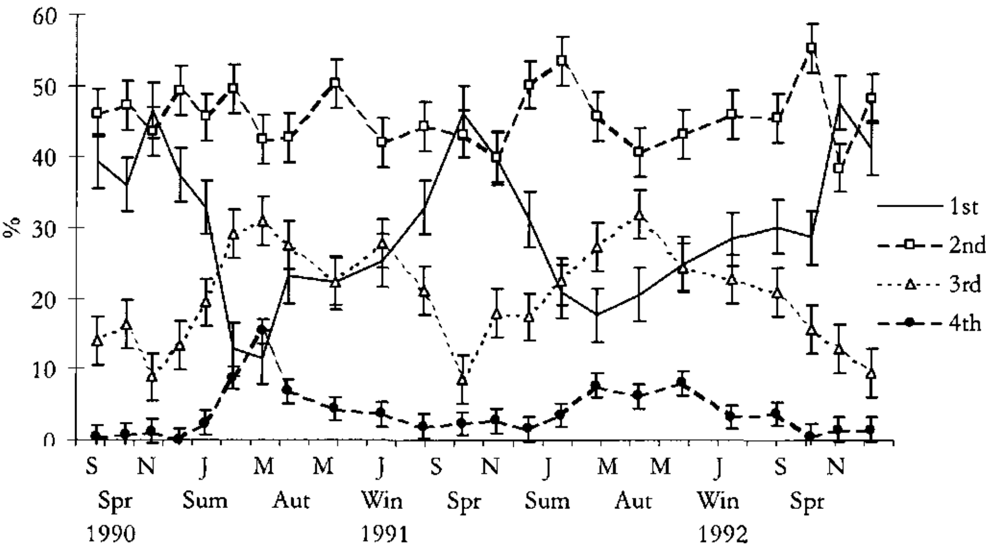


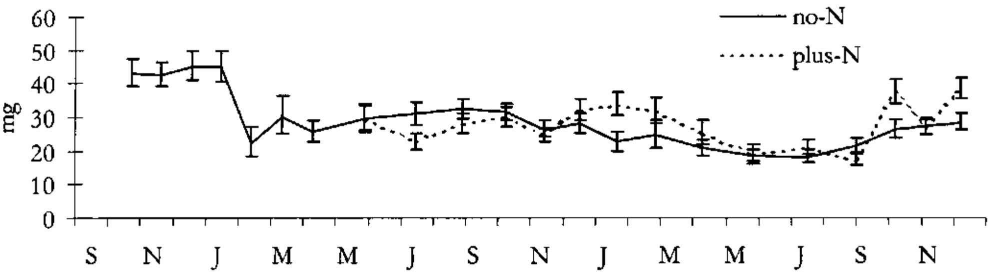
Figure 4-2 Seasonal variation of the distribution of clover plants among plant orders, expressed as percentage of the total plant population. Mean (±SE) of NN-data.

4.3.2 Total plant dry weight and partitioning of dry matter

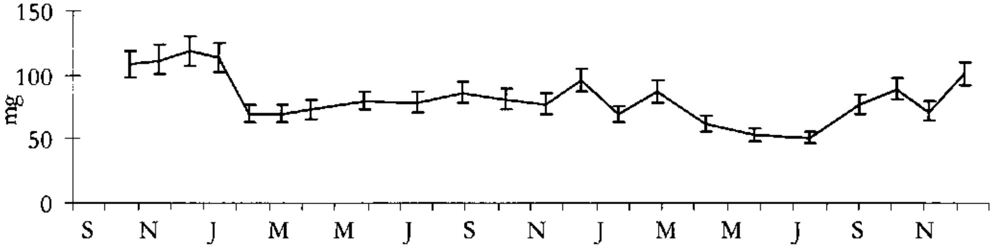
For 1st, 2nd and 3rd plant orders the average total DW per plant varied widely in the experimental period (Fig. 4-3). The main effect of sampling date was highly significant ($P<0.001$). The data set for 4th order plants was too unbalanced to estimate least squares means of each sampling date or of grazing or N treatments. The difference between years was significant for 1st ($P<0.001$), 2nd ($P<0.01$), and 3rd order plants ($P<0.05$), with the average DW being higher in the first year (30, 82 and 168 mg for year 1 and 23, 71 and 146 mg for year 2, respectively). In general total plant DW was higher in early summer and decreased in late summer. The decrease in January 1991 was very large for 1st and 2nd order plants, nearly halving the total plant DW.

The partitioning of plant DW to stolon, leaf and root material is shown in Fig. 4-4. Again seasonality was strong, with the main effect of sampling date being significant at $P<0.001$. The main effect of year was not significant for the partitioning of DW; hence the decrease in average total plant DW in the second year was evenly distributed over stolon, leaf and root material. The allocation of DW was very similar for 1st, 2nd and 3rd order plants, with stolon>leaf>root. The percentage allocated to leaf material was higher in late

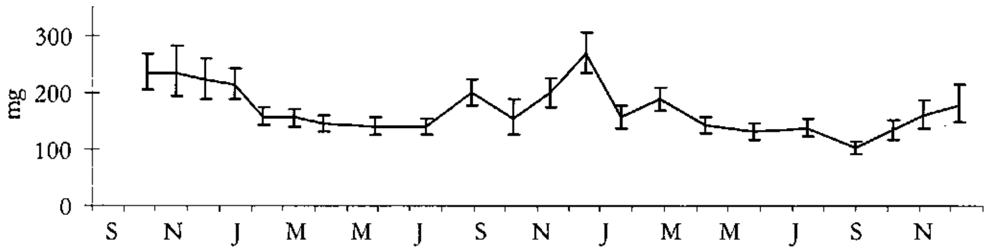
a) First order plant



b) Second order plant



c) Third order plant



d) Fourth order plant

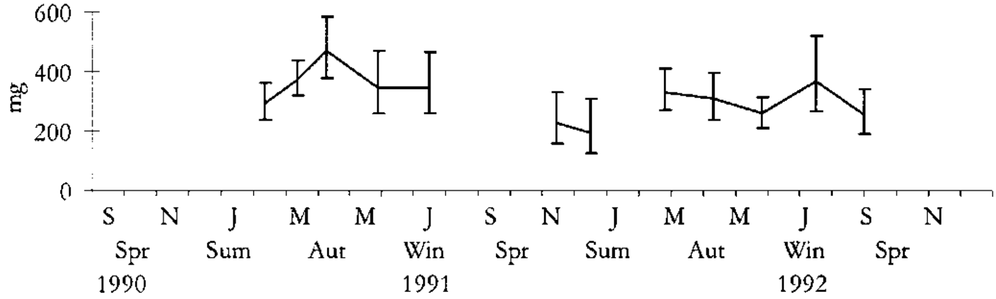
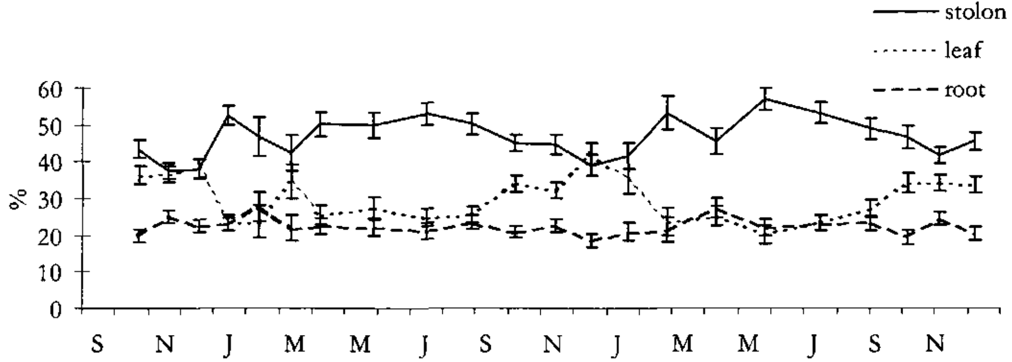
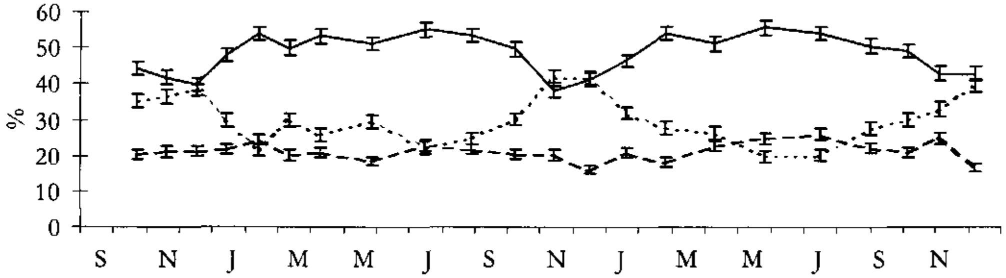


Figure 4-3 Seasonal variation of total plant dry weight (mg) per plant order and effect of N treatment on total dry weight of 1st order plants. Mean (±SE) of NN-data; for 1st order plants plus-N is also shown.

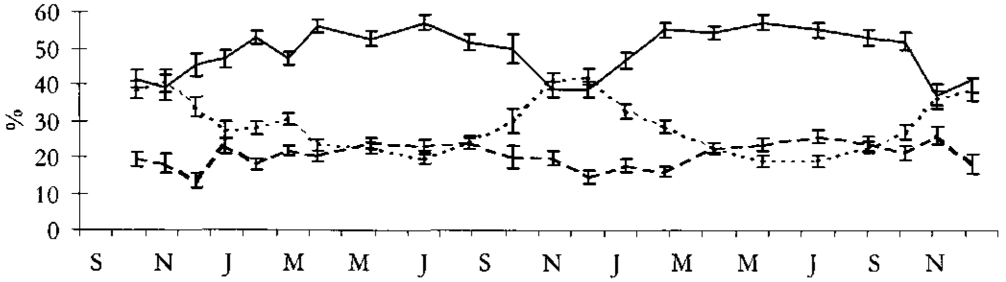
a) First order plant



b) Second order plant



c) Third order plant



d) Fourth order plant

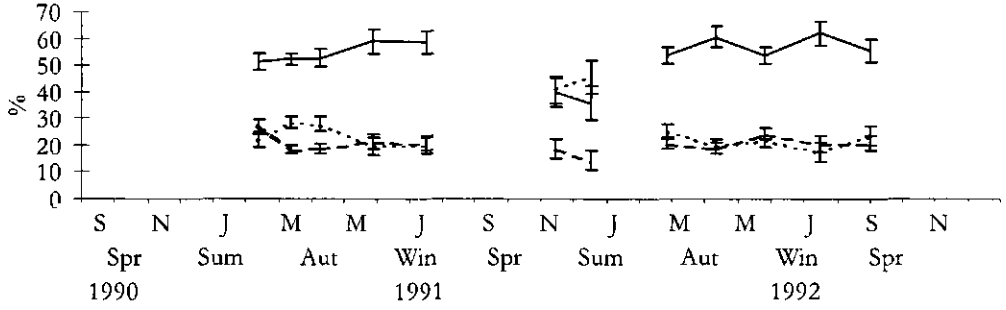


Figure 4-4 Seasonal variation of distribution of plant DW to stolon, leaf and root components per plant order. Mean (±SE) of NN-data.

spring/early summer (40%) and lower in late autumn/early winter (20%) for all plant orders. The partitioning of DW to stolon material showed the reverse and fluctuated between 40 and 55%. The percentage of DW allocated to roots was more stable, fluctuating between 15 and 25%. In winter the percentage allocated to roots was higher, in summer lower.

Table 4-2 Grazing treatment effects on a) average total plant DW (mg) and leaf, stolon and root DW per plant for each plant order, and biomass distribution (as % of total plant DW) to leaf, stolon and root per plant order and b) per stolon order within each plant order (primary stolon being the main stolon, secondary stolon originates from the primary stolon, etc.). Data from December 1990 to December 1992. The significance of differences between the grazing treatments (grazing system: CS4.9 *versus* RG4.9, or stocking rate within RG: RG4.9 *versus* RG7.4) is given by ** (P<0.01), * (P<0.05), and ns (not significant).

a) Total DW (mg/plant) and stolon, leaf and root DW (mg/plant and % of total DW)

Plant order		Total	Stolon		Leaf		Root	
		DW	DW	%	DW	%	DW	%
1st	CS4.9	22.2	11.0	50.7	5.2	24.9	5.2	24.4
	RG4.9	31.2	14.8	48.4	9.3	31.3	6.2	20.2
	RG7.4	26.8	11.4	43.4	9.0	34.0	5.9	22.5
grazing system		ns	ns	ns	*	*	ns	ns
stocking rate		ns	ns	ns	ns	ns	ns	ns
2nd	CS4.9	66.2	34.2	52.4	15.8	24.9	14.4	22.6
	RG4.9	90.6	43.5	48.7	27.3	31.2	17.4	19.7
	RG7.4	76.5	34.7	45.8	24.1	32.5	16.0	21.5
grazing system		*	ns	ns	**	**	ns	ns
stocking rate		ns	ns	ns	ns	ns	ns	ns
3rd	CS4.9	134.3	72.7	55.0	27.5	22.1	29.5	22.6
	RG4.9	187.1	91.2	50.0	51.1	28.8	35.7	20.5
	RG7.4	161.5	74.3	46.6	48.8	31.4	33.5	21.5
grazing system		*	ns	ns	**	*	ns	ns
stocking rate		ns	ns	ns	ns	ns	ns	ns
4th	CS4.9	253.9	138.6	55.5	55.2	23.3	51.6	21.1
	RG4.9	388.8	199.8	52.8	93.4	25.7	78.3	21.0
	RG7.4	340.7	167.5	49.9	92.1	28.3	69.6	21.6
grazing system		ns	ns	ns	ns	ns	ns	ns
stocking rate		ns	ns	ns	ns	ns	ns	ns

Table 4-2 Continued.

b) Percentage of total DW distributed to leaf, stolon and root per stolon order within plant order

		Primary		Secondary		Tertiary		Quaternary	
Plant order		Stolon	Leaf	Stolon	Leaf	Stolon	Leaf	Stolon	Leaf
1st	CS4.9	50.7	24.9						
	RG4.9	48.4	31.3						
	RG7.4	43.4	34.0						
grazing system		ns	*						
stocking rate		ns	ns						
2nd	CS4.9	39.6	11.0	12.8	13.9				
	RG4.9	37.6	16.0	11.0	15.2				
	RG7.4	34.4	15.7	11.5	16.8				
grazing system		ns	**	ns	ns				
stocking rate		ns	ns	ns	ns				
3rd	CS4.9	24.6	2.7	25.8	12.6	4.6	6.8		
	RG4.9	21.1	3.3	23.8	17.3	5.1	8.2		
	RG7.4	19.1	3.2	22.8	19.0	4.7	9.3		
grazing system		ns	ns	*	**	ns	ns		
stocking rate		ns	ns	ns	ns	ns	ns		
4th	CS4.9	13.8	0.4	25.0	5.4	15.3	12.6	1.4	4.8
	RG4.9	14.4	0.2	20.9	6.9	15.2	13.7	2.2	4.8
	RG7.4	9.9	0.4	17.2	4.7	19.6	16.4	3.2	6.9
grazing system		ns	ns	*	ns	ns	ns	ns	ns
stocking rate		ns	ns	*	ns	ns	ns	ns	ns

Grazing treatment affected the total plant DW and its partitioning (Table 4-2). There were no interactions between grazing treatment and sampling date. Plants within plant orders had on average a lower total DW under CS4.9 than under RG4.9, especially due to a lower leaf DW. The percentage of DW allocated to leaf was lower under CS4.9 than RG4.9.

The main N effect was not significant for total plant DW or the allocation of DW to the various plant parts. A few interactions occurred for 1st order plants between sampling date and N treatments. In winter following the first N application, the mean total DW of 1st order plants was lower for plus-N (Fig. 4-3a). In the following summer the mean DW was higher. Effects on 2nd and 3rd order plants were not significant. In the second year, the only difference found was a higher DW for plus-N, again for 1st order plants only, in spring/early summer (October and December 1992). The increased DW was evenly distributed over the plant parts. A significant effect of fertiliser N on DW allocation was found for only one sampling date: for 1st order plants the percentage allocated to leaves was lower for no-N than for plus-N in December 1990 (32.8 and 44.5%, respectively; $P<0.05$ for the interaction between sampling date and N treatment).

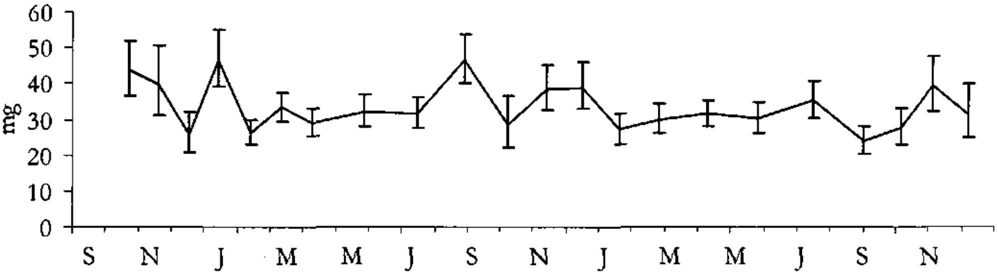
The average biomass allocation within the plants to the different stolon orders is presented in Table 4-2b. Within each plant order, more biomass was allocated to stolon tissue than leaf tissue in older stolon orders, rather than younger stolon orders. For example, the primary stolon of 4th order plants, which is the oldest plant part, consisted almost solely of stolon tissue, while the quaternary stolon (the youngest stolon) had more biomass allocated to leaf than to stolon. The main grazing treatment effect was significant for several variables, as is shown in Table 4-2b. A lower percentage of DW was allocated to leaf under continuous grazing on primary stolon of 1st and 2nd order plants and secondary stolon of 3rd order plants. On the youngest branches the difference was less. The only significant effect of stocking rate under rotational grazing was the lower percentage of DW allocated to secondary stolon material of 4th order plants under the higher stocking rate.

Biomass allocation to the different stolon orders for each plant order also varied widely between the sampling dates. This is shown for 3rd order plants in Fig. 4-5. Increase in leaf DW in spring was apparent for all three stolon orders. Some second and third order interactions occurred between grazing treatment, N treatment and sampling date for biomass allocation within stolon orders. However, these were generally one-off differences that did not occur in both years, nor did they occur for several stolon or plant orders at the same time.

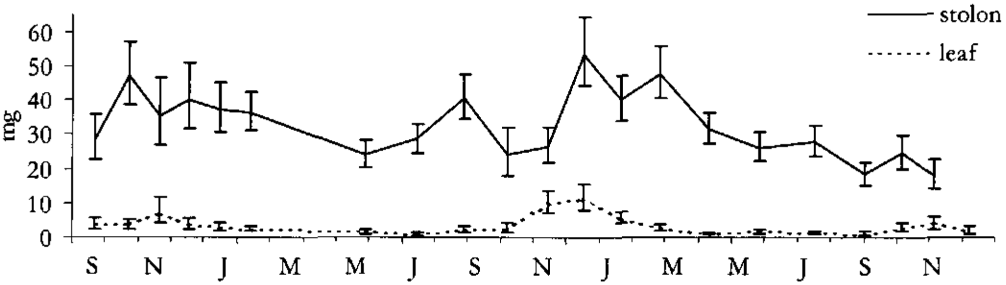
4.3.3 Morphological characteristics

Seasonal variation and interactions with N applications of plant structural characteristics are presented in Fig. 4-6 and Fig. 4-7, for 1st and 3rd order plants, respectively. The main effect of sampling date was highly significant for these plant orders and for 2nd order plants ($P<0.001$), but was not significant for 4th order plants. The various parameters showed similar seasonal trends. Highest values for most variables were found in summer, followed by a decrease. Lowest values were found in spring. For 2nd order

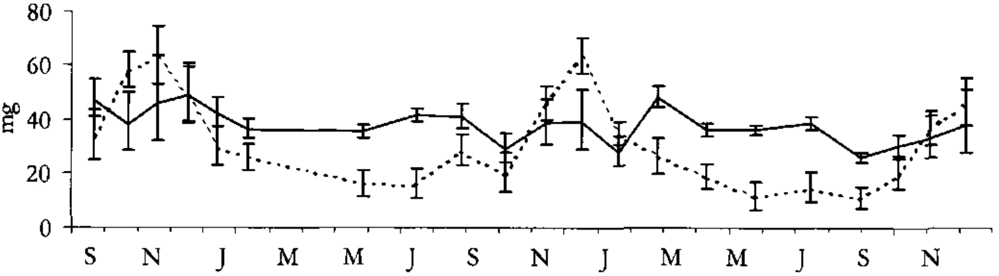
a) Root DW (total per plant)



b) Primary stolon DW



c) Secondary stolon DW



d) Tertiary stolon DW

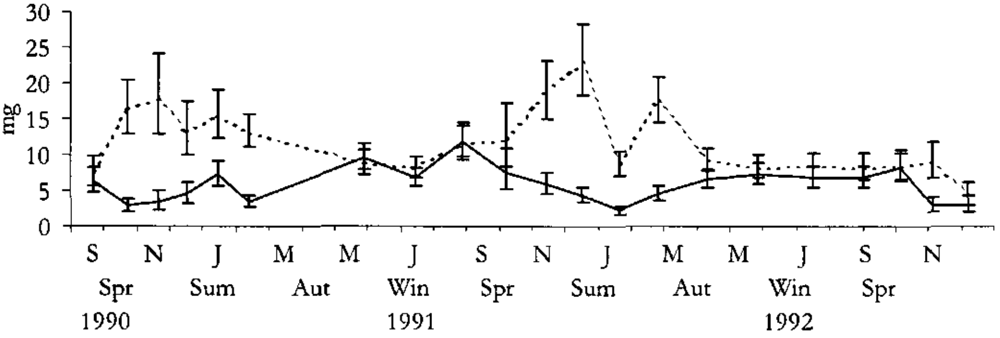


Figure 4-5 Seasonal variation of the distribution of plant dry weight (mg) to root, stolon and leaf components per stolon order for 3rd order plants. Mean (±SE) of NN-data.

plants seasonal trends were similar to those of 3rd order plants, but less pronounced. As with DW, numbers increased by about a factor two for each increase in plant order.

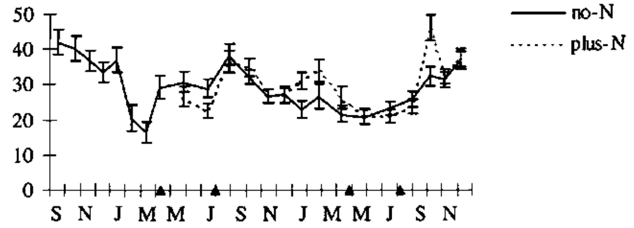
For 1st order plants N application significantly decreased the number of plants with a growing point (main effect $P<0.05$, 0.97 for no-N and 0.95 for plus-N), but this was most apparent in winter/early spring 1992. In November 1992, late spring, more 1st order plants had a growing point for plus-N. The number of leaves per 1st order plant was higher following the first N application. In spring fewer leaves per plant were found for plus-N, reflecting the lower percentage of plants with a growing point. The number of rooted nodes on 1st order plants was higher in summer 1992 (January and February) and lower in September 1992 for plus-N compared to no-N ($P<0.05$).

The number of stolons, growing points, nodes and leaves per 3rd order plant increased significantly following the autumn N application in 1991. In the following spring/early summer these numbers, as well as number of rooted nodes, were lower for plus-N. Flowering increased in summer 1991/1992 in the N fertilised swards ($P<0.001$).

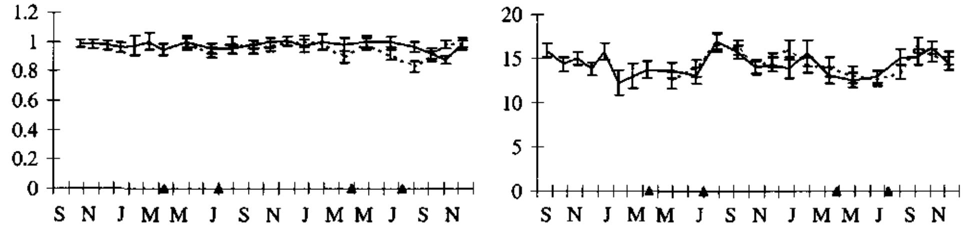
Grazing treatments affected a few parameters, as described in Table 4-3. Under continuous stocking more petioles per plant were found than under rotational grazing. The number of leaves was lower under continuous stocking, but only significantly so for 1st order plants. The stolon length of 2nd order plants was reduced by continuous stocking compared to rotational grazing and by the higher stocking rate under rotational grazing.

4.3.4 Estimation of clover plant density

Clover plant density per unit area can be estimated by two different procedures using the data collected (Table 4-4). The most accurate approach is to use data obtained from the tiller plugs collected to measure sward structural characteristics (Chapter 2), combined with plant data obtained from turf samplings. A second estimate is obtained by dividing total clover dry weight per turf (cut and uncut plants) by average plant DW. Tiller plugs were collected less often (3 times per year) than turves (8-9 times per year), and thus the first approach provides less detail of seasonal variations in estimated plant densities than the second approach.

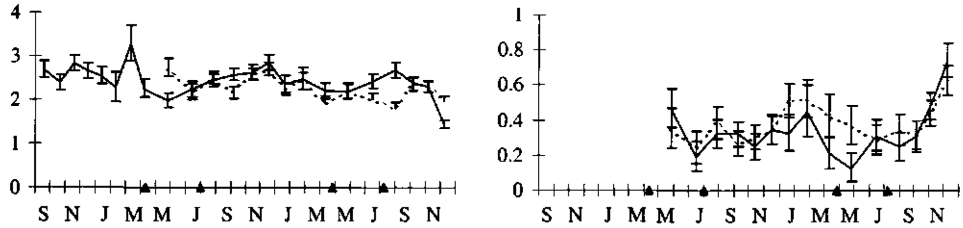


a) Stolon length



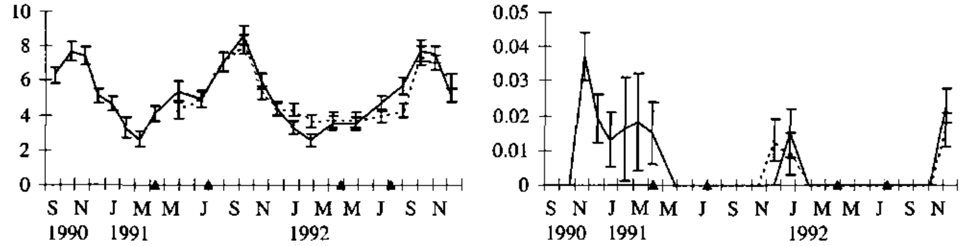
b) Number of growing points

c) Number of nodes



d) Number of leaves

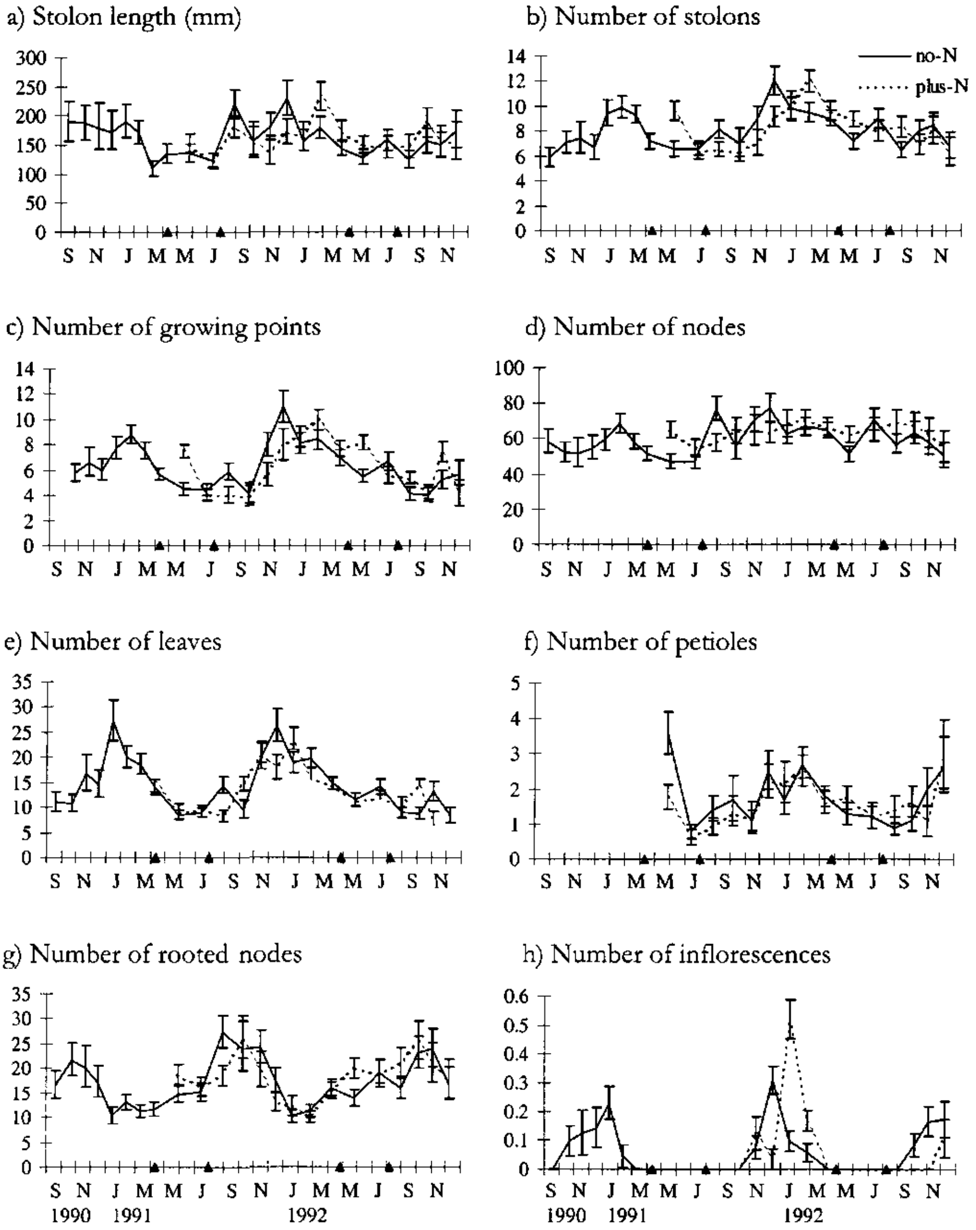
e) Number of petioles



f) Number of rooted nodes

g) Number of inflorescences

Figure 4-6 Seasonal variation of plant characteristics of 1st order plants and the effect of N application. Mean (± SE) of grazing treatments. Triangles show the timing of N application.



a) Stolon length (mm)
b) Number of stolons
c) Number of growing points
d) Number of nodes
e) Number of leaves
f) Number of petioles
g) Number of rooted nodes
h) Number of inflorescences

Figure 4-7 Seasonal variation of plant characteristics of 3rd order plants and the effect of N application. Mean (± SE) of grazing treatments. Triangles show the timing of N application.

Table 4-3 Grazing treatment effects on plant morphological characteristics per plant order. Data from December 1990 to December 1992. The significance of differences between the grazing treatments (grazing system: CS4.9 *versus* RG4.9, or stocking rate within RG: RG4.9 *versus* RG7.4) is given by ~ (P<0.10), * (P<0.05), ** (P<0.01) and ns (not significant).

	1st order	2nd order	3rd order	4th order
Stolon length (mm)				
CS4.9	27.1	75.3	150.3	294.0
RG4.9	32.6	83.5	162.5	330.4
RG7.4	27.2	71.8	153.2	317.0
grazing system	~	*	ns	ns
stocking rate	ns	*	ns	ns
Number of stolons				
CS4.9		3.7	8.0	15.5
RG4.9		3.9	8.8	17.7
RG7.4		3.7	8.5	16.7
grazing system		ns	ns	ns
stocking rate		ns	ns	ns
Number of growing points				
CS4.9	1.0	3.0	6.1	11.4
RG4.9	0.9	3.1	6.6	13.1
RG7.4	1.0	3.0	6.6	12.2
grazing system	ns	ns	ns	ns
stocking rate	~	ns	ns	ns
Number of nodes				
CS4.9	14.4	31.9	60.5	109.2
RG4.9	15.1	32.3	62.1	125.9
RG7.4	14.2	29.5	60.3	110.9
grazing system	ns	ns	ns	ns
stocking rate	ns	ns	ns	ns
Number of leaves				
CS4.9	2.0	6.0	12.1	24.5
RG4.9	2.4	6.8	15.4	29.6
RG7.4	2.5	7.0	17.2	32.3
grazing system	*	ns	ns	ns
stocking rate	ns	ns	ns	ns
Number of petioles				
CS4.9	0.5	1.7	2.7	2.6
RG4.9	0.3	0.7	1.1	2.0
RG7.4	0.2	0.6	1.4	1.5
grazing system	**	*	*	ns
stocking rate	ns	ns	ns	ns
Number of rooted nodes				
CS4.9	5.0	9.0	14.4	21.2
RG4.9	5.5	9.2	15.7	29.9
RG7.4	5.4	8.9	15.8	25.8
grazing system	ns	ns	~	ns
stocking rate	ns	ns	ns	ns

Table 4-4 Estimates of clover plant density derived from whole-sward and individual plant variables measured with tiller plugs ((1), (2) and (3)) and turves ((4) - (8)). Mean of grazing and N treatments.

Variable	Sampling date					
Whole-sward level	March 1991	July 1991	October 1991	February 1992	June 1992	September 1992
1 Growing points /m^2	4030	3070	2500	6840	5090	3130
2 Stolon length (m) /m^2	96	95	72	167	134	105
3 Stolon dry weight (g) /m^2	52	56	35	107	82	54
4 Total clover dry weight (g) /m^2	117	142	110	150	134	135
Individual-plant level						
5 Growing points/plant	6.3	2.9	3.8	6.2	3.9	2.6
6 Stolon length (m)/plant	0.137	0.091	0.085	0.140	0.089	0.112
7 Stolon dry weight (g)/plant	0.071	0.059	0.035	0.072	0.040	0.043
8 Total dry weight (g)/plant	0.147	0.103	0.089	0.132	0.076	0.087
Plant density (no./m^2) based on						
9 Growing point density (1/5)	640	1060	660	1100	1310	1200
10 Stolon length (2/6)	700	1040	850	1190	1510	940
11 Stolon dry weight (3/7)	730	950	1000	1490	2050	1260
12 Total clover dry weight (4/8)	800	1380	1240	1140	1760	1550

In general higher estimates of plant densities were obtained using total clover biomass per turf compared to using data from tiller plugs. The apparent overestimation of plant density resulting from use of total clover biomass in turves is probably a result of the turf sampling procedure. Bare patches or visible dung and urine patches were avoided when turves were taken, but were not avoided when tiller plugs were removed. Thus tiller plugs give a truer estimate of plant density on a whole sward basis, incorporating all within sward spatial heterogeneity.

The seasonal differences were similar, so Fig. 4-8 can be seen as a fair picture of fluctuations in clover plant density. Highest estimated plant densities occurred in winter/early spring and lowest densities in late summer/early autumn. Considerable variation between years is evident.

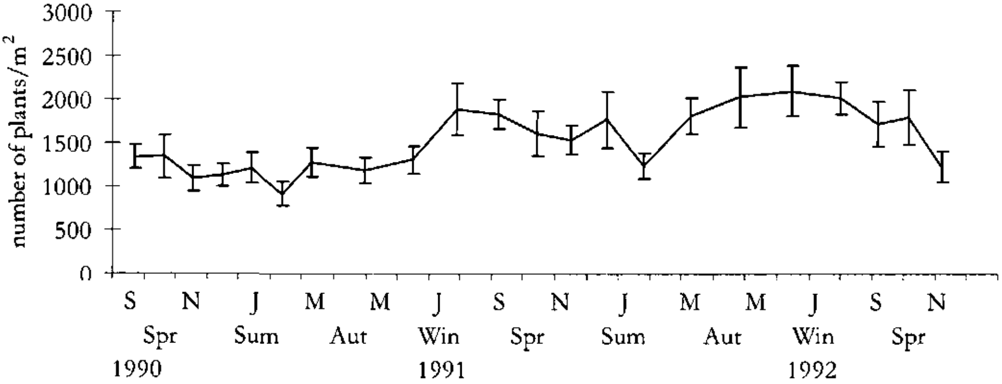


Figure 4-8 Seasonal variation of estimated density of clover plants. Mean (± SE) of grazing and nitrogen treatments.

4.3.5 Effects of treading and excreta return on plant structure

The proportion of the population constituted by 1st order plants was lower, and by 3rd order plants was higher, inside compared to outside the frames (Table 4-5). Total plant DW of 1st order plants was greater outside the frames. Third order plants showed the opposite effect and had greater plant DW inside the frames. The distribution of DW to stolon, leaf and root material was not affected for 1st and 3rd order plants. For 1st order plants, stolon length per plant was significantly less inside the frames (32.9 outside and 22.8 mm inside the frames; $P<0.05$), and fewer nodes had rooted (5.0 outside and 3.8 rooted nodes per plant inside the frames; $P<0.05$). For 3rd order plants stolon length was greater inside the frames (271 compared to 147.2 mm outside the frames; P<0.05).

Significant differences were observed for DW distribution between stolon orders for 2nd order plants (Table 4-6). Inside the frames more DW was allocated to the branches. This was not due to more branches, but the branches were bigger (Table 4-6). There were no N or grazing treatment interactions with frames.

Table 4-5 Effects of excluding a sward from treading and excreta return by a frame, on distribution of plants among plant orders and plant DW (mg) in spring. Mean of grazing and N treatments, per plant order. The significance of differences between the treatments is given by * (P<0.05), and ns (not significant).

Plant order	Treatment	Total plant dry weight	Percentage of population
First	no frame	27.4	40.3
	plus frame	17.3	35.8
		*	*
Second	no frame	86.0	47.6
	plus frame	79.1	44.1
		ns	ns
Third	no frame	143.7	9.8
	plus frame	261.4	17.7
		*	*

Table 4-6 Effects of excluding a sward from treading and excreta return by a frame, on distribution of DW as percentage of total plant DW and morphological characteristics of 2nd order plants. Mean of grazing and N treatments. The significance of differences between the treatments is given by *** (P<0.001), ** (P<0.01), * (P<0.05), and ns (not significant).

Treatment	Primary stolon	Primary leaf	Secondary stolon	Secondary leaf	Roots
no frame	32.5	26.4	6.9	13.7	19.2
plus frame	27.4	16.6	12.1	25.4	18.1
	ns	**	*	***	ns
Primary stolon	Stolon length	Growing points	Nodes	Leaves	Roots
no frame	68.6	0.9	18.4	2.1	7.5
plus frame	57.9	0.7	16.7	1.1	5.5
	ns	*	ns	***	ns
Secondary stolon	Stolon length	Growing points	Nodes	Leaves	Roots
no frame	23.7	2.4	11.5	3.0	1.7
plus frame	37.2	2.5	17.2	4.1	3.6
	*	ns	ns	ns	**

4.4 Discussion

4.4.1 Seasonality of plant characteristics

The population structure showed a strong seasonality. In sheep-grazed swards at a site near Palmerston North in the Manawatu (Brock *et al.*, 1988; Hay *et al.*, 1988) and in swards grazed by dairy cattle in the Waikato (Harris, 1994) a very rapid increase in the proportion of 1st order plants in spring (from September to October) was noted, with a concomitant decrease in 3rd order plants. This pattern was also noted in the present study, but here the shift to a less-branched plant structure started in autumn and continued steadily through winter. The proportion of 1st order plants was in spring greater under cattle grazing than under sheep grazing.

The proportion of DW distributed to stolon decreased in spring for all plant orders, per plant and per stolon order. As is shown for 3rd order plants, this was partly due to an increase in leaf DW for each stolon order, but also stolon DW decreased. It appears that in spring senescence of older stolon is faster than the production of new stolon material. Hence, the total stolon mass of the population is not maintained, as shown by the decrease in spring of clover stolon DW/m^2 in Chapter 2. Later in the growing season, new growth exceeded senescence, so the amount of DW distributed to stolon increased from early spring on, following an increase in leaf material. The great decrease in plant DW from January to February 1991 (Fig. 4-3) was not noticeable in clover stolon DW/m^2 in Chapter 2. The number of plants had not increased in this period; a decrease was even noticeable (Fig. 4-8). The only explanation of this is a rapid shift in the population structure to plants of a higher branching order. Indeed in Fig. 4-2 a steep decrease of percentage of plants of 1st order was recorded, with concomitant increase of percentage of plants of 3rd and 4th order. The number of growing points per plant within each plant order did not increase; appearance rate of branches was not especially high. Apparently the senescence of old stolon material was particularly slow in this period. This process was not recorded from the marked stolons (Chapter 3), and was also not obvious from the DW allocated to primary stolon material of 3rd order plants (Fig. 4-5). The variation in DW allocation to different plant parts of 4th order plants was too large, and the number of plants of this order in January 1991 was too small, to draw conclusions for this plant order.

There are very few published data on clover root DW in grazed swards. With the methods used in the present experiment, not all the root material would have been recovered. The turves were 5 cm deep; thus only roots in the top 5 cm were measured. Washing of the turves will have resulted in the loss of root hairs and a proportion of the finer roots. But since all turves were treated similarly, comparison of results between samplings and management systems is still valid. Root DW was approximately 20% of total plant DW, the same proportion as Young (1958) found per unit area in swards in Britain and Harris (1994) found per plant in Waikato dairy pastures.

The number of rooted nodes per plant increased from autumn to spring (Fig. 4-6 and 4-7). This indicates root formation occurred in this period. Root DW did not show this pattern, but was relatively stable (Fig. 4-4 and 4-5), thus growth of the developed roots was slow. In spring the number of roots was at a maximum, but many roots were small, considering the total root DW. The survival of these roots was not high, the number of rooted nodes was at a minimum at the end of summer. The surviving roots increased in size and were relatively large at the end of summer, with maximum values for root DW per rooted node.

4.4.2 Treatment effects

Several previous studies have found that clover plants are smaller under continuous stocking compared to rotational grazing (Carlson, 1966a; Briseño de la Hoz and Wilman, 1981; Wilman and Asiegbu, 1982; Brock *et al.*, 1988; Hay *et al.*, 1988). This was also apparent in the present experiment. A lower proportion of total plant DW was allocated to leaf under CS. Both the proportion allocated to stolon and to root tended to be higher under CS, but this was not statistically significant. Brougham *et al.* (1978) and Chapman and Robson (1988) observed earlier that clover invested a higher proportion of its photosynthates in stolon material when continuously stocked. However, in the current experiment part of the explanation of the difference between grazing treatments is that clover plants were sampled just prior to grazing in the RG treatments. Hence the regrowth period was maximal under this grazing management, while this was not the case under CS where grazing could have occurred just prior to sampling. This probably also caused the higher number of petioles and lower number of leaves per plant and per growing point under CS than under RG.

The application of fertiliser N did not affect the distribution of plants among plant orders, average total plant DW or DW allocation to stolon, leaf and root and plant morphological characteristics within plant orders. Similarly, Harris and Clark (1996) and Harris *et al.* (1996) did not record differences between non-fertilised dairy pastures and pastures fertilised with 200 kg N/ha/year, apart from a lower average root DW per plant for the whole population when 200 kg N was applied.

In the present experiment, N application in autumn at first increased the number of nodes, growing points and leaves per 3rd order plant. The most obvious explanation for this would be increased branching rates with N application in autumn, however this effect was not found in the marked stolon study (Chapter 3). The proportion of plants of higher branching order did not increase following N application either. This can be explained by a higher branching rate of secondary stolons. This was not analysed for marked stolons (Chapter 3), but a higher number of tertiary growing points would confirm this hypothesis. In Fig. 4-9 these numbers are shown. While in the statistical analyses the main N effect and the interaction between N and sampling date were not significant, on average more than one extra growing point was found on tertiary stolons in the sample taken after the autumn N application in both years. Their survival rate was not high, since the number decreased again to no-N levels in the following samplings.

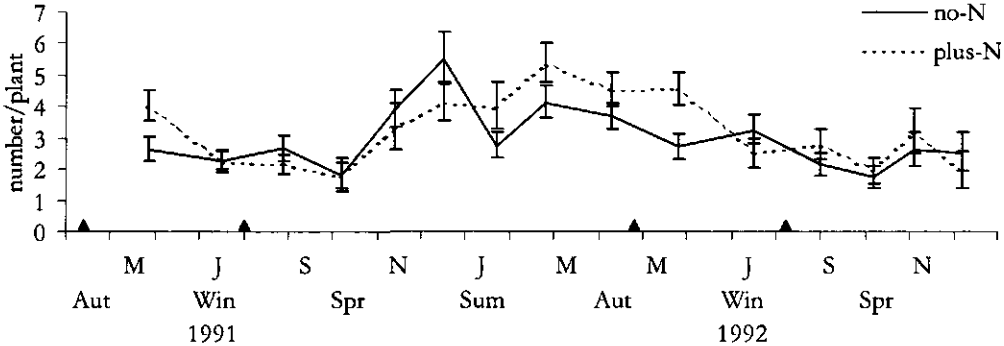


Figure 4-9 Number of growing points on tertiary stolon of 3rd order plants. Mean (± SE) of grazing treatments.

This increased branching, with low branch survival rate, results in a lower number of potential branching sites later, for example in winter or the following spring. A node never has a second chance to develop a branch.

4.4.3 Cattle *versus* sheep

No direct comparisons between cattle and sheep grazing were conducted in this study, but the availability of similar information on clover growth plant and population structure from sheep-grazed pastures located in the same region (Brock *et al.*, 1988; Hay *et al.*, 1989a, b, 1991) allows some broad conclusions to be drawn about the effects of sheep and cattle grazing on the environment for clover growth in a mixed pasture.

Average clover growing point densities recorded in this study (3,900/m^2) were higher than those reported by Brock *et al.* (1988) (2,100-3,250/m^2). This was despite the fact that grass tiller densities were also higher in the present study (average 13,800/m^2, compared to an average of 11,900/m^2 in the sheep-grazing treatments monitored by Brock *et al.*). This goes against the trend established by Brereton *et al.* (1985) toward lower clover growing point density as grass tiller density increases above about 5,000 per m^2 and suggests that cattle grazing may create a more favourable environment for clover to coexist with grasses than sheep grazing. One possible explanation for this is that cattle may select clover less selectively than sheep, (Briseño de la Hoz and Wilman, 1981; Cosgrove *et al.*, 1996), and do not have the jaw and biting characteristics to select within intermingled grass/clover patches as well as sheep.

The estimated density of clover plants in the present experiment was much higher than values for pastures grazed by sheep: 640-1310 plants/m^2 derived from data from tiller plugs and turves, compared to 200-800 plants/m^2 (Hay *et al.*, 1989b). This is due to higher growing point densities, and a higher proportion of 1st order plants in the present, cattle-grazed pastures in comparison to sheep-grazed pastures (Brock *et al.*, 1988; Hay *et al.*, 1988, 1989a). The number of stolons and growing points per plant within each plant order was less under cattle grazing in the present experiment than under sheep grazing in earlier experiments (Brock *et al.*, 1988). So not only was a larger proportion of the plant population in the present cattle-grazed pastures of simpler plant structure, within the branched plants the structure was simpler as well.

More severe treading damage inflicted on swards by grazing cattle, or differences in pattern of excreta return, may provide the explanation for this difference in population and plant structure between sheep- and cattle-grazed swards and its seasonal variation. Support for this hypothesis comes from the results of the study where treading damage and excreta return were excluded by frames. Here, a higher percentage of 3rd order plants, and a concurrent lower percentage of 1st order plants, were recovered for swards under the frames, in comparison to the sward outside the frames. Plants under the frames were sampled in late spring, when in general higher proportions of 1st order plants were found, and lower proportions of 3rd order plants (Brock *et al.*, 1988; Hay *et al.*, 1988; this chapter). The results from the study with the frames indicate that treading and/or excreta return contribute continually to the breaking up of plants in swards, and that this effect is stronger under cattle grazing compared to sheep grazing.

This difference in population and plant structure could make the clover population in cattle-grazed swards more vulnerable to adverse conditions such as a summer drought than under sheep grazing. Plants with a more complex branching structure have a greater capacity to buffer stress conditions by integrating the use of growth resources across the whole plant, and through greater ability to store reserves of energy and N (Chapman and Robson, 1992; Chapman *et al.*, 1992b).

In comparison to clover plants in sheep-grazed pastures (Brock *et al.* 1988; Hay *et al.*, 1991), within each plant order (except for 4th order plants), stolon length per plant was greater under cattle grazing, while there were fewer nodes per plant. This implies greater internode length under cattle grazing, as was found in work by Briseño de la Hoz and Wilman (1981). Also, more rooted nodes per plant, as well as more leaves per growing point were found under cattle grazing. A lower grazing frequency in the present experiment could explain this, possibly caused by higher herbage accumulation levels in the present experiment and lower stocking rates and partly by sheep grazing clover more selectively. A discrepancy in this respect is the lower DW of leaf material and higher number of leaves per plant in the present experiment. This implies lower DW per leaf in comparison to the plants in the sheep-grazed pastures used by Brock *et al.* (1988). In turn, this may be due to the relatively high proportion of small-leafed, acyanogenic genotypes in this pasture (Williams and Cornegé, 1979, Chapter 2), which may have derived from the Kent wild white clover ecotype introduced into New Zealand pastures in the early stages of agricultural development. Leaf size is generally found to be greater under cattle grazing than sheep grazing (Briseño de la Hoz and Wilman, 1981) when comparable clover varieties are used.

4.4.4 Conclusions

In the population of clover in these cattle-grazed swards, 2nd and 3rd order plants were the most important contributors. These plant orders provided the majority of growing points and rooted nodes of the population. It is also in these plant orders where a capacity for intra-plant integration in the use of growth resources (carbon, nutrients and water) can be expected: branches can support the main stolon and each other when conditions are adverse (Chapman *et al.*, 1992b), while old stolon material is not maintained for a prolonged time.

Grazing treatments and N applications did not alter the distribution of plants among plant orders, while seasonal fluctuations were large. N application resulted in temporary differences for plant DW, distribution of DW and morphology only. Interactions between grazing and N treatments were few and also temporary, thus the scope for influencing clover plant structure and morphology by adapting grazing management to improve clover persistence when using fertiliser N appears to be limited.

Chapter 5

DYNAMICS OF CLOVER IN RELATION TO CLIMATE

5. DYNAMICS OF CLOVER IN RELATION TO CLIMATE

5.1 Introduction

The dynamics of clover leaf and stolon growth, in response to season, grazing management and N application are described in Chapter 3. Seasonal variation was evident for nearly all clover growth components measured, and was often large. In this Chapter, the specific climate variables related to seasonal variation in growth are identified, and their relative importance compared to management or treatment effects is explored. A simple, conceptual growth model has been created to establish the relationship between the weather pattern in the various seasons and plant growth, and to relate the magnitude of these relationships to the magnitude of management and N treatment effects. Differences between the rotation periods are used to determine the relative importance of the various climate variables for measured variables of clover growth. The result is a synthesis from which comparisons can be made with similar studies conducted elsewhere, and from which general conclusions can be drawn regarding the opportunities for management manipulation of clover growth in the environment in which this study was conducted. The latter aspect (opportunities for management manipulation) will be developed more fully in Chapter 6.

Many previous publications have reported strong seasonal variation in clover growth (see the previous chapters), but few of these have related clover growth dynamics in a grass/clover sward to climate factors *per se*. Those that have done so concentrated mainly on rate of leaf appearance, which is also the birth rate of new nodes (for example Beinhart, 1963; Davies and Evans, 1982). Other work in controlled environments has determined the effect of single or multiple non-compounded environmental factors on clover behaviour, as for example temperature (Mitchell, 1956; Mitchell and Lucanus, 1962; Boller and Nösberger, 1983; Hoglund and Williams, 1984), light intensity (Beinhart, 1963; Solangaarachchi and Harper, 1987), light duration (Mitchell and Lucanus, 1962; Boller and Nösberger, 1983) or spectral quality of light (Solangaarachchi and Harper, 1987; Thompson and Harper, 1988).

Only one series of papers deals in detail with component growth responses to climate in a grazed grass/clover sward (Sackville-Hamilton and Harper, 1989; Sackville-Hamilton, 1990). Elgersma and Fengrui (1997) presented relationships between clover growth components and climate factors using data collected from mown swards in the Netherlands.

Sackville-Hamilton and Harper (1989) demonstrated that the quantitative response relationships obtained for specific growth components in the field differ markedly from those obtained in controlled environments. They noted that correlated variables (for example temperature and the intensity, duration and spectral quality of light) occurring in the field, but not in controlled conditions, are likely to explain this discrepancy. They highlighted the need for more information from field environments closer to farming practice to increase our knowledge of clover responses to environment. This Chapter will draw comparisons with Sackville-Hamilton and Harper's (1989) and Sackville-Hamilton's (1990) results, and extend their findings obtained from a single year in one paddock under common management by using results collected in two years and considering the effects of grazing management and fertiliser N applications.

5.2 Material and methods

5.2.1 Treatments

Three grazing treatments were applied: continuous stocking with 4.9 bulls/ha (CS4.9) and rotational grazing with either 4.9 (RG4.9) or 7.4 (RG7.4) bulls/ha. Each grazing treatment was assigned to two self-contained replicate farmlets. Within the farmlets, N treatments were no fertiliser N (no-N) and 50 kg N/ha as urea in autumn and again in winter (plus-N). More detailed information on the swards and treatments is given in Chapter 2.

5.2.2 Data

The data used are means for clover growth variables of each rotation period (Table 3-1) during the course of the experiment as described in Chapter 3. These variables are listed in Table 5-1. The climate data are shown in Fig. 5-1. Air (AIR, °C) and soil temperature at 10 cm depth (SOIL, °C), both expressed as mean of the maximum and minimum temperatures, total rainfall (RMM, mm), the coefficient of variation of rainfall (RCV, %) and the

a) Total rainfall per rotation period (RMM, mm) and coefficient of variation of rainfall (RCV, %)

b) Soil temperature at 10 cm depth (SOIL, °C) and air temperature (AIR, °C), both as mean of the maximum and minimum temperatures, and the mean radiation (RAD, MJ/m²/day) per rotation period.

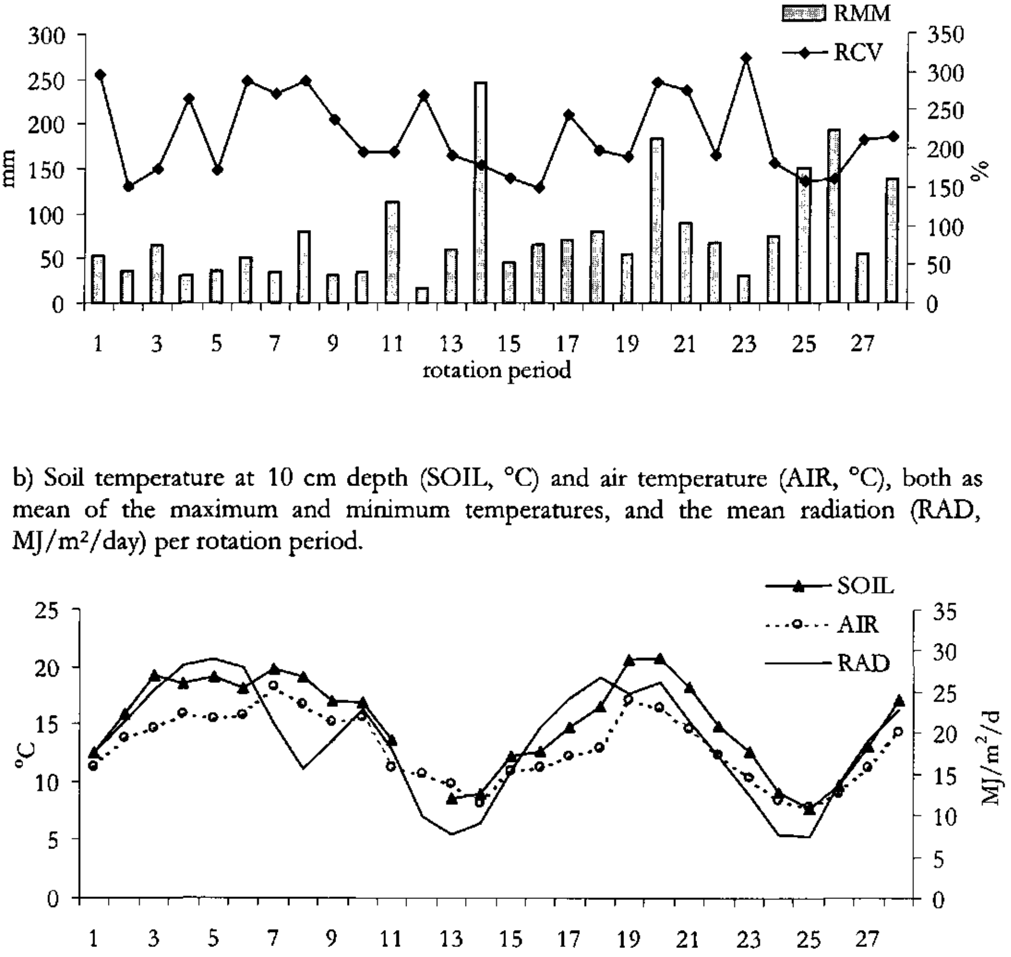


Figure 5-1 Climate data per rotation period during the course of the experiment.

mean radiation receipt (RAD, MJ/m²/day) were calculated per rotation period. The RCV was used to account for the distribution of the rainfall within a rotation period. For one rotation period, between 1 May and 20 May 1991, the maximum soil temperatures were not recorded every day. Hence this rotation period is missing in the data set.

Table 5-1 List of clover growth variables used in the analyses.

• Stolon elongation rate (cm/week)	SER
• Rate of leaf appearance (number per stolon/week)	RLA
• Leaf senescence rate (number per stolon/week)	LSR
• Leaf removal rate (number per stolon/week)	LRR
• Axillary bud appearance rate (number per stolon/week)	AAR
• Axillary bud senescence rate (number per stolon/week)	ASR
• Branch appearance rate (number per stolon/week)	BAR
• Branch senescence rate (number per stolon/week)	BSR
• Rooting rate of nodes (number per stolon/week)	RR
• Root senescence rate (number per stolon/week)	RSR
• Rate of loss of main stolons (due to senescence, grazing, dung, trampling or burial)	LOSS

5.2.3 Analysis

First, principal component analysis (PCP, Genstat 5 Committee, 1993) was performed on the correlation matrix (Table 5-2; Manly, 1986). This analysis was used to determine if clover growth could be described simply by a few components derived from the original clover growth variables (Table 5-1), since correlation between these variables was apparent (Table 5-2). A draw back of principal components is that the second and following components become increasingly difficult to interpret. Because of this, the individual variables were used for further analysis.

Second, a straightforward (multiple) linear regression of clover growth variables (expressed as means per rotation period and ignoring treatment effects and within treatment variation) against climate variables was performed to give simple models of clover growth. The RSELECT procedure of Genstat (Genstat 5 Committee, 1993; Goedhart, 1998) was used to find the model of best fit, defined as the model which explained the highest percentage of residual variation (adjusted for the number of parameters in the model, maximum R^2_{adj}) when only significant variables were included. Data transformations did not improve homogeneity of variance. Within the range of rainfall and temperatures of the current experiment, non-linear analyses, such as logistic or polynomial, did not explain any more of the variation than the linear model. Hence only linear models using untransformed data are presented. This does imply the models are valid only for the range of rainfall

Table 5-2 Correlation coefficients for clover growth variables (Table 5-1) and climate variables.

RLA	0.76														
LSR	0.10	0.22													
LRR	0.47	0.62	0.23												
AAR	0.61	0.56	0.04	0.56											
ASR	0.16	0.27	0.09	0.46	0.40										
BAR	0.69	0.72	0.17	0.61	0.70	0.30									
BSR	0.26	0.39	0.25	0.57	0.31	0.54	0.43								
RR	0.24	0.22	0.20	0.30	0.29	0.35	0.29	0.28							
RSR	0.26	0.22	0.13	0.56	0.45	0.55	0.38	0.52	0.56						
LOSS	0.14	0.29	0.05	0.23	0.03	0.01	0.18	0.20	–0.14	–0.05					
RMM	-0.32	-0.44	-0.05	-0.26	-0.35	-0.28	-0.21	-0.23	-0.17	-0.21	-0.02				
RCV	0.24	0.38	0.06	0.02	0.13	-0.05	0.19	0.07	-0.09	-0.09	0.32	-0.19			
AIR	0.54	0.82	0.33	0.62	0.44	0.41	0.57	0.51	0.05	0.20	0.56	-0.41	0.42		
SOIL	0.58	0.84	0.34	0.63	0.44	0.42	0.65	0.56	0.12	0.24	0.54	-0.34	0.41	0.97	
RAD	0.60	0.75	0.36	0.55	0.45	0.34	0.65	0.53	0.04	0.27	0.37	-0.33	0.21	0.77	0.84
	SER	RLA	LSR	LRR	AAR	ASR	BAR	BSR	RR	RSR	LOSS	RMM	RCV	AIR	SOIL

and temperatures encountered in the present experiment, extrapolation to more extreme climate conditions is not possible.

The linear relationships between rate of leaf appearance and other appearance rates were considered as well, because of the relatively large correlation between rate of leaf appearance and other growth variables. Also the relationships between senescence and appropriate appearance rates were determined.

Next, the Residual Maximum Likelihood (REML) procedure of Genstat 5 Committee (1993) was used to fit models including the grazing and N treatment effects and interactions between treatments and climate variables. Data used were means per rotation period and replicate of each treatment. With REML, random variance can be subdivided into different components. Apart from the variance unaccounted for by the fixed terms in the models, it gives an estimate of the unexplained variation between, amongst other variance components, the rotation periods. The VWALD procedure in Genstat (Genstat 5 Committee, 1993; Goedhart *et al.*, 1998) was used to determine which variables in the fixed model were significant. Only significant variables were included in the fixed models.

5.3 Results

5.3.1 Principal component analysis

A total of seven components was required to account for more than 90% of the variation in the clover growth variables as listed in Table 5-1. The first component (Z_1) explained 47 % of the variation, the second component (Z_2) 15%. These components were as follows:

$$Z_1=-0.33SER-0.36RLA-0.14LSR-0.38LRR-0.35AAR-0.28ASR-0.38BAR-0.31BSR-0.23RR-0.32RSR$$

$$Z_2=-0.44SER-0.37RLA+0.16LSR+0.04LRR-0.20AAR+0.40ASR-0.28BAR+0.29BSR+0.34RR+0.42RSR$$

The first component is basically an index of development, with stolon elongation, appearance and senescence rates all having negative coefficients. The second component shows a contrast between stolon elongation and appearance rates of leaves, axillary buds and branches on the one hand and rooting rate of nodes and senescence rates of roots, axillary buds, branches and leaves on the other.

5.3.2 Comparison of model type

As mentioned in section 5.2.3, non-linear analyses did not improve upon the results of a linear model. As an example, in this paragraph the results for rate of leaf appearance (RLA) are given. Sackville-Hamilton and Harper (1989) used a third order polynomial to describe the relationship between soil temperature (SOIL) and RLA. Using data from the present experiment, adding the squared and cubed soil temperature did not significantly improve the model. This is shown in Table 5-3, where the means per rotation period were used. Also for the second data set (means per rotation period and treatment) adding squared and cubed soil temperature did not improve the model, as tested with the Wald statistic in the REML procedure (Genstat 5 Committee, 1993).

Fitting other non-linear curves did not improve the model either. In Fig. 5-2 the resulting curves for a linear and a logistic relationship between RLA and SOIL are shown. It is obvious that the temperature was neither low enough nor high enough to fall much outside the linear phase of the curve for RLA. The percentage variation accounted for by using the different models, using the means per rotation period, was 68% (cubic, logistic and Gompertz), 69% (quadratic) and 70% (linear).

Table 5-3 Accumulated analysis of variance for rate of leaf appearance, using means per rotation period. Given are the terms added to the model (starting with a constant), degrees of freedom for the term (d.f.), mean square of the added term (m.s.) and the significance of adding the term to the model (F probability).

Change	d.f.	m.s.	F probability
+ SOIL	1	0.6300	<.001
+ $SOIL^2$	1	0.0019	0.680
+ $SOIL^3$	1	0.0040	0.548
Residual	23	0.0108	
Total	26	0.0340	

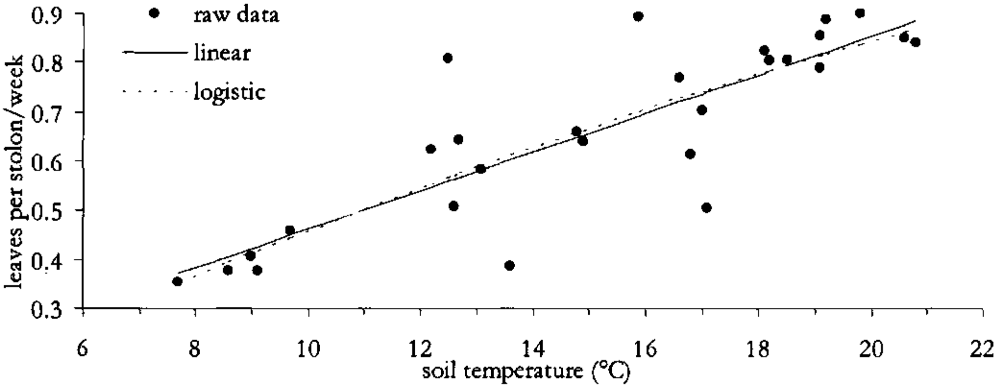


Figure 5-2 Relation between rate of leaf appearance (RLA; number of leaves per stolon/week) and soil temperature (mean of maximum and minimum temperature, SOIL, °C). Shown are the raw data, the linear relationship and the results of a logistic regression.

5.3.3 Relationships between clover growth and climate

Table 5-4 presents the models of best fit for each clover growth variable tested, using means per rotation period. Average soil temperature explained in all cases the largest part of the variation, showing the seasonality of growth. For all variables, the constant was not significantly different from zero and was deleted from the model. None of the other climate variables significantly improved the explanation of the variance.

Rate of leaf appearance showed the greatest response to soil temperature. The relationship between soil temperature and RLA was stronger than the relationship observed for all other variables. This relationship, RLA = 0.0436 SOIL ($P<0.001$), explained 70% of the variation in leaf production found, and predicts an increase of 2.3 leaves per stolon per year for every 1 °C increase in mean soil temperature. The relationship between leaf appearance, senescence and removal rates and soil temperature is shown in Fig. 5-3. Leaf removal and appearance rates had a relatively high coefficient of correlation (0.62, Table 5-2); 44% of the variation in removal rate of leaves could be accounted for by a linear model using only leaf appearance as a term (Table 5-4). Leaf senescence, however, was less directly related to rate of leaf appearance, and the coefficient of correlation between these variables was low (0.22, Table 5-2). In the model with rate of leaf appearance as an explanatory term, soil temperature still significantly increased the percentage of variation accounted for (Table 5-4). Leaf removal rate did not explain an additional part of the variation.

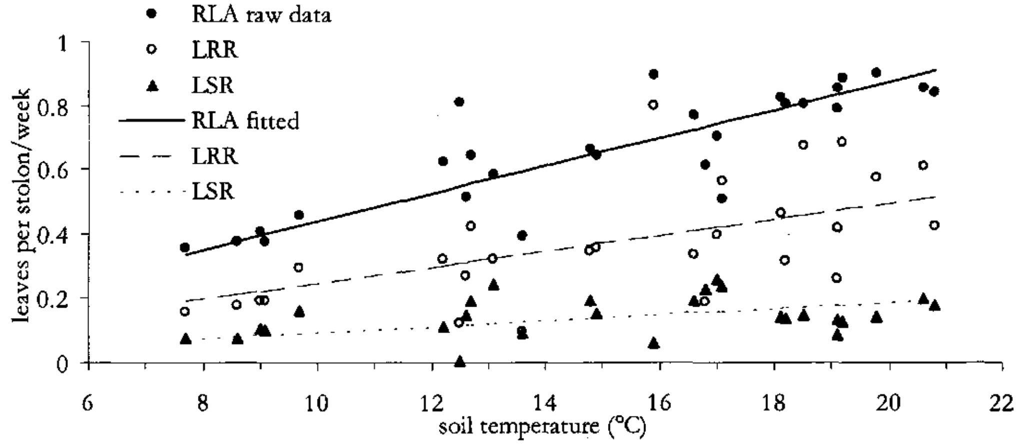


Figure 5-3 Leaf appearance, removal and senescence rates (RLA, LRR and LSR, respectively; number per stolon/week) in relation to mean soil temperature (SOIL, °C). Given are the raw data (means per rotation period, symbols) and the fitted linear regressions (lines).

Table 5-4 Best fitting models for clover growth variables (Table 5-1) with only significant parameters. Used data are means per rotation period. Variables are number per stolon/week, for stolon elongation mm/week and for loss of main stolon percentage of marked stolons. Total and residual mean squares and the percentage of variation accounted for (R^2_{adj}) are given.

	Mean squares			
	Total	Residual	R^2_{adj}	regression
SER	0.0164	0.0112	32%	0.0145 SOIL
RLA	0.0340	0.0101	70%	0.0436 SOIL
LSR	0.0037	0.0034	4%	0.0093 SOIL
LRR	0.0337	0.0213	38%	0.0246 SOIL
AAR	0.0092	0.0075	19%	0.0114 SOIL
ASR	0.0006	0.0005	17%	0.0012 SOIL
BAR	0.0142	0.0085	38%	0.0134 SOIL
BSR	0.0017	0.0012	25%	0.0032 SOIL
LOSS	0.0010	0.0004	55%	–0.0479 + 0.0078 AIR
		0.0005	49%	0.0305 + 0.0056 SOIL
SER	0.0163	0.0043	73%	0.8400 RLA – 0.0259 AIR
LSR	0.0037	0.0027	25%	0.0844 – 0.2690 RLA + 0.0158 SOIL
LRR	0.0340	0.0193	44%	0.5574 RLA
AAR	0.0089	0.0054	40%	0.2659 RLA
ASR	0.0006	0.0004	37%	0.1635 AAR
BAR	0.0142	0.0060	58%	–0.1370 + 0.5003 RLA
BSR	0.0017	0.0010	44%	0.2294 BAR
RSR	0.0023	0.0013	45%	0.4599 RR

For axillary bud and branch appearance rates, soil temperature accounted for a significant part of the variance, but at 19 and 38%, respectively, this was considerably less than for rate of leaf appearance. For these variables, the model with rate of leaf appearance as explanatory variable explained a larger part of the variation, 40 and 58%, respectively. For the axillary bud and branch senescence rates, the matching appearance rates explained considerably more than soil temperature. With the matching appearance rate in the model, none of the climate variables explained a significant additional percentage of the variation.

An important result was the lack of a relationship between climate variables and rates of rooting of nodes or root senescence. Differences between seasons were significant, with higher rates in spring and autumn (see Chapter 3), but these differences are not explained by the climate variables considered here. Polynomials did not account for the variation either. There was also no relationship with rate of leaf appearance. The positive correlation between senescence and appearance was also apparent for roots, with 45% of the variation in root senescence rate being accounted for by rate of rooting of nodes.

5.3.4 Interactions between treatment and climate

Table 5-5 shows results of the REML procedure with different factors in the fixed model: season, grazing and N treatments (the experimental treatments) and their interactions. Seasons are spring, summer, autumn and winter, with the rotation periods allocated to the seasons as described in Chapter 3. The second and third columns of the table give the rotation period component of the variance and the total variance (sum of all components) of the clover growth variables, for a model which only incorporates a constant. Note that for the regressions shown in Table 5-4 means per rotation period were used, for the regressions in Table 5-5 and Table 5-6 means per experimental plots (treatments x replicates) and rotation period were used. Hence the total variation for the data used in Table 5-4 is very similar to the rotation component of the variance in Table 5-5 where the model incorporates a constant only.

The fourth and following columns show the percentage of the variance components accounted for by three models, a model with season, experimental treatments and their interactions, another model with experimental treatments only, and a third model with season only. Where the percentage of variance accounted for is negative, the variance components could not be estimated properly due to ill-fitting of the fixed model. The importance of season was much greater than that of the experimental treatments for most variables. Interactions between season and management did not account for much of the variance either, since the percentage of variance accounted for was not increased much by the fixed model with these interactions, compared to the fixed model with only season as parameter. The biggest improvement was found for rate of rooting of nodes: experimental treatment effects and its interactions with season accounted for an additional 11% of the total variation compared to the model with season only. For this parameter, the model with experimental treatment effects only explained 5%

of the rotation component of the variance, the largest percentage found with this model.

Table 5-5 Variance components for the starting model (constant), and the percentage of the variance component accounted for by models with seasonal differences (SEASON, being spring, summer, autumn and winter, as in Chapter 3), or treatment effects (grazing (G) and nitrogen (N) treatments), and a model with season, treatments and their interactions. The random factors were rotation period, the experimental plots (treatments x replicates) and the interactions between experimental plots and rotation periods. Given are the rotation component (ROT) and the total of all components.

Fixed	Constant		SEASON		G*N		SEASON*G*N	
model:	ROT	total	ROT	total	ROT	total	ROT	total
SER	0.0160	0.0283	35	20	-2	0	38	29
RLA	0.0330	0.0485	60	41	2	1	62	44
LSR	0.0032	0.0112	7	2	-2	0	10	1
LRR	0.0302	0.0527	30	17	1	1	31	21
AAR	0.0082	0.0171	18	8	2	0	17	11
ASR	0.0005	0.0016	-4	-1	5	0	0	2
BAR	0.0135	0.0249	31	17	0	-1	31	18
BSR	0.0016	0.0032	13	7	3	1	15	7
RR	0.0043	0.0140	21	6	0	5	21	17
RSR	0.0021	0.0043	12	6	2	2	14	10
LOSS	0.0008	0.0024	64	23	0	-1	66	19

Table 5-6 shows the percentage of the same variance components accounted for by the models that include significant interactions between climate parameters and experimental treatments. Again mean soil temperature was the best fitting climate variable, with no additional significant contributions from the other climate variables used in the analyses. The interactions occurred for stolon elongation rate, leaf and branch appearance rates and axillary bud senescence rate. With N applications, the estimated stolon elongation rate was greater at higher temperatures, and less at lower temperatures than without N. The estimated stolon elongation rate was greater under RG4.9 than under CS4.9. In turn, under CS4.9 stolon elongation rate was greater than under RG7.4. These differences between grazing treatments increased with increasing soil temperature.

Estimated rate of leaf appearance was very similar under CS4.9 and RG7.4, being under RG7.4 0.02 leaves per stolon/week lower than under CS4.9 within the temperature range of the present experiment. Compared to the other two grazing treatments, the estimated rate was lower under RG4.9 at temperatures below approximately 10 °C, and higher at temperatures above approximately 20 °C. This resulted in estimated rate of leaf appearances at for example 7 °C of 0.30, 0.38 and 0.35 leaves per stolon/week under RG4.9, CS4.9 and RG7.4, respectively, and at 21 °C of 0.93, 0.88 and 0.86 leaves per stolon/week, respectively.

Table 5-6 Models of clover growth variables with statistically significant interactions (P<0.05) between climate parameters and experimental treatments. The distinguished components in the random model were rotation period, the experimental plots (treatments x replicates) and the interactions between experimental plots and rotation period. Given are the percentage of the variance accounted for by the models for the rotation component and the total of all variance components.

	Treatment		Model	rotation	total
SER	CS4.9	no-N	–0.0442 + 0.0167 SOIL	35%	26%
	RG4.9	no-N	–0.0816 + 0.0212 SOIL		
	RG7.4	no-N	0.0058 + 0.0115 SOIL		
	CS4.9	plus-N	–0.1500 + 0.0250 SOIL		
	RG4.9	plus-N	–0.1874 + 0.0303 SOIL		
	RG7.4	plus-N	–0.1000 + 0.0206 SOIL		
RLA	CS4.9		0.1233 + 0.0361 SOIL	71%	49%
	RG4.9		–0.0077 + 0.0445 SOIL		
	RG7.4		0.1011 + 0.0360 SOIL		
ASR		no-N	–0.0158 + 0.0030 SOIL	26%	9%
		plus-N	0.0023 + 0.0014 SOIL		
BAR	CS4.9		–0.0299 + 0.0152 SOIL	44%	25%
	RG4.9		–0.1641 + 0.0245 SOIL		
	RG7.4		–0.1081 + 0.0192 SOIL		

At lower temperatures (below approximately 14 °C) estimated branch appearance rate was lower under RG4.9 than under the other two grazing treatments. At higher temperatures the reverse was true, and was the estimated branch appearance rate higher under RG4.9 than under the other two grazing treatments.

Senescence rate of axillary buds at soil temperatures above 11 °C was greater for no-N than for plus-N, the difference getting bigger with increasing soil temperature.

In these analyses of interactions, effects of climate variables on rates of rooting of nodes or root senescence were not found, nor were there any interactions between climate variables and experimental treatments.

5.4 Discussion

In this chapter it is shown that during the present experiment the most important climatic variable for clover development was temperature. The soil temperature at 10 cm depth, expressed as the mean of the daily maximum and minimum temperatures, was the variable most closely related to the various growth components. It appears that temperatures experienced during the present experiment were mainly in the linear phase of the growth curve, since linear relationships were equal if not better than non-linear relationships in explaining variation in growth. It should be noted that the models presented are only applicable to temperatures within the range of the present experiment, and extrapolation to more extreme temperatures may not be valid.

Because of the lack of effects of rainfall or variability in rainfall on growth, it can be concluded that rainfall *persé* was not a limiting factor during the course of the study. On the contrary, the correlation between rainfall and all clover growth variables was negative, showing more often there was a surplus of water than a shortage (see also Chapter 2). Soil moisture, however, would probably have been a more appropriate parameter to relate clover responses to. Unfortunately as there are no data on evapotranspiration, soil moisture levels could not be calculated.

Solar radiation was correlated with temperature, and never explained a larger part of the variation in growth of clover components than soil temperature, nor did it explain a significant additional part of the variation.

5.4.1 Leaf appearance and soil temperature

Sackville-Hamilton and Harper (1989) used a cubic relationship between RLA and soil temperature, because for RLA there is an optimum (air) temperature at approximately 25 °C (Mitchell, 1956; Mitchell and Lucanus, 1962; Beinhart, 1963). In the present experiment adding the quadratic or cubic terms did not improve the model. The measured mean soil temperature ranged between 7 and 21 °C, being above the threshold value of clover growth (3 to 5 °C; Haycock, 1981; Chapman *et al.*, 1983; Frame and

Newbould, 1986), and below the optimum for RLA. Mean RLA ranged between 0.04 and 0.13 leaves per stolon/day. This is a narrower range than that measured by Sackville-Hamilton and Harper (1989; 0.02 to 0.17 leaves per stolon/day, with a temperature range of 4 to 19 °C). Their full model predicts zero leaf appearance at a soil temperature of 3.6 °C and lower, and a maximum of 0.20 leaves per stolon/day at 21.5 °C. Linearly, this represents a slope of 0.011 leaves per stolon/day per degree soil temperature. This is remarkably higher than the slope of 0.006 leaves per stolon/day per degree soil temperature in the present study. At lower temperatures the RLA is similar, but at higher temperatures the values found in the present experiment are lower than those found by Sackville-Hamilton and Harper (1989). In a situation where light intensity is limiting growth, rising temperatures, correlated with rising light intensity levels, may increase growth rates to a greater extent than in a situation where light intensity is uniformly high. When light intensity is not a limiting factor anymore, day length (light duration) becomes more important. In the UK days are longer in summer than in NZ, which could be the explanation of the greater RLA in summer found by Sackville-Hamilton and Harper (1989), compared to the present experiment. In agreement with this, Elgersma and Fengrui (1997) found significant cumulative effects of radiation and minimum air temperature in the Netherlands. In the present experiment, a model with minimum air temperature and radiation showed the latter term was not significant. In this model, the slope for the minimum air temperature was exactly the same as found by Elgersma and Fengrui (1997; 0.042 leaves per stolon/week per degree).

Removal rate of leaves was strongly related to the rate of leaf appearance and thus soil temperature. Variation in senescence rate, however, was not explained to a large extent by soil temperature, and no significant relationship existed with leaf appearance rate alone, nor with rates of both leaf appearance and leaf removal. The result of this is increasing total leaf dry weight and number of leaves per plant with increasing temperatures (Chapter 4).

5.4.2 Branch and root dynamics

The rate of branching is strongly related to leaf appearance rate, which determines the number of potential sites for branching. With leaf appearance rate in the model for rate of branching, no other climate variable significantly increased the percentage of variation accounted for. In Chapter 3 it was shown there is a difference between rate of branching and the percentage of nodes formed in a particular season that develop a branch. The former increased and the latter decreased from spring to summer. Hoglund and Williams (1984) found a reduced ratio of dry weight of secondary stolon to dry weight of primary stolon with increasing day/night temperature from 12.5 °C/6.0 °C to 27.5 °C/18 °C under a 12 hrs photoperiod. Beinhart (1963) found a decreasing percentage of nodes on the primary stolon formed branches when temperature increased from 10 to 30 °C under a 14.5 hrs photoperiod. Mitchell and Lucanus (1962) found an optimum temperature of 24 °C for the percentage increase per day in number of growing points at a 16 hrs photoperiod. With a photoperiod of 8 hrs, no significant differences were found in the range of 7 to 35 °C. Boller and Nösberger (1983) showed that the rate of branch appearance decreased when day/night temperature dropped from 18 °C/13 °C to 10 °C/7 °C with a photoperiod of 16 hrs. Because this temperature response was slightly less pronounced than the decrease in leaf appearance, the percentage of branching (number of stolons per total number of nodes) increased with the lower temperature. The linear regressions in this chapter also show branching rate did not increase to the same extent as leaf appearance rate did with increasing temperature. This explains the differing results when branching is measured as percentage of nodes forming branches, dry weight ratios or branch appearance rates. Since the number of growing points is the important factor for clover yield, and each growing point produces potential sites for new branches, the actual rate of appearance of new branches is the most important branching characteristic to measure.

Hoglund and Williams (1984) found large genetic variation in the branching of clover in response to changes in temperature and N. It is possible these genetic differences result in different responses to temperature in the various experiments. To investigate this and its importance in the field, development and survival of branches from primary as well as from secondary stolons of a range of cultivars should be screened in the field, in a grazed grass/clover sward. The large variation found in the present experiment illustrates that the clover population in these old swards has a great genetic diversity, making it resilient to a wide range of (climatic) conditions.

Persistence of clover depends on the survival of the branches. Senescence of branches was related to appearance of branches, without any additional effect of a climate variable. Also, adding season or the interaction between season and branch appearance rate did not explain an additional significant proportion of the variation found. It is not possible to fully explain the plant structure with the measured growth characteristics, because an important factor, the senescence of old stolon material, was not sufficiently recorded. Clover plants break up due to, amongst other things, senescence of old stolon material, resulting in a simpler branching structure. These processes are most rapid in spring. Within a plant order, changes were also apparent during the course of the experiment (Chapter 4). From spring through to early summer, the number of growing points per second or third order plant increased. From mid-summer through to the following spring, the number of growing points decreased. Indeed, since branch appearance rate increases more per degree increase in soil temperature than branch senescence, the number of growing points would be expected to increase with increasing temperature. The decrease in growing points per plant when the temperature decreases from mid-summer onwards is not explained by the relationships found for branch appearance and senescence rates, because at no point did the senescence rate of branches exceed the rate of branch appearance on the marked stolons.

5.4.3 Grazing and nitrogen treatment interactions with climate

Models incorporating the effects of grazing and fertiliser N treatments did not increase the percentage of variation accounted for to a large extent, compared to models including only the effects of soil temperature or season. However, some interactions between treatment and temperature occurred and should be taken into account. For example when looking at branching of clover, overall and even within season, no difference between the treatments was found (Chapter 3). However the analysis in this chapter shows differing slopes for the relation between branch appearance rate and soil temperature for the three grazing treatments. At a similar stocking rate, continuous stocking favoured increased branching at lower temperatures, whereas rotational grazing favoured branching when temperatures were rising. This agrees with work of Hay and Baxter (1989) who measured increased clover growing point density when sheep-grazed pastures in southern New Zealand were continuously stocked in late winter/early spring. Continuous stocking in spring could well be negative for branch survival, however. In Chapter 3 it was shown that under continuous stocking the percentage of nodes developing roots was lower than under rotational grazing. The presence of a root on the same parental node as a branch significantly increased longevity of the branch. This effect would be exaggerated when a drought occurs. Thus lower root production rate could drastically increase senescence of existing branches during a drought. On the other hand, however, Brock and Kim (1994) showed that the denser sward of a continuously stocked pasture was beneficial to clover in drought conditions: clover recovered more rapidly in continuously stocked pastures compared to rotationally grazed pastures. Future research could be directed to integrate beneficial traits of either grazing system. Branching of nodes, as well as survival of these branches, are important parameters to study further when research is aimed at maintaining a good clover component while increasing pasture production by the use of fertiliser N.

Chapter 6

GENERAL DISCUSSION

6. GENERAL DISCUSSION

6.1 The maintenance of clover populations

In New Zealand, the overall target for pasture management is increased animal production without losing the benefits of white clover. To bring total herbage production to a higher level, fertiliser N can be applied in the period late autumn/early spring when contribution of clover N or mineralisation is limited. The study presented in this thesis examines the effects of fertiliser N applied in this period on clover growth processes and plant and population structure in cattle-grazed grass/clover pastures, aimed to identify grazing management or plant breeding strategies that improve the growth and persistence of clover populations in the presence of moderate to high fertiliser N applications.

It is well-established that populations of white clover growing in moist-temperate environments are maintained primarily through vegetative growth (Turkington *et al.*, 1979; Chapman, 1983, 1987; Sheath and Hodgson, 1989; Chapter 3). The maintenance of clover populations, therefore, depends on the continued production and establishment of new branches. Plants break up due to senescence of older stolon material (Brock *et al.*, 1988; Hay *et al.*, 1988; Chapter 4), and branches may establish as new independent plants. Because of this, branching, and the branching structure of plants, were important points of focus in this study. To maintain a stable size population, only one replacement needs to be produced for every plant or stolon growing point (depending on which measure of population size is used) lost.

The best measure of the rate of loss from the population in this study is the turnover of white clover growing points, which was seemingly high at 3 to 11% of growing points on main (marked) stolons per week (Chapter 3). The rate of loss was highest in summer. To maintain the population of main stolons with a growing point, each week 1 node per 10 to 30 main stolons had to develop a branch with all the attributes necessary to enable it to establish successfully as a main stolon, i.e. independently of its parent stolon. Branches developed on 6 to 40% of main stolons (lower in winter and higher in spring/summer); 15 to 40% of these died before their parent stolons senesced and they became independent plants themselves. The highest senescence rates were found in summer and autumn, and in the spring of 1992, when relatively old branches also died. Thus, on average, each week 1 out of every 3 to 20 main stolons developed a potential main stolon (independent plant). This rate was clearly high enough to maintain the population of main stolons (it exceeds the estimated minimum rate necessary, 1 per 10 to 30 main stolons, above), an observation supported by the fact that there was no evidence of a decline in population density during the course of the study (Table 2-2).

An important factor for the successful establishment of a branch is the occurrence of a root on the same parental node where the branch develops. This significantly increases the life expectancy of the branch (Chapman, 1983; Chapter 3). On average, each week a root developed on 5 to 20% of main stolons. However, 18 to 80% of these roots died before an associated branch, if present, would have developed into a lateral stolon (Thomas, 1987a), i.e. the stage where it has a good chance of surviving independently as a main stolon. Only 2 to 10%, or 1 per 10 to 50 main stolons, produced per week a rooted node that had the potential to support a branch until that branch was established as a lateral stolon. This is lower than the number of main stolons producing a potential main stolon (1 per 3 to 20 stolons, above), illustrating that a certain proportion of the branches have to establish successfully without being supported by an associated root, to maintain the population of main stolons. This is possible if the conditions are favourable for rooting of nodes on the branch itself, making it less dependent on water and nutrients from the parental stolon.

The studied clover population seemed in equilibrium with its environment and the grazing management imposed upon it, and capable of replacing stolon growing points lost from the population. There is, however, evidence of some asynchrony between the occurrence of root and branch organs at nodes, which perhaps leaves the population open to increased plant or stolon losses when increased stress occurs, such as drought, defoliation or strong increased grass growth due to (local) increased soil inorganic N levels. While the overall picture is one of relative stability, this disguises the substantial seasonal variation in the growth and structure of clover plants and populations that occurred throughout the study, and which has some bearing on the outcome of competition between grass and clover for growth resources within the sward.

6.2 Seasonal variation

Seasonal effects dominated the growth of the pasture, and the growth dynamics of clover plants and populations, in the moist-temperate, fertile environment in which this study was conducted. Of the specific climatic variables associated with season that were examined in Chapter 5, soil temperature at 10 cm depth explained the greatest proportion of the variation in growth. Other studies also have identified the dominance of temperature in driving clover leaf appearance rate in moist-temperate climates (Beinhart, 1963; Davies and Evans, 1982; Chapman *et al.*, 1983; Sackville-Hamilton and Harper, 1989). It appeared that the soil temperatures experienced during the present experiment, averages per month (Chapter 2) or per rotation period (Chapter 5), of between 7 and 21 °C, were mainly in the linear phase of the growth curves. Apparently rainfall was not limiting clover development, however soil moisture availability could not be calculated. Since root primordia usually only develop when in contact with a moist medium (Thomas, 1987b), it seems likely that soil moisture is a crucial factor for root development. Rates of rooting or root senescence did not show any relation with the climate variables recorded (Chapter 5).

Death of older stolon material (Chapman, 1983; Sackville-Hamilton and Harper, 1989), treading by cattle, death of growing points, and development of branches, resulted in a population of clover plants which varied widely in size and structure. This showed a strong seasonal pattern, with small plants of relatively simple structure being abundant in spring and more complex plants present in early autumn (Chapter 4), similar to the findings of Brock *et al.* (1988) and Hay *et al.* (1988) for sheep-grazed pastures, and Harris (1994) for dairy pastures in New Zealand. In general, plants within plant orders were larger in late spring/summer; the DW per plant was higher as well as the number of nodes and leaves (Chapter 4). The best defined seasonal pattern was that of number of rooted nodes per plant, which was clearly highest in spring and lowest in the latter part of summer (January/February).

The importance of successful development of nodal roots for clover growth and survival has been stressed previously (Chapter 1, 3). Spring is a crucial season for this, since in this season the roots necessary for the clover plants to survive a possible summer drought will have to establish and survive. The success of establishment of roots developed in autumn was higher than in spring, considering the relatively lower senescence rates, resulting in an increasing number of rooted nodes per plant throughout autumn and winter. Even though rooting rates were relatively high in spring (Chapter 3), from mid-spring onwards and throughout summer the number of rooted nodes per plant or per stolon declined (Chapter 4). In this period the root senescence rate exceeded the rate of root development on nodes. However, this was not observed on the scored stolons, which indicates that an important part of the population was not monitored in that part of the study. Rooted nodes (mainly older ones) would also have been present on stolons without growing points (for example on a large part of the primary stolons of 3rd and 4th order plants), and these were not included in the sample of marked stolons.

6.3 Treatment effects

Treatment effects on pasture production, and on clover growth dynamics and plant and population structure, were generally much weaker than seasonal effects (Chapters 2, 3, 4 and 5). The swards responded to fertiliser N with an increased rate of herbage accumulation equivalent to around 15 kg DM/kg N applied; this extra growth occurred mainly in autumn and summer (Chapter 2). White clover content and accumulation were depressed when N was applied; this depression was seen mainly in spring. In terms of sward structure, the only measured effect of N application was a decrease in the tiller density of grasses other than perennial ryegrass.

Grazing treatments did not influence herbage accumulation or composition (Chapter 2). Tiller density of *Lolium* species was higher under continuous stocking than under rotational grazing, however the differences were smaller in the present study than in other studies comparing the two grazing methods under sheep grazing (for example Chapman *et al.*, 1983; Hay *et al.*, 1989b; Hay *et al.*, 1991; Brock and Fletcher, 1993). At only one sampling, in late summer, was the clover growing point density and stolon length/m^2 affected by grazing treatment; values for these variables were greater under continuous stocking compared to the rotational grazing treatments at this time.

The lack of great differences in (clover) herbage accumulation and sward density under the grazing treatments imposed can be explained by the similar rates of leaf appearance, senescence and utilisation found under these treatments (Chapter 3). The similar rate of leaf appearance for all three grazing treatments confirms previous reports of relative insensitivity of this important growth process to variation in defoliation treatment within the range normally found in grazing systems (Chapman, 1983; Chapman *et al.*, 1983). Leaf appearance rate was also relatively unresponsive to N application, with only the fertiliser applied in the first autumn resulting in a higher rate of leaf appearance. Stolon elongation rate was not affected by grazing treatment, but was increased by N application (average 27%), reflecting expected responses to increased shading of leaves and stolons through increased investment of growth resources in petiole and stolon extension to access light (Dennis and Woledge, 1982, 1985; Woledge, 1988; Robin *et al.*, 1992; Thompson, 1993, 1995). This response is often accompanied by a decrease in stolon branching activity (Solangaarachchi and Harper, 1987; Thompson and Harper, 1988; Jones and Davies, 1988; Davies and Evans, 1990; Thompson, 1995), a response not seen in the data collected from marked stolons (Chapter 3). The change in the pattern of resource allocation within the plant due to shading can also reduce allocation to root material (Thompson and Harper, 1988; Robin *et al.*, 1992). Rooting generally occurred before branching at a node (Chapter 3), but, for a proportion of the nodes, the reverse was true. For these nodes only, N application reduced the subsequent probability of a root developing. It appears that in the presence of a better nutrient supply for pasture growth and consequently increased shading, the greater apical dominance of the branch inhibits the development of a root at the same parental node.

Following leaf removal, translocation of assimilates from stolon, roots and remaining leaves occur in clover (e.g. Hoshino and Oizumi, 1968; Chapman and Robson, 1988; Marriott and Haystead, 1990). Root dry weight, nodule dry weight and N fixation capacity decreases following defoliation (Chu and Robertson, 1974). Culvenor *et al.* (1989b) measured in subterranean clover a 40% drop in root and nodule growth and maintenance respiration following removal of 70 or 80% of the shoot dry weight. Current photosynthate supply to roots in white clover decreases following one single defoliation (Gordon *et al.*, 1986) or with frequent defoliation (Chapman *et al.*, 1992b), and may result in a depressed root elongation (Evans, 1973). Also N for regrowth of leaves is mobilised from roots and branches (Culvenor *et al.*, 1989a). However large the effects of defoliation on plant physiological processes, the differing defoliation patterns between the grazing treatments applied in the present experiment did not result in differences in biomass allocation to stolon or root material of clover plants (Chapter 4). Also population and plant structure and plant morphology did not differ much between the grazing treatments.

6.4 Managing white clover in grazed pastures

6.4.1 Grazing management

Seasonal influences were much greater than the effects of grazing management on pasture production, clover population and plant structure in this study. Others have noted similar trends. It seems appropriate, therefore, to manage swards in accordance to these seasonal patterns. When plants are small they are more vulnerable to stress, so grazing should be carefully managed in late winter/early spring when plants are smallest (Brock and Hay, 1996).

Continuous stocking in late winter/early spring increased growing point density under sheep grazing in southern New Zealand (Hay and Baxter, 1989), and results from the present experiment suggest that this could also be the case under cattle grazing (Chapter 2, 5). However, for increased herbage production and proportion of clover, rotational grazing to low sward heights is generally recommended (Briseño de la Hoz and Wilman, 1981; Frame and Newbould, 1986; Brock and Hay, 1996). Furthermore, rotational grazing with a moderate stocking rate (RG4.9) increased the percentage of nodes rooting, and increased the rates of leaf appearance and branching at higher temperatures (Chapter 3). From this it can be derived that continuous stocking in late winter/early spring, followed by rotational grazing when the pasture growth rate increases with rising temperatures, could be the best way to manage a grass/clover sward. This is, indeed, very close to the grazing management employed on New Zealand sheep and beef properties, where animals are usually continuously stocked during lambing or calving in late winter/early spring, then moved into some form of rotational grazing when breeding animals plus lambs or calves increases the effective stocking rate on pastures as growth rate also increases. Conservation of excess herbage as hay or silage helps to maintain control of herbage mass across the property and good conditions for clover growth.

Despite adopting a grazing management that appears to strike a good balance between maximising total feed supply for animals and meeting the needs of the clover in the sward, New Zealand farmers are still faced with relatively low proportions of clover present in the total amount of pasture grown per year (Caradus *et al.*, 1996). Some increase in clover content through tactical grazing management seems to be possible (see above), but there is little evidence that large and sustainable gains can be achieved through changes to grazing management. The results of the present study reinforce this conclusion. The reasons for this have not been explicitly tested, though they are likely to relate to factors such as sward structural changes in response to changes in defoliation pattern that favour a stable equilibrium between leaf growth and removal (Chapman and Lemaire, 1993; Chapter 3). In short, the unique biological features of grass/clover associations used in moist-temperate regions appear to limit the extent to which the grass/clover balance can be shifted and sustained at some new level by grazing management manipulation.

6.4.2 Use of N fertiliser

The inability to change the grass/clover balance of pastures for increased herbage production through grazing management has led to increased use of N fertiliser to overcome N deficiencies for pasture growth in New Zealand. As explained in Chapter 1, N fertiliser use is commonly associated with reduced clover content in the mixture, making it more difficult to maintain the desired grass/clover balance. Schwinning and Parsons (1996a) showed by modelling the coexistence of grass and clover, that as soil inorganic N levels increase, the content of clover will decrease if the grass/clover system is self regulating these levels. However, the soil inorganic N level will not be elevated for a long time if N is fairly quickly utilised by the herbage and not applied frequently. It will mainly benefit the grass component, but also clover can benefit at times when N fixation is limiting growth.

In this study, the application of 100 kg N/ha/year decreased clover growth and content moderately for some time after application, but not permanently. Similarly, in dairy pastures relatively small reductions in clover content were measured when 200 kg N/ha/year was applied when stocking rate was high (4.48 dairy cows/ha; Harris *et al.*, 1996). The negative effect of N fertiliser in Harris *et al.*'s study was much greater at a lower stocking rate (3.24 cows/ha). In general, very few strong effects of N fertiliser on the clover attributes were found in the present study. However, this does not mean that morpho-physiological mechanisms or responses are not involved. Rather, it is possible that the range of N application used (0 and 100 kg N/ha/year) was too narrow to generate responses that were sufficiently large to detect as being statistically significant amidst the well-known variability that exists between plants and micro-sites in grazed pastures. In contrast, Harris *et al.* (1996) detected quite large differences in clover plant and population structure in pastures grazed by dairy cattle and receiving 400 kg N/ha/year. Almost all plant size attributes measured by Harris *et al.* (1996) were lower in the 400 kg N/ha/year treatment compared to treatments receiving no N or 200 kg N/ha/year. Harris *et al.* (1996) did not collect detailed information on stolon growth dynamics of the sort obtained in this study to identify how or when these differences developed.

The relatively moderate effect of the N treatment used here, and the 200 kg N treatment of Harris *et al.* (1996), on clover content shows that there is scope for increasing pasture production with the use of N fertiliser while maintaining something close to the clover contribution found in the absence of N, providing pastures are well utilised. However, it remains to be seen whether the clover in such systems is more prone to sporadic environmental stresses than in systems where fertiliser N is not used, or is used at still lower rates. Furthermore, a modelling study of population oscillations between a grass and a N fixing legume in a mixed sward showed sustained year-to-year variation in legume content at the field scale following a field-wide disturbance such as a fertiliser N application (Schwinning and Parsons, 1996b). Greater oscillations will make it more difficult to plan pasture management and achieve the desired balance between grass and clover in the mixture.

6.5 Implications for breeding

Breeding for clover varieties which have a higher N fixation capacity, or are more vigorous growers and stronger competitors in a grass/clover sward, may not necessarily lead to a higher production level of the whole sward nor a higher clover content in the sward (Ennik, 1981; Schwinning and Parsons, 1996a). However, when the aim is to increase production in the cooler seasons, there are various ways to achieve this. Firstly, plant breeding could be directed towards increased N fixation rates at lower temperatures. Secondly, as was outlined in the section above, the use of fertiliser N in the cooler periods results in increased production of the whole sward. When this method is applied, the continuing contribution of clover needs to be secured.

In Chapter 1 and 3 the importance of rooting and branching of nodes, to ensure maintenance of the clover population, has been stressed. Results in Chapter 3 indicate not branching itself, but survival of branches may be a key factor in clover persistence when fertiliser N is applied. The longevity of a branch increased when a root was present on the same parental node. Rooting of branched nodes was inhibited when N fertiliser was applied. Hence screening clover genotypes for variation in sensitivity of branching and rooting for the environment around the nodes, should make it clear if there is scope for selection and breeding of cultivars that are able to persist through successful establishment of branches in less favourable conditions. This would increase the range of management options for farmers, and protect the clover in swards to some extent from errors in management resulting in less-than-ideal conditions for nodal development.

Apart from a continuing contribution to the diet of the grazing animals when fertiliser N is used in cooler periods, clover should also maintain N fixation in the periods when the temperatures are sufficiently high, which is (late) spring through to (mid) autumn. The clover has to possess a sufficiently large rooting system from which a high rate of N fixation can be supported. Quantifying genetic variation in rooting rates of nodes and the rate of establishment of N fixation capacity, as well as higher N fixation efficiency, should indicate if it is possible to move to this goal through plant breeding. Hoglund (1973) suggested that selection for an increased rate of establishment of N fixation capacity is possible for lucerne. West *et al.* (1985)

showed the clover cultivar G18 (later renamed 'Grasslands Kopu') fixed more N than Huia, both in terms of kg N and % of total N accumulation in the plant, while inorganic N uptake was similar. Mytton and Rys (1985) showed genetic differences for nodulation and N fixation with applied N within the variety Aberystwyth S.100. Genotypic variation in root dry weight per plant when inorganic N is available was shown by Hoglund and Brock (1974). This does not necessarily mean variation in nodulation or N fixation, but availability of sites for nodulation does vary.

6.6 Main conclusions

The target for this study was to increase knowledge of clover plant growth processes and plant and population structure in grazed N fertilised pastures. This should help identify management strategies and plant breeding goals aimed to successfully combine fertiliser N applications and sustained clover contribution in grazed pastures. The results of this study show herbage production increases by the use of fertiliser N in autumn and late winter, and at a rate of 100 kg N/ha/year clover content and production are not permanently compromised. The grazing treatments applied did not result in great differences in herbage production or composition, and clover was maintained under all grazing treatments. Comparison with studies under sheep grazing indicates the environment under cattle grazing could be more favourable for clover in a grass/clover sward, than under sheep grazing, but that breaking up of plants through treading and/or excreta return is stronger under cattle than under sheep grazing. Especially in spring plants are small, and probably most vulnerable to stress. In this season rooting appeared to be inhibited when fertiliser N was used. It is possible that this decreased rooting makes branches and clover plants more susceptible to periods of stress. The results suggest continuous stocking in the cooler times of the year, with a shift to rotational grazing when temperatures rise, may help to increase occurrence of branching and rooting, and therefore increase persistence of clover. For improved herbage production at lower temperatures, plant breeding could be directed towards increased N fixation at lower temperatures, or maintenance of the clover contribution when fertiliser N is used at lower temperatures. For the latter goal, development and longevity of branches and roots are key factors, as well as N fixation capacity and efficiency at higher temperatures.

6.7 Future research

This thesis clearly showed the large variation between clover plants in a grazed grass/clover sward. Small-scale differences in the environment define the dynamics of each individual growing point and node, be it from a main stolon or a branch. This variation that exists in the grass/clover sward should not be seen as undesirable, it can moderate the otherwise large differences that might be seen between years in clover content of the pasture when the pasture is relatively uniform with respect to factors such as inorganic N availability (Schwinning and Parsons, 1996b).

The large variation implies that observations on a large number of plants or stolons are necessary to establish significant differences between treatments, or between seasons. Since the variation between seasons was larger than that between the applied treatments, the seasonal differences could be better established than differences between treatments. For future research, the total residual variances, which can be derived from Chapter 5, are a good guideline to determine the number of observations needed to establish the required differences. Also, in future research greater contrast between treatments could be used. In the present experiment extreme treatments were not used, so that the results would relate more directly to farming practice. The lack of large differences, and the problems of generating sufficient discriminatory power when background variation in the sample unit is high, are significant risks in this kind of applied research.

The discrepancies in the results of scored clover stolons and dissected clover plants, showed that the method of scoring main stolons with a growing point present did not give a full description of processes in the whole clover plant population. For example, part of the population consists of stolons without a growing point, which also bear rooted and branched nodes. These were not included in the sample for the stolon observations described in Chapter 3. As soon as the branch became a main stolon itself, it would behave similarly to the marked stolons and could have been included in the sample. While intuitively it seems likely that a combination of results from marked stolons and census sampling of whole plants will generate greater explanatory power (as was expected at the outset of this study), there are important requirements for sample selection and representation, and sampling frequency and intensity, that must be identified and met if this dual approach is to succeed.

Further research is necessary to establish relationships between temperature, inorganic N levels in the soil, and the ability of clover roots to establish or maintain sufficient N fixation capacity in a grazed grass/clover sward. When temperatures rise in spring, the grasses respond first with higher growth rates, followed by the clover. Hence, for a time, clover faces increased competition from grasses for inorganic N and other nutrients, as well as competition for light. Simultaneously, increased death of old stolon leads to fragmentation of larger plants and an increase in small clover plants in the pasture with limited root capacity. Maintenance of the *Rhizobium* symbiosis at this time when pressure on the energy supply of the clover plant is great, and the root system of plants is somewhat weakened, could be problematic. Such research should link with parallel work on genetic variation in the speed and efficiency of establishment of functional nodules (section 6.5).

The importance of 2nd and 3rd order plants for the persistence of the clover population (either as number of plants or number of growing points per unit area), warrants more detailed measurements on these classes. Monitoring whole plants in a grazed sward, to determine the fate of branches and roots, would increase the understanding of the seasonality and various impacts on the plant, and hence on the population. However, it will be difficult to determine in which class a plant belongs, since much of the stolon material is usually buried. The fate of buried nodes and stolon material is hard to monitor in time with repeated measures. The method of Sackville-Hamilton (1989) to excavate plants after a set period of time, will show the state of the material at that point in time, but clearly it is not possible to measure the same plant again at a later date.

A better option might be to mark main stolons, record the events on the whole plant, also after the growing point has disappeared, and to monitor also all new growing points, i.e. the secondary and tertiary branches. Once the branch itself has developed around eight nodes and has rooted itself, it will probably behave similarly to main stolons monitored in the present experiment, but this still remains to be established. The importance of plant structure for the survival of plants and branches should be established. For example, do tertiary stolons of 3rd order plants survive better in adverse conditions than secondary stolons of 2nd order plants? Severance of the roots can be used to determine the effects of rooting of the parental node. The vulnerability of main stolons and branches to local or temporal stresses should be tested. Monitored plants can be subjected to single stress factors, or to combinations of these, such as N fertiliser application, shading, surplus or shortage of water, hard or lax grazing.

Reference was made earlier in this Chapter to the relative insensitivity of the grass/clover balance in pastures to management manipulation, and some possible reasons for this (section 6.4). Chapman *et al.* (1996) argued that expectations of more than 50% clover in the pasture (a commonly stated goal) may be unrealistic under grazing, and advocated that more attention be focused on determining the 'optimum' clover content from productivity, profitability and sustainability perspectives to set realistic goals for future research. The actual content will fluctuate seasonally, as seen from the results of this study and many others. It is important therefore to establish to what levels clover can decrease in winter and spring, and still be able to come back to such an extent in summer that it contributes significantly to the production and N supply of the pasture. The effect of added N on this recovery capacity should also be examined. For example, an optimal and attainable clover content for a grass/clover sward could be one that fluctuates between 10% of total DM production in winter and 50% in summer. Higher contents in summer may cause bloat, while the utilisation of proteins in the rumen is inefficient when there is an imbalance between carbohydrates (energy) and protein in the diet. A lower content in summer may be suboptimal for N supply to the sward. A lower content of clover in winter decreases the protein content of the animals' diet undesirably.

In conclusion, field experiments are useful in determining practical implications of results from fundamental research, and to create new hypotheses about growth processes. However, they are not suitable for establishing the working of processes, because too many factors are involved in field experiments, which can not be controlled by the researcher.

It is clear that variation in clover growth processes and population and plant structure is very large in the field. This variation makes determination of statistically significant differences between treatments difficult, especially so when moderate treatments, close to farming practices, are used. A large number of observations is then required. This can be achieved by long term trials, running for several years, or by trials running at various locations. A further step towards this could be to bring together all the presently available data, from New Zealand and other countries, from cattle- and sheep-grazed pastures. Such a data set also gives a range of soils and climates, further improving its power to detect influences of environment. At present powerful statistical methods are available to tackle this kind of data set.

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SUMMARY

Introduction

The overall target for the present study was increased herbage production through the use of fertiliser N in the cooler times of the year, while maintaining the benefits of the white clover (*Trifolium repens* L.) component of the herbage. The continued use of fertiliser N generally leads to a decrease in the clover content. The mechanisms behind this decrease in clover are not fully understood.

The experiment presented in this thesis studied clover population and plant structure and plant growth processes. Seasonal dynamics in cattle-grazed pastures, the effects of fertiliser N applications, grazing management, and their interactions, were investigated. This helped to identify management strategies and plant breeding goals aimed to successfully combine fertiliser N application and sustained clover contribution in grazed pastures.

The grazed sward, setting the scene

The study was conducted at Aorangi field research station, an experimental area of AgResearch Grasslands, near Palmerston North, New Zealand. The grazing treatments imposed on the pastures were rotational grazing with either 4.9 or 7.4 Friesian bulls/ha (RG4.9 and RG7.4, respectively), and continuous stocking with 4.9 bulls/ha (CS4.9). Within the grazing treatments, fertiliser N (urea) was applied to strips within the pastures twice each year, in mid autumn and late winter, at 50 kg N/ha per dressing.

Total herbage accumulation was 16.6 tonnes DM/ha/year in 1991 and 16.0 tonnes DM/ha/year in 1992 (Chapter 2). Clover content was lowest in winter at 10%, increasing to 30% in summer. Grazing management did not affect the herbage accumulation or composition. The average efficiency of the applied fertiliser N was 17.5 kg DM/kg N in 1991 and 12.9 kg DM/kg N in 1992, with the greatest response of herbage accumulation occurring after the autumn applications. On average N tended to reduce clover DM accumulation by 15% and clover content by 3.6%. The greatest reductions were recorded in spring.

The tiller density of *Lolium* species was on average 8800 tillers/m^2 under CS and 6420 tillers/m^2 under RG. The density of other grasses was on

average 7270 tillers/m^2 without N and 6320 tillers/m^2 with N. Seasonality was apparent, with highest grass tiller densities in winter. Clover density was on average 4000 growing points/m^2, stolon length 110 m/m^2, and stolon weight 63 g/m^2. Clover density increased greatly in the February 1992 sampling (summer), which was the only sampling showing an effect of grazing treatment, with a greater density under CS4.9 than under RG7.4. N application did not affect clover density.

Growth dynamics of stolons

Seasonality and the effects of grazing and N treatments on clover growth dynamics are reported in Chapter 3. Growth dynamics were characterised by loss of growing points at the main stolon, appearance, removal and senescence of leaves, axillary buds, branches, roots and inflorescences, and stolon elongation of marked main stolons in the pastures described above.

Seasonal effects were in general much stronger than treatment effects. In Chapter 5 growth dynamics are related to climatic variables. Growth was positively related to mean soil temperature at 10 cm depth, which explained the greatest part of the variation. The relations were linear with soil temperature, with monthly averages between 7 and 21 °C. However, the rates of rooting and root senescence could not be explained by the climatic variables recorded. These rates were highest in spring and autumn. Senescence rates of axillary buds, branches and roots were more closely related to appearance rates of the respective plant parts than to climatic variables.

To maintain the population of main stolons each week 1 node per 10 to 30 main stolons had to develop a branch that could survive independently from the parental stolon. The longevity of a branch increased when the parental node had rooted, and a root showed an increased longevity when a branch was present at the parental node. Each week only 1 per 10 to 50 main stolons produced a rooted node that had the potential to support a branch until it could survive independently from the parental stolon. Thus, to maintain the population of main stolons a certain proportion of the branches has to establish without the support of an associated root.

Grazing treatments did not differ in rates of leaf removal, appearance, or senescence. Thus, utilisation of leaf growth was also similar for the grazing treatments, with an average of 46%. With the same stocking rate, the percentage of nodes that developed roots was lower under CS than RG. Under RG, the higher stocking rate resulted in a lower percentage of nodes rooting. Rate of rooting of nodes was suppressed in spring for plus-N, compared to no-N, particularly on branch bearing nodes. It appears the greater apical dominance of the branch inhibits rooting of the parental node. In periods with adverse conditions, such as drought, this may have implications for branch survival.

A few interactions between climatic variables and treatments were found (Chapter 5). The rates of leaf and branch appearance were lower under RG4.9 at low mean soil temperatures than under the other two grazing treatments, while at higher temperatures the reverse was true.

Population and plant structure

Plants were described by branching complexity, plant dry weight (DW), allocation of biomass to different plant parts (stolon, leaf, root and inflorescence), stolon length and numbers of stolons, growing points, nodes, leaves, petioles (without leafy material), roots and inflorescences per plant and per stolon order (Chapter 4). The plants were extracted from intact turves removed from the swards.

The estimated plant density, based on growing point density and average number of growing points per plant, varied between 650 and 1300 plants/m^2. In winter/early spring the estimated density was highest and in late summer/early autumn lowest. The population of clover plants varied widely in size and structure, and this showed a strong seasonal pattern. On the whole, plants of 2nd and 3rd order branching structure were most important in this clover population. In early autumn plant structure was most complex. From autumn on, the population shifted to a less branched structure. In spring the plants were small and of relatively simple structure, with mainly 1st and 2nd order plants. Within plant orders, plants were generally larger in late spring/summer.

Effects of grazing or N treatments were few. Plants under CS had a lower leaf DW than under RG. N application tended to decrease the number of rooted nodes per plant in spring. Treading and/or excreta return contributed to breaking up of plants.

Conclusions

The target for this study was to help identify management strategies and plant breeding goals aimed to successfully combine fertiliser N application and sustained clover contribution in grazed pastures. For clover persistence, branches have to develop and establish successfully as independent plants. Increased rooting improves the chance of branch establishment. Continuous stocking in the cooler periods of the year, with a shift to rotational grazing when temperatures rise, may help to increase branching and rooting. Pasture production can be increased in the cooler times of the year by the use of fertiliser N. Clover content and production are not permanently reduced with 100 kg N/ha/year. However, rooting appeared to be inhibited in spring following N applications. Especially in spring plants are small, and probably most vulnerable to stress. It is possible that the decreased rooting makes branches and clover plants more susceptible to periods of stress, when N is applied.

Apart from maintenance of the clover population, N fixation should be maintained in warmer periods. For both goals, root development and longevity are key factors. Genetic variation in root development and longevity, N fixation capacity and efficiency, and the rate at which N fixation capacity is established when temperatures rise, should be quantified. This should indicate if improvement of these characteristics could be achieved through plant breeding.

Future research

In the present experiment main stolons were only included in the sample of marked stolons when a growing point was present. However, part of the population of main stolons consists of stolons without a growing point. The branches and roots on these stolons were not monitored, but these also play a significant role in the clover population. Also, branching and rooting of nodes on secondary stolons were not considered in the present experiment. These parameters should receive attention in future research, to fully explain the structure of a clover plant population. The importance of plant size and structure for persistence of clover should be established. Monitored plants could be subjected to stress factors such as shading, defoliation, N fertiliser application, and surplus or shortage of water, to establish vulnerability of main stolons and branches to local or temporal stress.

SAMENVATTING

Dynamiek van witte klaver in beweid grasland in Nieuw Zeeland

Introductie

Een doelstelling van graslandmanagement in Nieuw Zeeland is een hogere productie in de koelere tijden van het jaar te bewerkstelligen door gebruik van kunstmest N, terwijl toch voldoende witte klaver (*Trifolium repens* L.) aanwezig blijft om nog een belangrijke bijdrage aan het rantsoen en de N voorziening van het grasland te leveren. Regelmatig gebruik van kunstmest N leidt veelal tot een daling van het klaveraandeel in het grasland. Hoe dit precies in zijn werk gaat is nog niet bekend.

Dit proefschrift presenteert een studie van de populatie- en plantstructuur en de groeiprocessen van klaver in grasland dat wordt beweid door rundvee. De dynamiek over de seizoenen heen wordt beschreven, en de effecten van beweidingmanagement, het gebruik van kunstmest N, evenals interacties tussen deze factoren. Het doel is om mede door dit werk strategieën vast te stellen voor gras/klaver management en veredeling van klaver, voor een succesvol gebruik van de combinatie van klaver en kunstmest N in beweid grasland.

Beweid grasland, een schets van de omgeving

De studie is uitgevoerd op Aorangi, bij Palmerston North in Nieuw Zeeland. Aorangi is een proefbedrijf van DSIR Grasslands (nu AgResearch Grasslands). Er werden drie typen beweiding toegepast, omweiden met 4,9 of 7,4 Friese stieren/ha (RG4.9 en RG7.4), en standweiden met 4,9 stieren/ha (CS4.9). In de weilanden werden stroken aangelegd waar kunstmest N (ureum) werd gestrooid. Dit gebeurde twee keer per jaar, in het najaar en de winter. Per keer werd 50 kg N/ha gebruikt.

De totale droge-stofproductie van het grasland was in 1991 16,6 ton/ha/jaar en in 1992 16,0 ton/ha/jaar (Hoofdstuk 2). Het aandeel klaver in de droge stof was het laagst in de winter, met 10%, en steeg tot 30% in de zomer. De verschillende typen beweiding resulteerden niet in verschillende opbrengsten of aandelen van de verschillende graslandcomponenten (raaigras, overige grassen, witte klaver, overige kruiden en dood materiaal). Gemiddeld was de efficiëntie van de gebruikte kunstmest N 17,5 kg droge stof/kg N in 1991 en 12,9 kg droge stof/kg N in 1992. De grootste respons van de graslandproductie op de kunstmest N werd gemeten na de toediening in het najaar. Gemiddeld bleef de klaveropbrengst achter met 15% en daalde het aandeel klaver in de droge stof met 3,6% door toediening van kunstmest N. De grootste achteruitgang in klaver werd in het voorjaar gemeten.

De spruitdichtheid van de *Lolium* soorten was gemiddeld 8800 spruiten/m^2 bij standweiden en 6420 spruiten/m^2 bij omweiden. De dichtheid van andere grassen was gemiddeld 7270 spruiten/m^2 zonder N en 6320 spruiten/m^2 met N. De spruitdichtheid van grassen was hoger in de winter. De dichtheid van klaver was gemiddeld 4000 groeipunten/m^2, met een stolonlengte van 110 m/m^2 en een stolongewicht van 63 g/m^2. De klaverdichtheid was zeer hoog in februari 1992 (zomer). Dit was ook het enige tijdstip met verschillen tussen de typen beweiding: de dichtheid onder CS4.9 was hoger dan onder RG7.4. De toediening van kunstmest N had geen invloed op de dichtheid van klaver.

Groeidynamiek van stolonen

Het seizoensverloop en de effecten van beweidingmanagement en kunstmest N op de dynamiek van klavergroei zijn beschreven (Hoofdstuk 3). De groeidynamiek werd gekarakteriseerd door het verlies van groeipunten, het verschijnen, verwijderen en afsterven van bladeren, okselknoppen, zijscheuten, wortels en bloeiwijzen, en de verlenging van gemarkeerde hoofdstolonen.

De verschillen tussen de seizoenen waren over het algemeen veel groter dan de verschillen tussen de toegepaste behandelingen. Relaties tussen de groeidynamiek en klimaatsfactoren werden berekend (Hoofdstuk 5). De groei was positief gerelateerd aan de gemiddelde bodemtemperatuur op 10 cm diepte, wat de belangrijkste verklarende factor was. De relaties waren lineair tussen de groeidynamiek en bodemtemperatuur, met gemiddelden per maand tussen 7 en 21 °C. Alleen het verschijnen en afsterven van wortels konden niet verklaard worden met de gemeten klimaatsfactoren. Meer wortels verschenen en stierven af in het voor- en najaar. Het afsterven van okselknoppen, zijscheuten en wortels was meer gerelateerd aan het verschijnen van deze plantendelen, dan aan de gemeten klimaatsfactoren.

Om de populatie hoofdstolonen te behouden, moest gemiddeld 1 op de 10 tot 30 hoofdstolonen een zijscheut ontwikkelen die zich onafhankelijk van de hoofdstolon kon vestigen. Het benodigde aantal is afhankelijk van het seizoen. Een zijscheut leefde langer wanneer de knoop waarop deze zijscheut zat ook beworteld was. Andersom leefde een wortel ook langer door de aanwezigheid van een zijscheut. Op maar 1 op de 10 tot 50 hoofdstolonen ontwikkelde zich een wortel die lang genoeg leefde om een zijscheut te ondersteunen totdat het zich onafhankelijk van de hoofdstolon zou kunnen vestigen. Om de populatie van hoofdstolonen te behouden, moest daarom een deel van de zijscheuten zich kunnen vestigen zonder de ondersteuning van een wortel op dezelfde knoop.

De verschillende typen beweiding resulteerden niet in verschillende snelheden van verwijderen, verschijnen en afsterven van bladeren. Dus de benutting van de bladgroei was ook gelijk voor de verschillende typen beweiding, met een gemiddelde van 46%. Met een gelijke veedichtheid wortelde een lager percentage van de knopen onder standweiden dan onder omweiden. Onder omweiden wortelde een lager percentage van de knopen bij de hogere veedichtheid. In het voorjaar was de beworteling minder na toediening van N, vooral van knopen waarop al een zijscheut was ontwikkeld. Het lijkt er op dat de grotere dominantie van het groeipunt van de zijscheut het wortelen van de knoop, waarop deze zijscheut gevestigd is, remt. In perioden met slechtere groeiomstandigheden, zoals droogte, kan dit consequenties hebben voor de overlevingskansen van de zijscheut.

Een paar interacties zijn gevonden tussen klimaatsfactoren en beweiding- en N-behandelingen (Hoofdstuk 5). De snelheid van verschijnen van bladeren en zijscheuten was bij lagere temperaturen lager onder RG4.9 dan onder de andere twee beweidingbehandelingen. Bij hogere temperaturen was dit juist omgekeerd en waren de snelheden hoger onder RG4.9.

Structuur van populatie en plant

Klaverplanten werden beschreven aan de hand van de mate van uitstoeling, het drooggewicht van de verschillende plantendelen (stolon, blad, wortel en bloeiwijze), de stolonlengte en de aantallen stolonen, groeipunten, knopen, bladeren, bladstelen, bewortelde knopen en bloeiwijzen per plant (Hoofdstuk 4). De planten werden uit plaggen gehaald die regelmatig werden gestoken uit het grasland.

De geschatte plantdichtheid, gebaseerd op de dichtheid van groeipunten in het grasland en het aantal groeipunten per plant, varieerde tussen 650 en 1300 planten/m^2. In de winter en het vroege voorjaar was de geschatte plantdichtheid het hoogst, in de late zomer en vroege najaar het laagst. De populatie van planten varieerde sterk in grootte en structuur, met een duidelijk seizoenverloop. Over het algemeen waren planten van de tweede en derde orde van uitstoeling het belangrijkst voor de huidige klaverpopulatie. In het vroege najaar was de structuur van uitstoeling het meest complex. Daarna nam de complexiteit af. In het voorjaar waren de planten klein en van een relatief simpele structuur, met voornamelijk planten van de eerste en tweede orde van uitstoeling.

De beweiding- en N-behandelingen hadden maar weinig effect op de structuur van de populatie en de planten. De planten onder standweiden hadden een lager bladgewicht dan onder omweiden. Met toediening van N, waren minder knopen beworteld in het voorjaar. Het bleek dat betreding en/of uitwerpselen bijdragen aan het opbreken van planten in kleinere planten.

Conclusies

De doelstelling van deze studie was een bijdrage te leveren aan strategieën voor gras/klaver management en veredeling van klaver, waarmee klaver en kunstmest N in beweid grasland met succes zijn te combineren. Voor persistentie van klaver moeten zijscheuten de mogelijkheid krijgen zich te vestigen als jonge planten. Een goede beworteling verbetert de kansen voor vestiging van zijscheuten. Standweiden in de koelere perioden, gevolgd door omweiden als het warmer wordt, lijkt de vorming van zijscheuten en wortels te bevorderen. De graslandproductie kan in de koelere perioden van het jaar verhoogd worden door toediening van kunstmest N, waarbij klaver bij matige hoeveelheden kunstmest N niet blijvend onderdrukt wordt. Maar gebruik van kunstmest N resulteert in slechtere beworteling van klaver in het voorjaar. Juist dan zijn de planten klein en waarschijnlijk het meest gevoelig voor stress. De slechtere beworteling bij gebruik van kunstmest N kan deze gevoeligheid vergroten, en daarmee de persistentie van klaver verminderen in perioden van stress.

Behalve de persistentie van klaver, is ook de N binding in de warmere perioden belangrijk. Voor beide aspecten is het van belang dat veredeling zich richt op het wortelstelsel: de beworteling, de levensduur van de wortels, de capaciteit en efficiëntie van N binding, en de snelheid waarmee de capaciteit om N te binden wordt opgebouwd als de temperatuur stijgt. De genetische variatie in deze karakteristieken moet worden bepaald om vast te stellen of verbetering mogelijk is.

Toekomstig onderzoek

In dit onderzoek is gekeken naar hoofdstolonen met een groeipunt. Een deel van de populatie van hoofdstolonen heeft echter geen groeipunt meer. De dynamiek van zijscheuten en wortels aan deze stolonen is niet gevolgd, maar speelt wel een belangrijke rol in de klaverpopulatie. Ook werden de uitstoeling en beworteling van secundaire stolonen niet meegenomen in het huidige onderzoek. Deze aspecten dienen in toekomstig onderzoek aandacht krijgen om de structuur van de populatie en van de planten volledig te kunnen verklaren. Daarnaast dient het belang van de grootte en de structuur van de plant voor de persistentie van klaver te worden bepaald. Daarvoor kunnen gemerkte planten worden gevolgd terwijl er stress wordt uitgeoefend op de planten, zoals beschaduwing, ontbladering, gebruik van kunstmest N en een tekort of teveel aan water.

CURRICULUM VITAE

Jentina Bertha (Ina) Pinxterhuis werd op 21 juli 1965 geboren in Meppel. De eerste vijf jaar groeide ze op in De Wijk en eind 1970 verhuisde het gezin naar Pesse. In 1984 behaalde zij het atheneumdiploma van de openbare scholengemeenschap De Groene Driehoek in Hoogeveen. Aan de landbouwuniversiteit in Wageningen studeerde zij van 1984 tot 1990 Landbouwplantenteelt, oriëntatie Graslandkunde. Voor een afstudeervak Natuurbeheer bracht ze een half jaar door in Burkina Faso, waar begrazing door rundvee werd bestudeerd. Daarna vertrok ze naar Nieuw Zeeland voor een afstudeervak Graslandkunde. Hier bestudeerde ze klaver, begraasd door schapen, bij DSIR Grasslands in Palmerston North, Nieuw Zeeland.

In augustus 1990 keerde zij terug naar DSIR (later AgResearch) Grasslands om tot begin 1994 promotieonderzoek te doen dat tot dit proefschrift heeft geleid. Zij keerde voor korte tijd terug naar Wageningen om haar proefschrift af te ronden, maar al spoedig kreeg zij een aanstelling bij het Praktijkonderzoek Rundvee, Schapen en Paarden (PR) in Lelystad. Daar werkte ze bij de afdeling Weidebouw drie jaar aan analyses van databestanden, onder andere van het Bedrijfslaboratorium voor Grond- en Gewasonderzoek (nu Blgg Oosterbeek). In september 1997 kreeg ze een vaste aanstelling bij het PR als projectcoördinator biologische melkveehouderij voor Aver Heino. Dit proefbedrijf schakelde eind 1997 om naar biologische melkveehouderij. Alles viel weer op zijn plaats: rundvee en klaver!