Structure, Morphogenesis and Patterns of Defoliation of *Brachiaria decumbens* Stapf. Pastures

Juan Busqué Marcos

Thesis submitted for the degree of Doctor of Philosophy

Institute of Ecology and Resource Management
University of Edinburgh
October 2001

Declaration

This thesis has not been submitted in any other application for a degree and is the result of my own work and composition.

Juan Busqué Marcos
Edinburgh, August 2001

Abstract

This study aimed to contribute to the understanding of the morphogenesis and structure of signal grass (*Brachiaria decumbens* Stapf.) pastures, and how they are affected by the environment and cattle defoliation. A diagrammatic model was adopted to identify and relate the major morphogenetic and structural characteristics at different levels of organisation of the sward: phytomer, tiller, plant and population. The morphogenetic characteristics studied were leaf elongation rate (LER), internode elongation rate (IER), leaf senescence rate (LSR), leaf appearance rate (LAR), tiller birth rate (TBR) and tiller death rate (TDR). The structural characteristics analysed were length of phytomer components (blade, sheath and internode), number of phytomers per tiller, total length and number of live blades per tiller, number and size-age distributions of tillers per plant, and number and size distribution of plants and tillers per area. Experiments to analyse the effects of temperature, nitrogen application, season and defoliation intensity on sward structural and morphogenetic variables were performed under controlled and field conditions, and using destructive and non-destructive measurements.

An initial experiment under controlled environments showed highest LAR, LER and IER values at 30°C, and strong mutual regulations between morphogenetic and structural characteristics at the phytomer and tiller levels. The effects of cattle grazing and nitrogen fertilisation on the dynamics of signal grass morphology, and the patterns of defoliation were monitored during one year in long established pastures in Santa Cruz, Bolivia. Plant and population demography were mainly determined by the season: high number of seedlings and new vegetative tillers at the beginning of summer; new vegetative tillers, plant fragmentation, and flowering during summer (wet season); and low tiller and plant densities with low proportions of live leaf towards the following winter. Defoliation affected the phytomer and tiller levels of sward organisation, mainly through increases in the population of young tillers. All morphogenetic characteristics were mainly affected by season, with higher values during summer. LSR, IER and LAR were partly regulated by tiller size or age characteristics, while TBR and TDR were directly affected by the intensity of defoliation. During winter, soil water availability affected tiller growth more than temperature. The low doses of nitrogen applied in the field were effective in increasing LER and TBR after short spells at the end of winter, but not during the main growing season. Tillers of signal grass showed very high turnover rates, mainly because of their pattern of growth with internode elongation, the production of weak aerial tillers, and the speed in developing flowers during the reproductive season. The pattern of defoliation by cattle was best explained at the tiller level, due to the sward heterogeneity in tiller structure and the physical barrier imposed by the tiller stem.

A conceptual model integrating the information obtained from the experiments was developed as a synthesis for discussion. The applicability of information on sward morphogenesis and structure as a pasture management decision support tool, especially in the diagnosis of pasture degradation is finally considered.

Acknowledgements

Scholarship and other financial support for this research were granted by the Instituto Nacional de Investigación Agraria, Spain. CIAT Bolivia provided the experimental site and cattle. DFID-LPP Project R6606 provided the logistic and technical support for the experiments.

Special thanks are given to Mario Herrero for his friendship, supervision, support, advice and patience.

Acknowledgments are paid to Rose Mary Illescas, Paulina Chaverri, Beatriz Poquechoque, Isabel Velez, Wilson Lazarte, Victor Hugo Gestemberger, Ronald Palma and Juan Carlos Severiche for helping with the laboratory, field and digitising work.

To my friends and colleagues at the Institute of Ecology and Resource Management of the University of Edinburgh, at the Scottish Agricultural College, and at the Centro de Investigación Agrícola Tropical of Santa Cruz, Bolivia. Thanks to Salman, for helping me especially in the last moments of putting everything together.

Finally, thanks to my family for their continuous support wherever I was.

Content

List of Tables

List of Figures

Chapter 1 Signal grass. Description and justification of morphogenetic and structural studies

1.1 Introduction

Signal grass (*Brachiaria decumbens* Stapf.) is a C_4 grass species originally from central-east Africa but nowadays widely distributed in tropical and subtropical areas of the world (Hacker and Jank, 1998). Its high potential productivity and adaptability to a broad range of climatic and soil characteristics (CIAT, 1999) explains its wide current distribution and utilisation as forage. On the other hand, its relative recent introduction in many tropical areas, as in the "cerrado" savanna ecosystem of Brazil and Bolivia, and the scarcity of information available on its ecology and responses to grazing, have resulted in accelerated processes of pasture degradation (Macedo, 1997).

Up to date, research on signal grass has focused mainly on its agronomic characteristics (Fisher and Kerridge, 1996) relating dry matter yields to management treatments and soil nutrient conditions. Germplasm selection has also been based on these agronomic characteristics (do Valle *et al.,* 1993), and simulation models consider total aerial biomass per area as the sole unit of pasture productivity (Giraldo *et al.,* 1998). The use of area-based physiological models can initially be capable of predicting yields with changing external conditions, but are unable to explain the processes involved in the determination of pasture productivity. The failure of area-based models to distinguish between processes of pasture degradation and reversible fluctuations in productivity can lead to erroneous medium- and long-term yield estimations.

A more detailed approach to define sward productivity and state considers pastures as made of successive hierarchical levels of organization (Briske, 1989). The integration of these levels defines the same variables used in agronomic studies (i.e. dry matter yields and leaf area index). However, the study of the factors affecting the growth and fitness of each of the hierarchical levels gives a greater insight in self-regulation processes within the sward and in limits of plasticity of pastures to recover from external disturbances (Lemaire and Chapman 1996).

1.2 Signal grass: origin, morphology and agronomic characteristics

Signal grass has its centre of diversity in the surroundings of Lake Victoria: Kenya, Rwanda, Burundi, Uganda, Tanzania and Zaire (Keller-Grein *et al.,* 1996). The cultivar used in this study is probably *Brachiaria decumbens* cv. Basilisk, originally from Uganda, first evaluated by CSIRO, Queensland, and later selected by EMBRAPA, Brazil.

Signal grass adopts a caespitose growth form under low to medium aboveground biomass values (see figure 4.2). Its capacity to elongate its internodes from the initial stages of growth can result in long stems, 0.60-1 m high, if left to grow undisturbed. With long stems it tends to lie down, producing the decumbent growth form that gives it the name. The stem nodes can produce new tillers and roots, thus being capable of colonising new space vertically and horizontally through stolons. Blades are 1-20 cm long, usually glabrous and with spinulose margins. The inflorescence is a raceme of 1-4 spikes of 1-5 cm long each. Flowers of 4-5 mm of length are disposed in two rows at the sides of a flat rachis. Reproduction through seeds is apomictic.

Agronomic characteristics of *Brachiaria* forage species are well documented (Miles *et al.,* 1996). Signal grass is adapted to tropical areas with dry seasons of up to six months and minimum annual precipitation regimes of 800-1000 mm. It usually forms an aggressive, high yielding sward and for this reason it is not easy to maintain in association with legumes. It is also well adapted to low fertility acid soils through its root/shoot growth plasticity, nitrogen fixation through *Azospirillum* bacteria and phosphorus acquisition through the association of its roots with vesicular-arbuscular mycorrhizae (Rao *et al.,* 1996). It is highly susceptible to spittlebug (several genera of Homoptera: *Cercopidae*) attack (Valerio *et al.*, 1996) and can also develop hepatogenous photosensitization in cattle when consumed as a sole diet for long periods.

Signal grass in optimal conditions and low levels of fertilisation can produce 8-10 Mg DM ha^{-1} (McIvor, 1978; do Valle *et al.* 1993), but with high nitrogen fertilisation inputs it can reach more than 20 Mg DM ha^{-1} (Whiteman, 1980; Okeagu *et al.* 1989). The most commonly limiting nutrient for signal grass swards is nitrogen (Rao *et al.* 1996). Crude protein content is especially sensible to nitrogen fertilisation, increasing linearly from 0 to 150 kg N ha^{-1} (Alvim *et al.* 1990). *Brachiaria* species have much lower requirements of other nutrients, especially P and Ca, than other tropical forage grasses such as *Panicum maximum* (Rao *et al.* 1996).

1.3 Degradation of improved pastures in the tropics

Degradation of improved pastures is a generalised problem in livestock farms of tropical and sub-tropical areas, causing a major negative economic and environmental impact at the farm and at the regional level (Boddey *et al.,* 1996). Pasture degradation is linked to an inadequate grazing management (Jones and Jones, 1997), leading in the medium and long term to soil nutrient depletion and lack of regeneration capacity of the introduced forage plants. Though pasture degradation is normally detected after a few years (Macedo, 1997), it is mostly originated from incorrect management practices leading to changes in the dynamics of the pasture in the short term. These changes may lead to sward structures that produce soil degradation through erosion and compaction, and pasture changes through the invasion of weeds and lost or decrease in the participation of desired species. Pasture degradation can thus be studied at different temporal scales. While long-term studies relate more to soil nutrient depletion, short-term studies should consider morphogenetic and structural characteristics at the different levels of sward organisation (Rickert, 1996).

1.4 The use of population studies in grasslands

A pasture can be studied at the whole population or at the individual plant level. These approaches coincide, at the plant community level, with the two general views of structure in plant ecology: Clements' and Gleason's views (Crawley, 1997). Plant populations develop under constraints of limited resources that are 'area' determined (see figure 2.1 under sward patch), with the result that holistic properties of community performance, such as productivity, can be recognised (Harper, 1978). However, the persistence of a species in a pasture community is the result of the fitness of its individuals, which normally shows little correlation with productivity. As J.L. Harper put it: "Fitness does not depend on maximizing physiological function but in leaving *more* descendants than neighbouring plants are able to do" (Harper, 1978). These ecological principles can partially explain the short persistence of highly productive forage species after establishment if fertilisation is not frequent (Macedo, 1997). Models that predict pasture productivity and livestock utilisation per area of land (Herrero *et al.,* 1998) are unable to forecast pasture persistence and thus fail to predict pasture yield if external conditions affect plant fitness. Modelling pasture persistence needs to consider plant demography and population dynamic processes explicitly (Hay *et al.,* 2000). In addition to environment and management conditions, the existence of intrinsic factors regulating the performance of the individual units is best recognised if the pasture is considered made of successive hierarchical units (Briske and Silvertown, 1993).

The objective of this thesis is to provide evidence of the effects of intrinsic and extrinsic factors upon the structure and morphogenesis of successive demographic units of signal grass pastures, and how they ultimately affect pasture persistence and productivity. The scarcity of this type of information in tropical grasses (Humphreys, 1991) will permit the comparison of these results with those of temperate grass species. It will also help in interpreting other experiments made on signal grass of a more agronomic nature, and will hopefully serve as a reference in future research on pasture production and livestock management in tropical grazing systems.

1.5 Structure of the thesis

This thesis can be divided in three main parts. The first part (chapter 2) corresponds to a literature review on morphogenetic and structural characteristics of grasses. The second part (chapters 3 to 6) comprises the experimental work of the thesis. The last part (chapter 7) synthesises the information obtained from the different experiments.

Chapter 2 provides a comprehensive literature review on morphogenetic and structural characteristics of grass species at the phytomer, tiller, plant and population levels. The information mainly relates to temperate species, though available data from tropical species is shown.

The experimental part of the thesis can be initially divided according to the location of the experiments. The first set of experiments (chapter 3) were performed in growth rooms under controlled environments, while the second group of experiments (chapters 4 to 6) were performed in the field.

Chapter 3 describes the effects of nitrogen and temperature on morphogenetic and structural variables of leaves and tillers of signal grass grown in microswards under controlled environments. Measurements were made very frequently and at the leaf level. Regulations by intrinsic tiller and population characteristics were also considered.

Field experiments were performed in well established signal grass pastures in Santa Cruz, Bolivia. These experiments can be classified in two groups according to the nature of sampling: destructive (chapter 4) and non-destructive (chapters 5 and 6).

Chapter 4 considers the structure dynamics of established signal grass pastures at all its morphological hierarchical levels, and under different grazing intensities for nearly a year. The destructive nature of the experiment allowed for the special consideration of the dynamics and structure of the plant level of pasture organisation.

In chapter 5 the patterns of defoliation by cattle are described for different grazing intensities, seasons of the year and levels of organisation in an established signal grass sward.

Chapter 6 details the effects of nitrogen fertilisation, grazing intensity and period of the year upon morphogenetic characteristics of signal grass leaves, tillers and populations in established swards.

Chapter 7 gives a general discussion of the experimental results of chapters 3 to 6 within the framework used in chapter 2. Implications for research and pasture management are commented and further discussion is made on the advantages and limitations of the study and on possible future improvements.

Chapter 2 A review on morphogenesis and structure of grasses at different hierarchical levels[*]

2.1 Introduction

Demographic processes and architectural attributes are of greater importance than physiological processes in defining the tolerance and persistence of a grass species to hervibory (Hendon and Briske, 1997). The persistence of a forage species in a sward depends on its longevity and its reproductive performance (Humphreys, 1991). These two characteristics can be noticed at most of the levels of the pasture organisation: phytomer, tiller, plant and sward patch (Figure 2.1). Within a tiller, phytomers have a precise life duration, and new phytomers appear successively until the tiller flowers or dies. A plant is made of interconnected tillers of variable life durations, and new tillers can appear vegetatively. A patch sward is made of a number of plants that vary according to their longevity and the appearance of new plants through seed germination or fragmentation of existing plants. A patch of sward can also be considered within this structure: it can be cleared of plants under punctual disturbances (e.g. dung deposition, pests) and be recolonised by new seedlings or by rhizomes or stolons from adjacent sward patches.

Climate and management can change the persistence strategy of a plant species in a pasture depending on its plasticity (Figure 2.2). The amount of precipitation during the dry season proved to be a major limiting factor in the vegetative reproduction of the tropical legume *Macroptilium atropurpureum* cv. Siratro (Thailand in figure 2.2; Gutteridge, 1985). In the case of wetter dry seasons (Australia in figure 2.2; Walker, 1980) stocking rate defined the importance of the vegetative or seedling regeneration pathways in the persistence of the pasture. This shows that, within certain limits, the different plant regeneration strategies and their survival rates compensate to produce plant densities allowing the persistence of the species in the pasture.

---

[*] Based on Busqué, J. and Herrero, M. Atributos funcionales y su implicación en el manejo de pasturas. *Pasturas Tropicales* (in press).

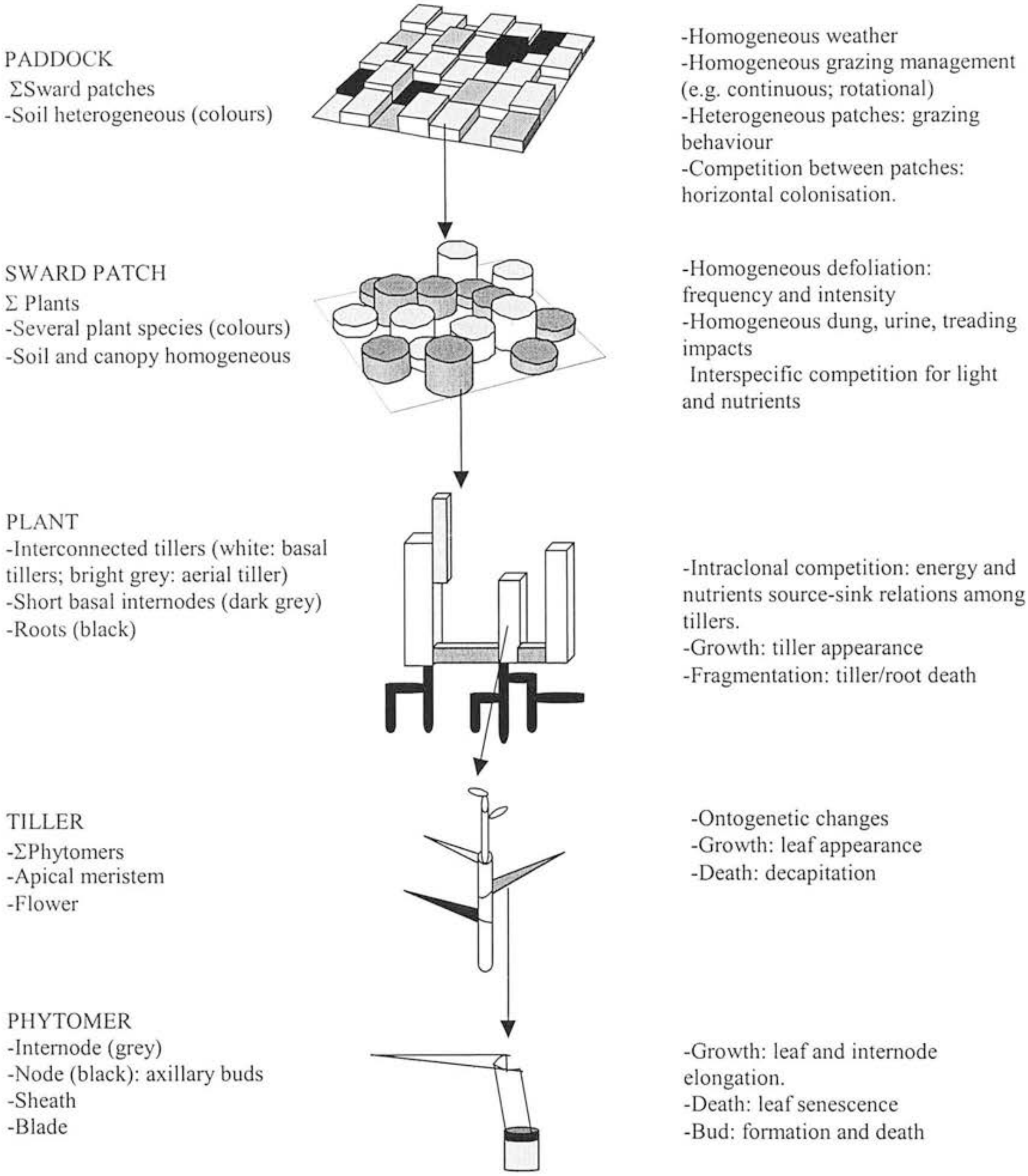

Figure 2.1. Hierarchical model of pasture organisation. The left column defines the components of each level. The right column defines the morphogenesis of each level and the factors affecting it at the same level.

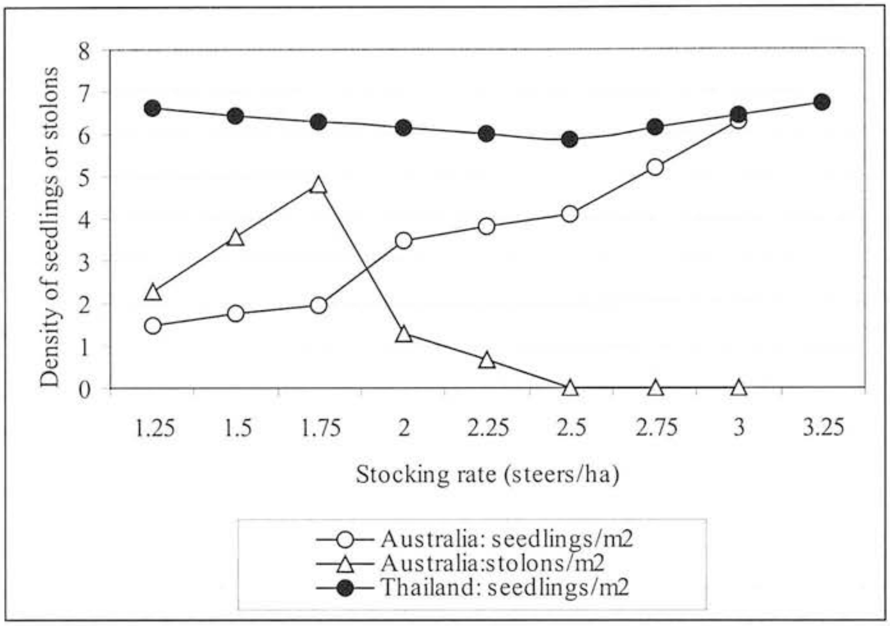

Figure 2.2. Comparison of persistence strategy of the tropical legume *Macroptilium atropurpureum* cv. Siratro to grazing intensity in two locations with contrasted intensity of drought during winter: Australia, with 30% of annual rain during winter; Thailand, with 15% of annual rain during winter. Adapted from Gutteridge (1985) and Walker (1980).

This review deals with the longevity and vegetative plant replacement of forage grass species. Information on tropical grass species will be presented when available, though most of the knowledge on the subject comes from experimentation with temperate grasses. Clonal growth is the common reproductive strategy of forage species in grasslands not subjected to extreme disturbances, especially drought and severe grazing (Briske and Silvertown, 1993); thus it prevails in tropical areas unless dry periods are too long or extreme, or overgrazing occurs. Sward structure at different levels is also considered in this review, as, apart from affecting morphogenetic characteristics, it also defines the architectural attributes of the grasses, of great importance in its tolerance to herbivory.

The following sections describe the morphogenetic variables acting at the different levels of organisation of the sward, their relationship with the structure at that and contiguous sward levels, and the intrinsic and external sources regulating their values.

2.2 The Phytomer

The phytomer is the smallest level of organisation considered in this work (Figure 2.1). It is an iterative unit consisting of differentiated organs: internode, node, axilar meristematic bud, adventitious root primordia, blade and sheath. Its growth and senescence is a direct consequence of the multiplication, elongation and death of cells initially located in meristematic tissue next to the node. The growth of a phytomer can occur at both sides of its node, elongating the blade and sheath in one side or the internode in the opposite side. The main morphogenetic variables considered at this level are leaf elongation and senescence rates and internode elongation rate. From now on, leaf will be used when considering blade and sheath together. The main structural variables derived partially from the above morphogenetic characteristics are blade, sheath and internode lengths.

2.2.1 Leaf elongation

Leaf growth in a grass is the result of two different cell growth processes spatially and temporally delimited: cell division in the meristem at the leaf base, and cell elongation in a zone immediately above the meristem (Volenec and Nelson, 1983). Changes in the rates of both processes affect directly the leaf elongation rate (LER), but while cell elongation affects LER immediately, cell division only affects measurable LER after a delay of several hours (Durand *et al.,* 1999). Four stages in the elongation of a grass leaf are normally observed (Skinner and Nelson, 1995): (i) cell division only, with establishment of the epidermal cell division zone, (ii) cell division and cell elongation with the establishment of the entire elongation zone, (iii) cell elongation predominant until cell division ends, and (iv) cell elongation only until all cells are mature. The first two stages can have similar duration to the last two, but they end at very small leaf lengths: 1-2 mm and 20-30 mm respectively. The third stage is characterised by the highest absolute LER along the leaf development, and thus contributing most to the final leaf length. In spite of their small absolute contribution to leaf length, the first two stages define the magnitude of the latter leaf growth. LER is more related to the number of cells elongating (i.e. cells produced in the first two stages) that to the rate of elongation per cell (Skinner and Nelson, 1995).

LER has been observed to be stable with leaf insertion level in the tiller (Bultynck *et al.,* 1999; Duru and Ducrocq, 2000a), or to decrease in some tropical grasses (Murtagh *et al.,* 1987).

External effects

LER is the leaf morphogenetic variable more sensible to the tiller external conditions. Water (Schnyder and Nelson, 1988), nitrogen (Gastal and Nelson, 1994) and temperature (Durand *et al.,* 1999) affect LER through their effect at the initial stages of leaf development. The response of LER to water stress is curvilinear, with cessation of leaf growth for tropical grass species at predawn leaf water potential values of over -1 MPa (Toft *et al.,* 1987; Jones, 1988). Leaf elongation under water stress periods lasts longer in frequently defoliated swards due to their lower transpiration surfaces and thus their longer soil water availability (Toft *et al.,* 1987). Nitrogen affects much more the rate of cell division than that of cell elongation (Gastal and Nelson, 1994). An increase in temperature leads to an increase in the activity of the leaf meristem through a response in the rate of cell division and a co-ordinated response of cell expansion rate (Lemaire and Millard, 1999). The response of meristems to increasing temperature is under the control of the concentration of substrate nitrogen and, reversibly, the maximum rate of substrate nitrogen use for leaf growth is a saturating function of temperature (Sheehy *et al.,* 1996).

Coordination between blade and sheath elongation

The relative length of blade and sheath in a leaf is an important characteristic in grasses, as it affects directly the structure of the sward and its way of utilisation by livestock. From the point of view of animal nutrition the leaf blade is the most interesting part of the plant because of its higher quality, digestibility and accessibility to the grazer. From the point of view of pasture productivity, the leaf blade is the main photosynthetic organ of the plant. The sheath is characterised by lower forage quality (Deinum *et al.,* 1996) and a more rigid anatomy, appropriate for its supportive function in the tiller.

A light signal is the most probable explanation in the shift from blade to sheath elongation in a growing leaf. This shift is related with the length of the subtending sheaths, and acts upon the cell division and elongation zones of the growing leaves (Casey *et al.,* 1999). In addition to the regulation of the timing of blade/sheath elongation, the length of subtending sheaths also exerts a direct positive effect on the total duration of leaf elongation, with the production of a variable number and size of cells (Casey *et al.,* 1999). In this way, a stable relationship between final sheath and blade lengths for each leaf is established, at least during the vegetative phase of the tiller growth, though there are factors like the external light quality, capable of changing this relationship. When comparing leaf lengths under different light qualities, Casal *et al.* (1987) observed that under low red far-red ratios, as those inside close canopies, tillers of warm-season grasses showed higher sheath lengths while blade lengths remained constant. This indicates that the subtending sheath length regulates blade length (Begg and Wright, 1962), while the amount of elongation of the sheath is also affected by the external light quality. In this case, the plasticity in sheath length could response to changes in LER or in leaf elongation duration, or in both at the same time. Allard *et al.* (1991) showed that increases in leaf area with increasing shade involved increases both in LER and the duration of the elongation period. Duru and Ducrocq (2000a) also point out the possibility of certain plasticity in leaf blade length for equal lengths of subtending sheaths. They suggest that this plasticity depends on the external conditions (e.g. temperature, nitrogen) at the time when the growing leaf is in its cell division stage. If growth conditions are favourable cell multiplication should be greater, and consequently the lamina length should be greater for the same sheath length.

The last leaves appearing in a tiller during its flowering stage are characterised by acute changes in the lengths of blades and sheaths. Blades turn to be consecutively shorter while sheaths are longer. These changes are not well understood, but they are probably related with the overall physiological changes starting at the onset of the reproductive stage, well characterised by the increase in internode elongation and leaf elongation rate (Parsons and Robson, 1980; Gastal *et al.,* 1992).

2.2.2 Internode elongation

Internode elongation in clonal species is considered a process to colonise favourable light environments (Hutchings, 1997). It occurs as an integrated part of vegetative growth in many grasses, whilst in others it is only associated with reproductive development (Silsbury, 1970). In temperate grasses it is usually associated with stoloniferous species and even caespitose species whenever disturbances like nodes burial occur (Brock and Fletcher, 1993). In the case of tropical species internode elongation is more widespread and can be found in grass species with any type of growth form. Its occurrence is of great importance in the definition of the sward structure and in the use herbivores will make of it, as leaf and stem vertical disposition will determine the grazing behaviour (Stobbs, 1973). The mechanisms associated with the growth rate of the internodes (IER) and its duration are unknown in stoloniferous grass species. Light quality is considered to be the major factor regulating internode lengths, as it occurs in dicotyledonous species (Casal *et al.,* 1987).

2.2.3 Leaf senescence

The rate at which leaves die (leaf senescence rate: LSR) determines, along with the rate of elongation or appearance of new leaves, the instantaneous amount of live leaf existing per tiller (Chapman and Lemaire, 1993). It also affects the amount of litter in the ground and its rate of decomposition in the soil (Thomas and Asakawa, 1993; Rezende *et al.,* 1999). The determination of the patterns of leaf senescence is required to predict the dynamics of gross and net herbage production of the pasture. These aspects have straightforward implications both in animal productivity (i.e. quality of the forage) and pasture productivity (i.e. nutrient cycling of plant dead material through the soil).

Most of the studies consider leaf senescence rates exclusively for the leaf blade. In this section leaf and blade will be considered synonymous. Depending on the detail of the analysis, leaf senescence can be studied as the rate at which whole blades die (i.e. leaves d^{-1}) or as the rate of death per leaf (e.g. mm of blade d^{-1}). The first type of study permits, along with the calculation of leaf appearance rate, the determination of the maximum number of green leaves per tiller (Vine, 1983). This number is genetically determined (Lemaire and Chapman, 1996) and does not change much along the year. The number of green leaves per tiller can serve as a fast and easy measurement tool useful for grazing management decisions (Fulkerson and Slack, 1994).

The life of a leaf can be divided in three stages: the time from initiation to total expansion, the time in which the leaf remains green and expanded, and the time from the initiation of senescence until the total death of the leaf. Normally LSR is calculated from the time when the leaf reaches it total expansion. The duration of maximum expansion with no sign of senescence has been negatively related in temperate grasses with the temperature during the last part of the elongation stage (Vine, 1983). The timing of water availability seems to be more important than temperature in the rate of leaf senescence of tropical grasses (McIvor, 1984). High water stress following wet periods accelerates senescence, while sustained water deficits delays it (Wilson and t'Mannetje, 1978). Also the level of insertion of leaves in a tiller affects the LSR, with leaves of high insertion number staying green for a longer period and senescing later more slowly (Wilson, 1976). These changes of LSR for leaves within a tiller are more plausible for first-order tillers (i.e. seedlings and the first to appear vegetatively afterwards) than for older tillers.

2.2.4 Leaf appearance

Leaf appearance rate (LAR) is considered the most important leaf morphogenetic characteristic because of its effects on different structural variables of the sward (Chapman and Lemaire, 1993). LAR is related with tiller density by the presence of a tiller bud in each new phytomer. It is related to the number of green leaves per tiller when balanced with LSR considered at the whole leaf level. Finally, the relation of the phyllochron (i.e. inverse of LAR) with the plastochron (i.e. interval between the initiation of two consecutive leaves), and the regulations existing between elongating and initiating leaves within a tiller (Skinner and Nelson, 1994a) links LAR and LER to determine maximum leaf lengths. Knowing the tiller density, the number of green leaves per tiller and their length, it is possible to determine whole sward structural variables like leaf area index or green leaf herbage mass.

The relatively recent recognised importance of LAR (Chapman and Lemaire, 1993), coupled with its ease of measurement (Laca and Lemaire, 2000), has resulted in its consideration in many studies of sward dynamics, especially in the last decade.

Coordination of leaf appearance within the tiller and the length of a leaf

The phyllochron depends mainly on the lapse of time between the initiation of the stage of high leaf elongation (stage iii under leaf elongation; section 2.2.1) of two successive growing leaves, and on the difference in length of their respective subtending sheaths (Grant *et al.,* 1981). Different types of regulation between growing leaves in a tiller have been documented for different grass species (Skinner and Nelson, 1995). Skinner and Nelson (1994b) found coordination among three successive growing leaves in tall fescue: while the youngest leaf started initiation (stage i), the preceding leaf changed from rapid elongation of the blade to that of the sheath, and the oldest growing leaf ceased its cell division (stage iv). It is possible to predict LAR and leaf size considering this coordination and leaf elongation duration related to the environmental conditions at the initial stages of leaf growth (Durand *et al.,* 1999). Depending on the grass species, it may be feasible to establish simple empirical relations between LAR and LER that can explain satisfactorily leaf size (Chapman and Lemaire, 1993; but see Bahmani *et al.,* 2000). The increase of phyllochron with leaf insertion number, observed in many studies, has usually been related to increasing sheath lengths (Yin and Kropff, 1996; Bonnett, 1998; Duru and Ducrocq, 2000a).

Leaf appearance rate and tiller density

When vegetative reproduction is the main path of persistence of a grass species in a sward, linear positive relations can usually be found between LAR and tiller density. The intermediate processes existing between the appearance of a phytomer and the appearance of the tiller associated with it may define different strategies of regeneration that will be discussed in the next section. The existence of a relation between LAR and tiller density for different grass species is more remarkable when average values for whole seasons or years are considered. Figure 2.3 shows that this relationship was similar for three warm-season grass species cohabiting in the same sward. The added contrasted sensibility of LAR to tiller age, depending on the grass species, suggests that it may be a factor determining species balance in multispecific pastures (Agnusdei *et al.,* 1997).

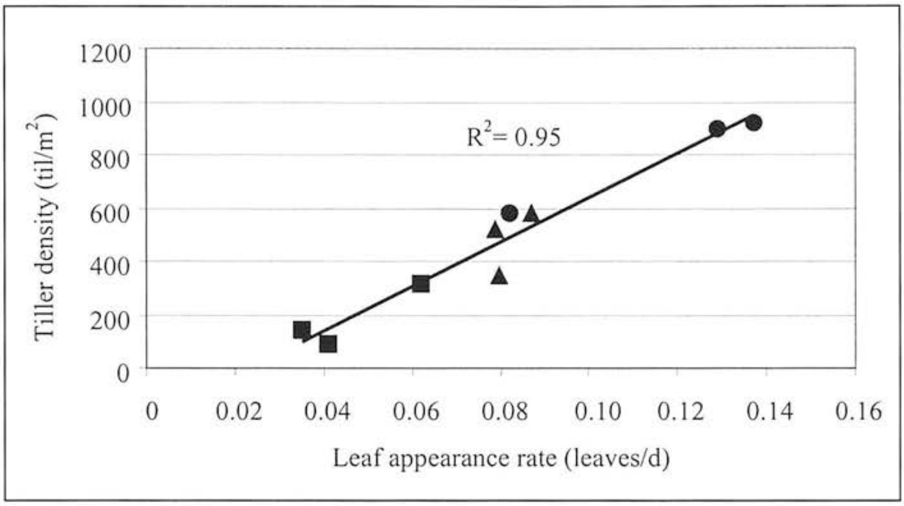

Figure 2.3. Relationship between average leaf appearance rate and tiller density of three cohabiting warm-season grasses of the humid pampa: *Leersia hexandra* (■), *Paspalum dilatatum* (▲), and *Cynodon dactylon* (●) (adapted from Agnusdei *et al.,* 1997).

External regulation

LAR is more affected by temperature than by any other environmental factor (Anslow, 1966; Ong and Baker, 1985). While in temperate grasses maximum leaf appearance rates are obtained at 20-25°C (Parsons and Chapman, 2000), in tropical grasses optimum temperatures lie around 30°C (Ivory and Whiteman, 1978; Herrero *et al.,* 2000). Soil water and nitrogen availability also affect LAR, usually to a lesser extent than they affect LER, except when reaching very low values.

2.2.5 A model of leaf growth in the tiller

Once the leaf is initiated, the rate of cell division (stage i) is mainly a function of the meristem temperature (Durand *et al.,* 1999) and the nitrogen status (Gastal and Nelson, 1994). The more the number of cells produced the more the potential length of the mature leaf. Once cell elongation starts (stage ii), it will depend mainly on the leaf water status (Schnyder and Nelson, 1988) and on temperature. At one point in the leaf elongation, the activation by a light quality switch, function of the length of the subtending sheath, changes cell division from blade to sheath. From this point on the blade will finish its elongation with lower relative elongation rates, while the sheath will start to elongate rapidly. The final blade length will be mainly determined by the previous sheath length, but some plasticity will exist depending on the final number of cells it had when the light quality switch occurred. The amount of sheath growth will depend on different factors. The number of cells available to elongate will determine how much the sheath can potentially elongate. If there is a low number of cells available, the sheath will be shorter than it could be expected from the external conditions existing. That is the case of leaves initiating (stages i and ii) with low temperatures, and after elongating (stage iii) with higher temperatures. Morphologically this is represented by a newly developed leaf with a sheath shorter than the sheath of its previous leaf. If the number of cells is large enough, sheath elongation will continue up to a point regulated intrinsically by the initiation of a new leaf (two younger according to Skinner and Nelson (1994a)), or extrinsically by the light conditions in the sward canopy. If the second hypothesis rules, then the end of sheath elongation would be the signal activating the initiation of the new leaf. Either case (alternative cause and effect) would produce the same result. Schematically the alternative processes are:

-Light change→Cessation of sheath cell division→Initiation of new leaf (2 younger)
-Light change→Initiation of new leaf (2 younger)→Cessation of sheath cell division

The successive increases in sheath length that under normal growing conditions are produced by this intrinsic tiller regulatory process, are usually accompanied by successive increases in blade length. This trend changes with tiller ontogeny (i.e. from vegetative to reproductive growth) or with defoliation. Tiller defoliation by livestock implies in many cases the excision of its youngest sheaths. If defoliation is severe it also produces a short-term halt in growth until the plant substrates and reserves are directed towards the aerial meristems (Richards, 1993). These changes produce a sharp decrease in the length of the next leaf to appear, but the increasing length trend will be regained afterwards if no other disturbances occur. The plasticity a plant has to reduce its leaf length in response to defoliation is considered as an adaptive trait of a grass species (or variety) to grazing, as this is a way to position more meristems out of reach of the herbivore (Chapman and Lemaire, 1993; Briske, 1996).

2.3 The Tiller

The tiller is the largest clonal unit easily recognisable in the sward. It comprises successive leaves and finally an inflorescence. Leaves and flowers are originated in a shoot apex consisting of a meristematic apical dome continuously differentiating leaf primordia. Tillers can appear from seed or vegetatively from axilar buds of phytomers. They are usually connected to other tillers, forming a bigger sward unit: the plant (Figure 2.1). The net growth of a tiller is the sum of the net growth of the units it comprises: the phytomers. The morphogenetic characteristics of the tiller would then be its rates of leaf elongation and senescence and internode elongation, measured at the tiller level, its rate of appearance and its rate of death. Leaf elongation or senescence are not usually the same when measured at the leaf or at the tiller level. The occurrence of synchronous growth or senescence of two leaves at the same time, or on the contrary, the existence of a lapse of time without noticeable growth or senescence between consecutive leaves explains the different values obtained measuring at the tiller or leaf level (Duru and Ducrocq, 2000a). In any case, both types of measurements are usually very correlated while the tiller remains in the same ontogenetic state, as the type of coordination between leaf growth initiations are stable (see section 2.2.4). The structure of a tiller can be well represented by its total length of live leaf (or its number of live leaves) and its stem length. The consideration of these variables serves also to define the vertical distribution of live leaf in the tiller.

As the rates of leaf and internode elongation and leaf senescence were already reviewed in the previous section, this section will cover the processes involved in the regulation of tiller appearance and the causes of tiller death. Figure 2.4 represents the morphogenetic and structural variables involved in these processes.

2.3.1 Tiller appearance

The path from the appearance of a leaf with its corresponding axilar tiller bud to the appearance of a new established tiller is regulated by multiple mechanisms that act at different stages of the tiller development (Figure 2.4).

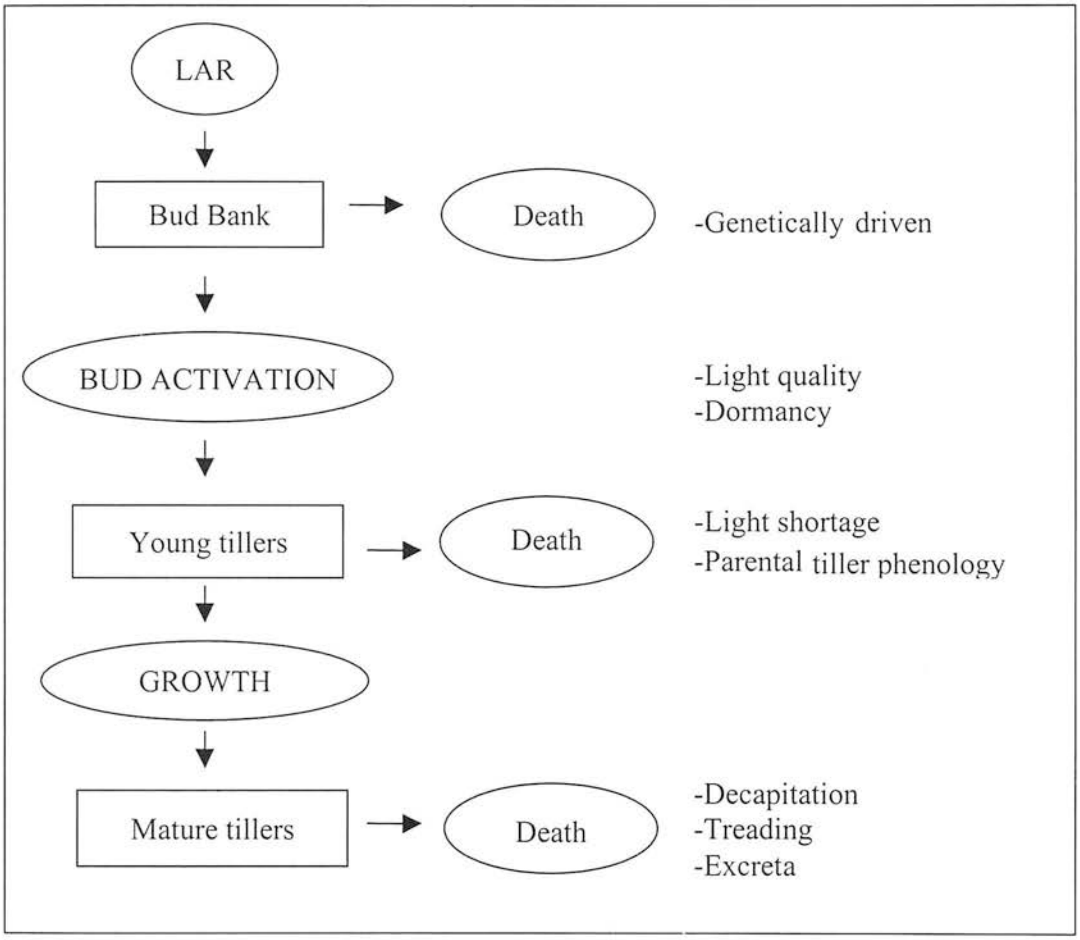

Figure 2.4. Processes and states involved from the appearance of a leaf (LAR) until the establishment of a new vegetative tiller (left column), and possible causes of death at each of the states (right column).

The bud bank

Axilar buds develop along with the phytomers but they usually don't progress towards the formation of new tillers immediately. Instead they incorporate into the bud bank (Harper, 1977), equivalent concept to that of the soil seed bank in the case of the sexual reproductive path. The magnitude of the bud bank in the sward and its seasonality depends on the density of established tillers, the number of buds per tiller and the longevity of the individual buds. The population of established tillers and their age determines the potential number of active meristems in the sward. Two contrasted patterns in their dynamics can be described (Briske, 1996). The first pattern refers to a synchronous tiller development, characterised by a wide fluctuation in the potential number of active meristems under grazing, depending on the time of the year in which the sward is grazed. Thus, low fluctuations occur while grazing coincides with tillers in a culmless state, while high fluctuations can occur if the sward is grazed when tiller culms are developing synchronously and defoliation involves apical meristem decapitation. The second pattern involves grass species in which tiller development is asynchronous, producing a much less pronounced seasonal variation in tiller populations in response to grazing. Examples of both patterns are found in different tropical grass species (Mott *et al.,* 1992).

The second variable defining the magnitude of the bud bank refers to the longevity of the buds. Again, two contrasting interpretations exist (Hendrickson and Briske, 1997). One interpretation is that longevity of axilar buds exceeds that of parental tillers, while the other interpretation is that buds that do not grow out following maturation of the parental tiller rapidly senesce. The first interpretation leads to bud banks of long duration. Hendrickson and Briske (1997) found that buds of parental tillers of grass species of semi-arid ecosystems remained viable or dormant 12 months after the death of the parental tiller. They suggest that this trait would be more adaptive of species of arid or semiarid environments or of those of systems subjected to long-term intensive grazing.

Bud activation

In potentially active axillary buds, activation is a function of the quality of the light (ratio of red to far-red radiation) reaching the bud (Deregibus *et al.,* 1983; 1985), irrespective of the existing amount of photosynthetic active radiation (Briske and Silvertown, 1993). The ratio of red to far-red radiation seems to act as a sign to the whole plant (see also the effects on leaf growth under section 2.2.), informing of the availability of light and space resources in its microenvironment. This effect is reflected in the relation found between sward leaf area index (LAI) and tillering in different temperate grass species, with tillering rates approaching zero for values of

LAI just over 3 (Simon and Lemaire, 1987). The effect of shading on axillary bud activation is also regulated by the plant, affecting much less buds belonging to the main shoots than those of higher order tillers (Bahmani *et al.,* 2000). Tiller ontogenetic changes can also affect tillering rates, with tillering before and after, but ceasing at the time of flowering. This occurs in temperate (Colvill and Marshall, 1984) and tropical grass species (*Panicum maximum* var. *trichoglume*; Humphreys, 1991).

Tropical grass species that elongate their culms from early stages of their development do so to position their leaves in favourable light environments of the sward canopy. The appearance of tillers from axilar aerial nodes is common in these species, especially when defoliation produces an excision of the sheath and the bud is readily exposed to a different light quality. The production of aerial tillers is an added source of plant assimilating tissue in an advantageous light position, when regeneration from basal tillers may not be possible due to lack of bud activation under closed canopies. Aerial tillers may also serve as future rooted tillers in many species with a tendency to fall down and adopt a stoloniferous growth habit if bare ground is available in its vicinity (e.g. *Brachiaria decumbens*). As compensation to the advantages, sward structures that promote aerial tillers have a higher proportion of their herbage dry matter as stem, and presumably have higher energy costs to cover their nutritional and metabolic requirements. Aerial axilar buds differentiate from basal axilar buds in that they usually show short lifespans, normally coincident with the lifespan of their attached leaves. Once the leaf dies and separates from its node the bud is negatively exposed to the environment and will die fast if it does not receive the adequate light quality.

In many graminaceous species there is a large proportion of viable axilar buds that do not activate when the adequate light requirement is met and other environmental conditions are favourable (Noble *et al.,* 1979). This pool is known as dormant buds. There are still no clear explanations as to what are the environmental conditions and the physiological processes required to break bud dormancy (Murphy and Briske, 1992).

The classical concept of apical dominance, stating the inhibition of axillary bud activation while the apical meristem of the tiller remains present, has proved to be inconsistent for grasses, as in many cases tillering occurs in plants with intact apical meristems (Murphy and Briske, 1992).

The higher tillering response to frequent defoliation of forage grass species adapted to mesic, fertile environments, independently of the time scale considered (Briske and Silvertown, 1993), has facilitated the adoption of indexes of bud activation efficiency, like the site filling ratio (Davies, 1974), or site usage (Skinner and Nelson, 1992). These indexes relate leaf appearance rates with tillering rates, and have proved valuable to compare different species and varieties under equal low sward LAI values. For some temperate species negative genetic correlations between LAR and tillering rate have been found in different varieties (Van Loo *et al.,* 1992).

2.3.2 Tiller death

As the causes of death are usually different, tillers are distinguished as new and mature (Figure 2.4).

New tillers

The scarcity of resources in their microenvironment is the main cause of death in newly born tillers of vegetative swards. Among the environmental factors, light is usually more limiting for the survival of new tillers than soil nitrogen (Ong, 1978; Ong *et al.,* 1978). This is related with the increase of sward mass and is part of a tiller density dependent regulation (see section 2.5.1).

Tillers born from parental tillers about to flower do normally suffer also from shadowing, as swards have high herbage mass at that stage. The supply of assimilate from the parental flowering tiller to the new daughter vegetative tiller is minimum (Colvill and Marshall, 1984), and these small tillers are prone to die unless decapitation of parental tiller occurs before the flowering stage finishes. If decapitation occurs, assimilates that were aimed at seed development are directed towards the small daughter tillers and the chances for their survival increase. The survival of these daughter tillers is important in the establishment of the new tiller base for the following year (Matthew *et al.,* 1991).

For grass species characterised by producing large bud banks, death of young tillers has also been associated with the age of the parental tiller node from where they appeared, with less vigorous tillers from older proximal buds compared to those developed from more distal buds (Mueller and Richards, 1986).

Mature tillers

In grazing systems the common causes of death of mature tillers are related to the action of the herbivores: trampling, dung and urine excretion and defoliation. Mature tiller death may also come when limiting environmental conditions are reached, impeding the normal physiological metabolism of plants. Finally, death may also be the result of the end of the natural tiller life, following flowering and seed maturation.

In grazing and cutting systems, death of mature tillers by decapitation of their apical meristems is common in erect grass species with developed internodes. It is considered the main cause of tiller death in temperate grasses during spring, when a large proportion of tillers is reproductive and thus with elongated stems (Woodward, 1998). In tropical grass species with culmed vegetative tillers, tiller death rate due to apical meristem decapitation will be a function of the intensity and frequency of defoliation, and of the architectural plasticity of the plant (Humphreys, 1991; Briske, 1996). Some grasses of erect habit (e.g. *Pennisetum purpureum*, *Brachiaria brizantha*, *Panicum maximum*) show low architectural plasticity to change the vertical height of their apical meristems, and a linear relationship between grazing intensity and tiller death rate exists. This relation has been established for tropical and temperate legume species with different growing point heights (Clements, 1989). Other grasses of greater plasticity (e.g. *Cynodon dactylon*, *Pennisetum clandestinum*) accommodate the majority of their growing points to heights under those of grazing, after a pattern of grazing frequency is established. It is important to establish the degree of architectural plasticity of grass species and varieties to vary their apical meristem heights, considered as an avoidance strategy to grazing. But this should always be matched with the quantification of the tolerance strategies of the plant to grazing (Briske, 1996), expressed mainly through its tillering capacity. The result, the seasonal balance between tiller births and deaths, and the effects of grazing and other management practices, gives an indication of the sensibility of the sward population to the different actions and their timing (Bullock *et al.*, 1994a).

2.3.3 Tiller structure

The length of live leaf per tiller is the balance between leaf elongation and leaf senescence. As leaf senescence lags in time with respect to leaf growth a duration equivalent to the leaf lifespan, the initial net growth of a tiller is a function exclusively of its leaf elongation rate (Chapman and Lemaire, 1993). Once leaf senescence starts, net leaf growth will decrease and will reach a plateau whenever leaf senescence rate equals leaf elongation rate. Net leaf growth can also be negative if leaf senescence is greater than leaf elongation. This is the case when leaf (i.e. blades) senescence and elongation rates are measured at the tiller level and changes towards reproduction occur in the tiller, with the consequent decrease in the blade to sheath length ratio.

If the sward is assumed to be composed of tillers similarly developed, a logical management aim would be to set a grazing frequency equivalent to the time taken for the tiller to reach its plateau in live leaf length. In this way leaf losses to senescence would be minimised and green leaf herbage harvested would be maximised in the long term.

2.4 The Plant

The plant is an integrated physiological unit of tillers of the same genetic origin (Figure 2.1). The number of tillers comprising a plant is a function of their rates of birth and death and of the fragmentation of the plant at any of its connective internodes. The aboveground structure of a plant can be defined using its total live leaf length (or weight, or area) and its total length of other organs (e.g. stems). The growth of a plant will then be the result of the individual growth of tillers (see section 2.2.) and of the recruitment of new tillers and death of existing ones (see section 2.3.). The balance between these two processes has traditionally been studied at the sward level, without considering the plant (Nelson and Zarrough, 1981). The main reason for the oblivion of the plant level comes from the difficulty to distinguish individual plants in swards of caespitose or stoloniferous grass species (Hay *et al.,* 2000). The interest in studying the plant lies in the existence of intraclonal regulations that may explain certain processes not detectable at the sward patch level (Briske and Silvertown, 1993; Brock and Hay, 1993).

The ability of grass plants to accumulate nutrients and assimilates in their basal nodes and principal roots, and their plasticity to mobilise those resources to different parts of the plant allow them to buffer external stressful conditions. This buffering capacity is a function of the size of the plant, measured as weight of its different components: crown, leaves, stolons; and not as a function of its number of tillers (Brock and Hay, 1993). The physiological integration of tillers helps to minimise competitive interactions, increase residence times of limiting resources and reduce soil nutrient demand (Derner and Briske, 1998). The dynamics in the size of plants is a consequence of the heterogeneity in the size or age of their tillers. Changes in tiller ontogeny, or death of main tillers of the plant produce plant fragmentation, resulting in new plants of normally less vigour. These changes are very accused in temperate and mesic swards, with plant minimum sizes and highest plant mortalities registered at the start of spring (Brock *et al.,* 1988; 1996).

Young tillers are especially susceptible to plant fragmentation, as they depend on assimilates from their parental tillers for growth (Bullock *et al.,* 1994b), except in the case of flowering parental tillers (Colvill and Marshall, 1984; see section 2.3.2). On the other hand, plant fragmentation seems to be beneficial for older daughter tillers, as intraclonal competition for resources with their parental tiller depresses their growth (Bullock *et al.,* 1994b).

Plants tend to adjust to changes in availability of resources more through tiller recruitment than through tiller death and tiller growth (Derner and Briske, 1999). Tiller death is hampered by the physiological integration of tillers and only will occur under extreme limitations of resources, as in self-thinning for lack of light.

2.5 The sward patch

The sward patch is made of a number of plants of the same or different species subjected to the same environmental conditions (i.e. soil type and light environment) and biotic disturbances (e.g. those related to the livestock grazing activity: trampling, urine and dung deposition, and defoliation). The sward patch has proved a convenient unit to simulate and understand spatial processes occurring at the paddock level through the connection and integration of the individual processes occurring at its level (Schwinning and Parsons, 1996a,b). Considering monospecific swards, the structure of the sward patch is best characterised by its leaf area index or its leaf herbage mass and the leaf vertical distribution in the canopy. These structural variables define the productivity of the sward (Ludlow and Charles-Edwards, 1980), the optimum grazing management (i.e. stocking rate in continuous systems and regrowth duration in rotational systems) and the pattern of defoliation by the livestock (Boval *et al.*, 1999).

2.5.1 Tiller size-density relationship

Tiller density has often been considered an indicator of vigour or persistence in forage swards, but it cannot be considered an unambiguous indicator because of the known compensation existing between tiller density and tiller size (Bircham and Hodgson, 1983). In this way, temperate vegetative swards of the same or different grass species are characterised by being able to achieve the same growth rates by markedly different combinations of tiller size and density (Parsons and Chapman, 2000). This plasticity is ruled by the logarithmic relation existing between the tiller density and the average tiller size, known as the -3/2 self-thinning or power rule, common for all monospecific plant stands (Westoby, 1984). This rule states that average tiller size (w) and tiller density (d) are linked by the relation:

$$w = \mathrm{k}\, d^{-3/2} \qquad \text{(eq. 2.1)}$$

where k is a constant whose value depends on tiller growth form and environment. The extreme values of this logarithmic tiller size-density relationship for a species determine its tiller size-density plasticity. The knowledge of these limits may define the adaptability of the species to different defoliation regimes or environmental conditions (Chapman and Lemaire, 1993). Figure 2.5 shows that the tropical grass "Kikuyu" (*Pennisetum clandestinum*) can reach higher tiller weights with lower tiller densities than the temperate perennial ryegrass. On the contrary, ryegrass may be adapted to more frequent hard grazing due to the plasticity it shows to reach very low tiller sizes and high tiller densities.

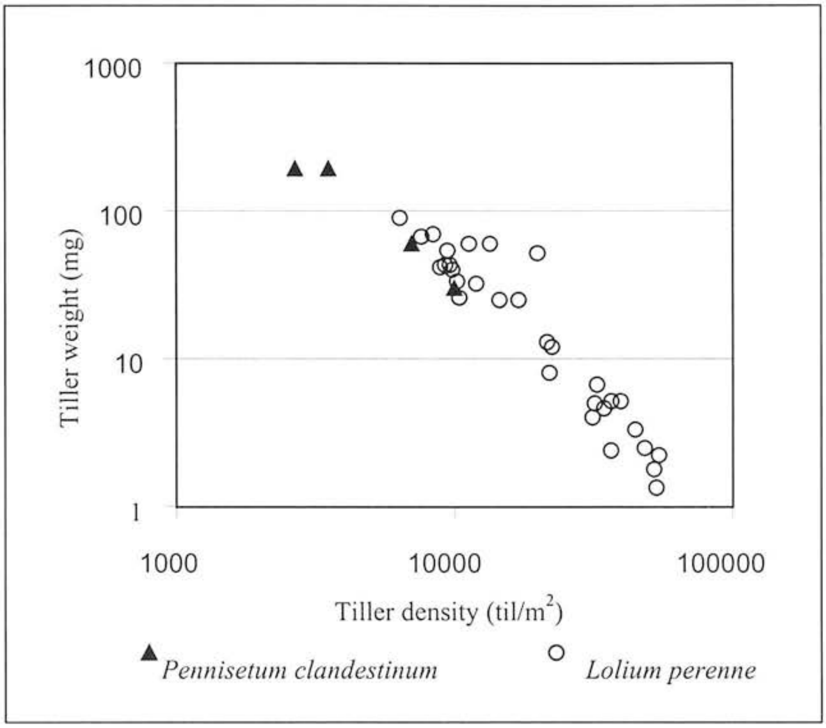

Figure 2.5. Values of average tiller weight and tiller density of productive swards of *Pennisetum clandestinum* and *Lolium perenne* (slope line -3/2). Adapted from Mears and Humphreys (1974), and Davies (1988).

The -3/2 self-thinning rule holds for swards maintained at ceiling leaf area index (LAI_c) and with constant tiller geometry (Sackville-Hamilton *et al.,* 1995), which is an uncommon scenario in continuously grazed swards. For frequently defoliated swards maintained at different levels of constant herbage mass, Matthew *et al.* (1995) devised a multiphasic size-density compensation line comprising four phases depending on the amount of herbage mass. The first phase corresponded to very low herbage masses, with low tiller density and size values due to the lack of reserves to produce new tillers. The second phase followed a self-thinning line with a slope higher than -3/2 and variable LAI. Most of the monospecific temperate pastures grazed at a constant sward height would lie within this line. With higher increases in LAI, the former line would intersect with a line with a -3/2 slope at the point of reaching LAI_c. This point of intersection is regarded as defining the mature size of tillers of the analysed species for the existing environmental conditions (Matthew *et* *al.*, 1995). Finally, a point will be reached from which herbage mass will remain constant and the self-thinning line slope will decrease to -1.

In order to include grazing managements and environmental conditions in which constant herbage mass is not achieved (e.g. most of the tropical grassland systems), the simple -3/2 self-thinning line is more useful than the multiphasic line (Matthew *et al.*, 1995). The applicability of the -3/2 self-thinning line rests on considering it as a boundary line of potential pasture productivity. If this line is defined for a species and a fixed environmental condition (e.g. a season of the year), it is possible to relate it to other swards of the same species and environment. This comparison allows the determination of the productivity state of a sward and helps in taking decisions on the adequate management to improve its productivity. Figure 2.6 shows average tiller size/density values of Kikuyu swards under different management regimes Mears and Humphreys, 1974). In this case, high productivity states are only achieved under high fertilisation regimes, except in the case of excessive stocking rate (17 animals ha^{-1}), in which the lower tiller size limit of Kikuyu grass was probably surpassed, producing the death of many tillers. Within the swards with low fertilisation regimes, those with lower stocking rates laid further away from the size/density boundary line, indicating a possible sward overgrowth because of undergrazing. Hernández Garay *et al.* (1999) indicates that an increase in the tiller leaf area/volume ratio at high sward leaf masses (i.e. increase in the parameter k of equation 2.1) must theoretically result in a higher decrease in tiller density over and above that expected by the size/density compensation line. The very different mature tiller structures of tropical grasses compared to vegetative tillers of temperate grasses does not allow to ascertain that the geometry of the tillers develops towards higher leaf area/volume ratios with tiller age, but the results of Figure 2.6 may suggest so.

Following the same theoretical principles, Hernández Garay *et al.* (1999) proposed a sward productivity index using an arbitrary logarithmic line of -3/2 slope (compensation line), which laid below all the tiller density-weight co-ordinates of any sample of sward patches. The sward productivity index was defined as the length of the perpendicular distance from a sward co-ordinate to the compensation line. This variable showed very high correlations with sward leaf mass and daily herbage production values.

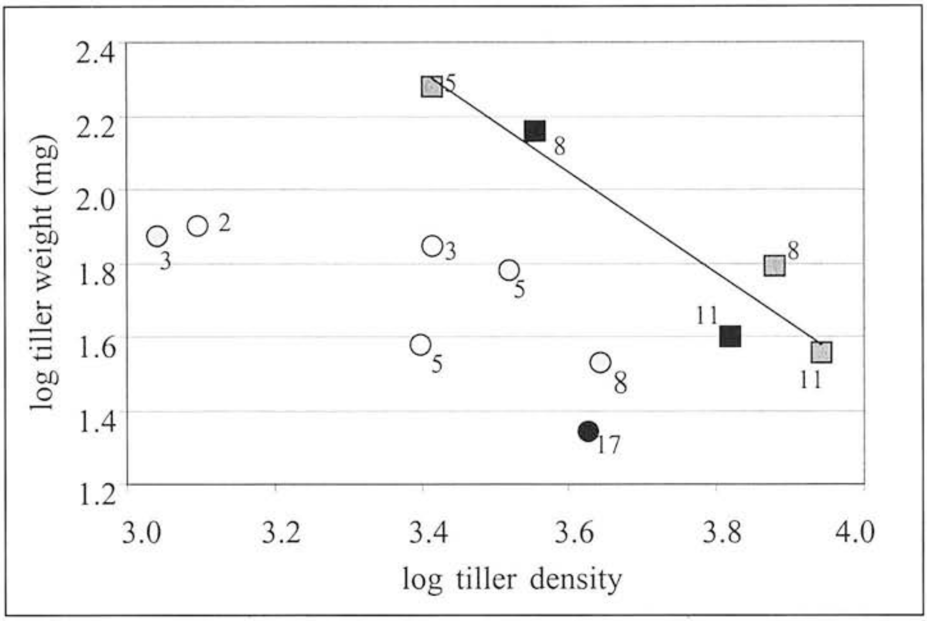

Figure 2.6. Tiller size-density relationships in Kikuyu (*Pennisetum clandestinum*) swards with different combinations of nitrogen fertilisation and stocking rate levels. Nitrogen fertilisation levels are indicated by different colours (white: 0; bright grey: 134; dark grey: 336; and black 672 kg N ha^{-1}). Numbers by the dots correspond to stocking densities (animals ha^{-1}). The straight line is the size/density boundary line calculated through a linear regression for the swards with higher tiller size/density values (square dots). The slope of the straight line is -1.37. Adapted from Mears and Humphreys (1974).

2.5.2 Distribution of plant sizes in the sward

As it was shown in section 2.4, the growth of a tiller is partially regulated by the plant in which it is integrated. The strength of this regulation will be both a function of the stage of development of the tiller and the size of the plant. In order to integrate this information to the sward patch level, it is important to know the existing distribution in plant sizes.

The sizes of individuals in plant populations are far from uniform. Usually a marked size hierarchy exists, with a small number of large plants, accounting for most of the population biomass, and many small plants. Several factors combine to produce this asymmetric, positively skewed distribution of adult plant weights. Even under absence of inter-plant competition, a size hierarchy can develop if relative growth rate changes faster than linearly with plant size (Hutchings, 1997).

In temperate swards plant size distribution is very resilient to grazing management, and only shows changes in spring, with an increase in the number of small plants resulting from larger plants' fragmentation (Brock and Fletcher, 1993; Brock *et al.*, 1996). This is the time when the sward is considered to be most fragile, though under normal conditions it recovers very fast due to the favourable climate.

2.6 The paddock

The paddock is the smallest spatial grazing management unit of the farm. As that, it has to be subjected to a structural time-based schedule of decisions, from daily to multiyear decisions (Sheath and Clark, 1996). The multiyear decisions consists mainly on the size of the paddock and the species and variety to use. These decisions will have to be taken in accordance to the characteristics of the region and the farm. Annual decisions concern stocking rate, grazing-cutting management (e.g. continuous, rotational, conservation), fertiliser input, and annual activities as burning and slashing. Knowledge of pasture annual and seasonal production and its inter-annual variability according to long-term climatic data is of great help in limiting the range of possible annual decisions. These decisions have also to couple those of parturition and general stock buying and selling policy. Monthly decisions are those already considered at the year time scale. The aim here is to adjust year decisions according to the inter-annual climate and market variability of the area. Predicted pasture growth rates over weeks or months can be used in conjunction with production targets. Daily decisions concern rotation length, stocking rate adjustments and feed supplementation. Key indicators at this level are daily lactation yield, short-term live-weight gain and pasture residual mass and state (Sheath and Clark, 1996).

This array of decisions needs a regular monitoring of pasture and livestock characteristics. Pasture characteristics to monitor must show correlation with livestock performance (measured by the livestock characteristics) and pasture vigour, or sustainability in the long term. In temperate pastures some very simple measurements, such as sward height, have been widely adopted because of their high relationships with animal performance parameters (Hodgson, 1990). For tropical pastures there is still little agreement in the usefulness of universal simple measurements that can predict leaf herbage mass and productivity (Humphreys, 1991). The different and more variable tropical sward structures with respect to temperate ones (e.g. development of tiller stems during all the year, aerial tillering, higher litter biomass, longer reproductive season), need further investigation on the relationships among structural variables of different hierarchical levels which can be useful for management purposes.

Chapter 3 — *Morphogenesis and structure of signal grass phytomers grown in controlled environments. Effects of temperature and nitrogen* *

Abstract

Morphogenetic and structural characteristics of phytomers of forage grasses can explain the state, dynamics and productivity of swards. The effects of temperature and nitrogen fertilisation on morphogenetic and structural characteristics of signal grass (*Brachiaria decumbens* Stapf) phytomers were studied. Signal grass microswards were grown in controlled environments under different temperatures (25°C, 30°C and 35°C) and nitrogen application regimes (0, 100 and 200 kg N ha^{-1} yr^{-1}). Leaf elongation rate was the morphogenetic variable more responsive to the treatments and more independent of the structure of the tiller. It also was strongly related to the duration of the leaf elongation and to the rate of leaf appearance. The strength of these relationships resulted in a lower response of the number of simultaneous growing leaves to the external environment, with values always close to one. The pattern of internode elongation differentiated signal grass from other grass species and proved to be significantly related with important structural characteristics of the phytomer.

---

[*] Based on Busqué, J. and Herrero, M. Morphogenesis and structural characteristics of signal grass phytomers at various temperatures and nitrogen nutrition levels. *Crop Science.* (submitted).

3.1 Introduction

Signal grass (*Brachiaria decumbens* Stapf) is a C_4 perennial grass originally from central-east Africa and today widely distributed in many tropical and subtropical areas of the world (Hacker and Jank, 1998). Only in Brazil it occupies nearly 40 million hectares of "cerrado" savanna land (Macedo, 1997), mainly due to its adaptation to the infertile acid soils of the region (CIAT, 1999).

Improved tropical pastures are characterised by grass species of high productivity under optimal soil nutrient levels but with low forage quality (Fisher *et al.,* 1996). Their high potential productivity and over-utilisation are the main factors causing soil degradation and low livestock productivity (Lascano, 1991). On the other hand, their low forage quality is mainly the result of their high proportion of stem and dead material. According to Humphreys (1991), animal production from tropical pastures must be sustained in the long term through adequate forage allowance, plant growth and persistence, maintenance of cover against erosion and desirable botanical composition. These factors are affected by the dynamics of plant growth components of the pasture which determine the sward green leaf area and biomass per unit area, considered the most important sward variables for animal and plant productivity (Stobbs, 1975; Hodgson, 1985a).

Green leaf biomass per unit area and leaf area index can be obtained by combining the average size of green leaves, their number per tiller and the density of tillers. Each of these structural variables is directly affected by the three main leaf morphogenetic characteristics of the plant: leaf elongation, appearance and senescence (Chapman and Lemaire, 1993). For many tropical grass species, signal grass included, it is also necessary to consider stolon elongation, as this process is active during most of the tiller's ontogeny and can interact with leaf growth processes (Cowan and Lowe, 1998; Cruz and Boval, 2000). In this chapter we will be focusing exclusively on the morphogenetic and structural characteristics of signal grass that define the phytomer's growth.

Leaf elongation rate (*LER*) is mainly affected by the amount of resources available to the plant, especially nitrogen and water (Gastal and Durand, 2000), and by temperature (Lemaire and Agnusdei, 2000). When studied at the cell level, external environmental conditions affect *LER* altering different growth processes, namely cell production and expansion rate, and duration of cell expansion (Skinner and Nelson, 1995; Schnyder *et al.*, 2000). *LER* is considered to be constant during the main phase of leaf development (Skinner and Nelson, 1995; Bultynck *et al.*, 1999).

Internode elongation rate (*IER*) may increase with nitrogen availability (Cruz and Boval, 2000), but internode lengths are strongly regulated by light quality in the canopy (Casal *et al.*, 1987). The processes relating *IER* and internode lengths are not yet well understood.

Leaf appearance rate (*LAR*), or its inverse the phyllochron is very responsive to temperature. It is closely related to leaf initiation rate (*LIR*), or its inverse the plastochron, under non-external limiting conditions and for mature tillers (Nelson, 2000). Differences in consecutive sheath lengths can deviate considerably LAR values with respect to *LIR* values (Duru and Ducrocq, 2000a). Cruz and Boval (2000) suggest that the increases observed in *LAR* with nitrogen availability for some tropical species with ability to elongate their internodes may be the result of the high sensitivity of *IER* to nitrogen availability. This suggestion assumes that internode elongation should occur at the same time as leaf elongation.

The relation between the phyllochron and the duration of leaf elongation (*LED*) defines the number of leaves growing in a tiller at the same time. This relation has been considered quite stable as a consequence of the co-ordination existing between the end of elongation and initiation in consecutive leaves (Chapman and Lemaire, 1993; Skinner and Nelson, 1994a). However, some plasticity has been found in that relation both in tropical (Cruz and Boval, 2000) and temperate grasses (Duru and Ducrocq, 2000a). It has been argued that the number of simultaneously growing leaves in a tiller is a coarse or long-term source of phenotypic plasticity in comparison with short-term responses like those of *LER* (Nelson, 2000).

Finally, within the leaf, the length of the blade has been demonstrated to be a function of the length of the previous sheath in temperate grass species (Grant *et al.*, 1981; Casey *et al.*, 1999). Leaf blade and sheath elongation processes have also been seen to be co-ordinated with other growth processes within the tiller (Skinner and Nelson, 1994a).

The aim of this work was to study the responses of the leaf morphogenetic characteristics *LER*, *LAR* and *IER* to nitrogen fertilisation and external temperatures under controlled environments for signal grass. A further objective was to analyse the mechanisms of size regulation of the phytomer's organs: leaf, blade and internode lengths.

3.2 Materials and methods

General experimental design

Fifteen signal grass microswards were grown on wooden trays of 0.24 m^2 x 0.10 m deep. The soil type used was compost (UC II type mix: 75% peat and 25% sand plus added limestone and a base fertilizer). Microswards were established using certified seeds (90% germination rate) at a sowing rate of 8 kg ha^{-1}. A preliminary period of growth of 50 days at constant day/night (12h/12h) temperatures of 30°C/25°C produced plant populations mainly consisting of first order tillers. After this initial period microswards were cut at a height of 5 cm (fixed cutting height for the experiment) and allocated to one of three controlled environment rooms. Photoperiod (12h light/12h dark), relative humidity (75%), radiation (total radiation of 330 W m^{-2} at tray level using metal halide lamps: 760 μmol m^{-2} s^{-1} of *PAR*) and irrigation regime (watered daily to field capacity) were common for all the microswards during the experiment.

Leaf and tiller growth and tiller population structures were studied during 3 consecutive periods characterised exclusively by vegetative growth. The number of days in each consecutive growing period was 36, 50 and 48 days. In all cases tillers were of a high order rank, similar to the majority found under tropical field conditions, and most of them were born at the beginning of each regrowth period. Microswards in each growth room were changed in their relative position twice a week to avoid special microenvironments. At the beginning of period 2 a trace element solution and phosphorus (equivalent to 400 kg P_2O_5 ha^{-1}) were applied to all the microswards. Each period ended with the cutting of the microswards and their random allocation to a new temperature regime. Day temperatures of 25°C, 30°C and

35°C were used, corresponding to each of the growth rooms. Night temperatures of 25°C were common for all the periods and growth rooms.

Each microsward was assigned to a fixed nitrogen fertilisation regime throughout the whole experiment. Nitrogen treatments consisted of equivalent to yearly applications of 0, 100 and 200 kg N ha^{-1} yr^{-1} (0N, 100N, 200N), applied as $(NH_4)_2SO_4$ diluted in water at the beginning of each regrowth period in equal doses. Five microswards were allocated to each growth room in each period (three of 0N, one of 100N and one of 200N).

Measurements

At the beginning of each period, 8-10 newly appeared tillers were selected randomly in each micro-sward. These were marked and identified at their base using telephone wire loops. If any of the tillers died during the period, they were replaced and marked in the same way. Non-destructive measurements of leaf length and appearance were carried out on each tiller throughout each growing cycle, while different morphological components (length, area and weight of internodes, leaf blades and sheaths) were measured destructively at the end of the last growing period. A common ruler was used in all the length measurements.

The results presented correspond only to the last period of regrowth, as this was the only period with a complete record of destructive and non-destructive measurements.

Measurements at the leaf level

The length of a growing leaf was estimated measuring the distance from its tip to the ligule of the second youngest fully emerged leaf (Figure 3.1: *RM*). This method is recommended as a straightforward leaf length measurement for temperate vegetative tillers, as no significant internode elongation occurs during this phenological phase (Davies, 1993). However, most tropical grasses have a different vegetative ontogeny, developing their internodes from early growth stages (Cowan and Lowe, 1998). True leaf length could be calculated from the length of the other components of the leaf as:

$$Leaf_4 = RM + Sh_2 - IN_3 - IN_4 \qquad \text{(equation 3.1)}$$

where *Leaf₄* is the length of the leaf being measured (blade + sheath), Sh_2 is the sheath length of the leaf two below the one being measured, and IN_4 and IN_3 are the internodes corresponding to the leaf being measured and the one below respectively (Figure 3.1). IN_4 is effectively non-existent in growing leaves of signal grass and can be considered equal to 0.

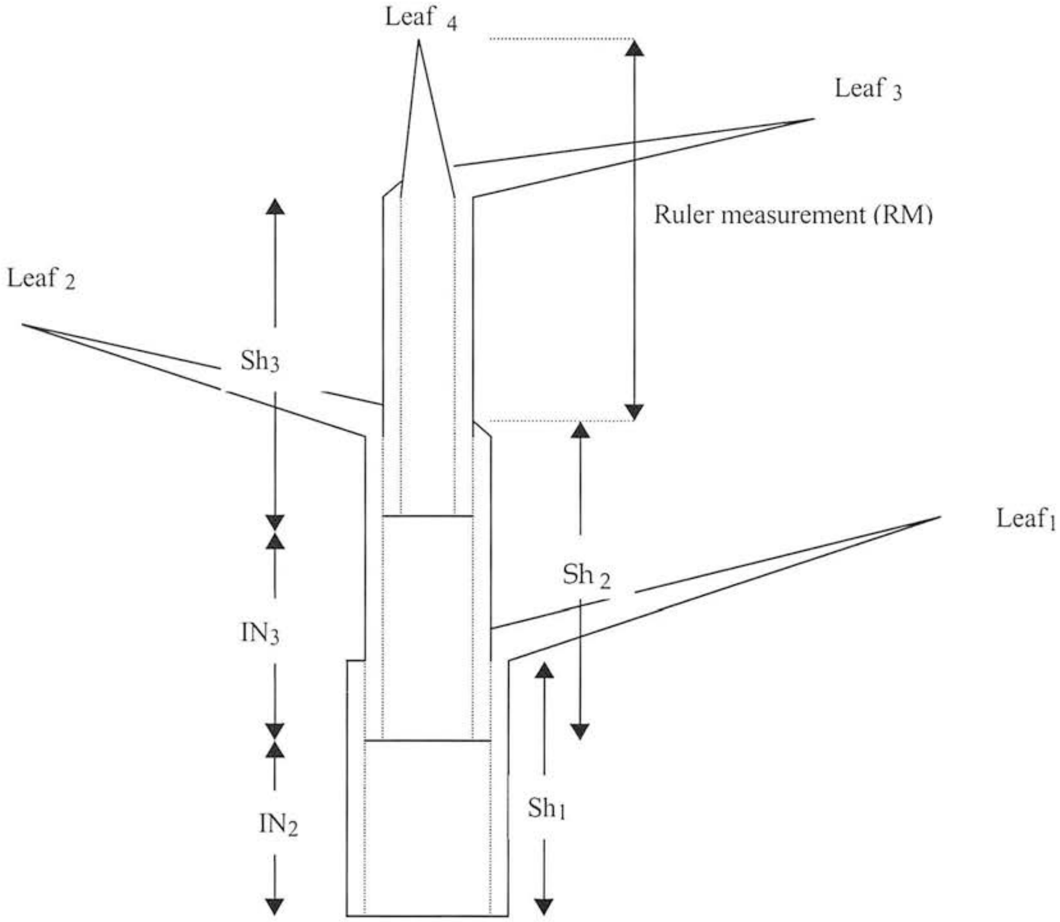

Figure 3.1. Leaf arrangement in a signal grass vegetative tiller. Sh_i, sheath length; IN_i, internode length.

According to equation 3.1, estimation of true leaf length requires the knowledge of exact length values of different components of the tiller (sheaths and internodes). The main purpose of measuring leaf lengths at the 2-3 day intervals used in this experiment was to obtain estimates of *LER*. *LER* was calculated from the difference in leaf length (*RM*) between two consecutive dates of measuring. It was not considered necessary to determine true leaf lengths, as the difference between consecutive ruler measurements could be used if some considerations were made. Figure 3.2 represents a series *RM* lengths of three successive leaves from the same tiller. Arrows point out the *RM* length of fully extended leaves calculated using equation 3.1. Only ruler measurements before the arrow (Figure 3.2) could be used for *LER* calculation, as it was assumed that after the arrow internode elongation was occurring. The general pattern of growth slope change before and after the arrow for all the leaves (Figure 3.2) supports the assumption of separation in time of internode and leaf growth. On the other hand, the first *RM* measurements of each leaf may be over-estimated if the internode corresponding to the previous leaf has not completed its elongation (Figure 3.2). Correcting these measures and considering only the first 2 ruler measurements in each leaf (always corresponding to leaf extension and not to internode extension) allowed the calculation of *LER* for a greater number of observations than if *LER* were calculated using true leaf length estimates (as in equation 3.2). Using only the first 2 leaf length values to calculate *LER* was justified by the growth linearity observed (Figure 3.2), following the usual pattern of leaf growth in grasses (Bultynck *et al.*, 1999).

Analysis of morphogenetic characteristics

Only initial *LER* values were considered in each leaf, as leaf growth was seen to be linear between appearance and total extension. *IER* was calculated in the same way as *LER*, but considering ruler measurements per leaf occurring after the point marked with an arrow in Figure 3.2. As for *LER*, it was observed that internode growth was linear (*IER* constant) and so only the initial value for each leaf was chosen.

The general model used in the analysis of *LAR*, *LER* and *IER* was:

$$Y_{ijr} = T_i + N_j + N \times T_{ij} + e_r \qquad \text{(equation 3.2)}$$

where T was the temperature effect (i = 25, 30 and 35°C), N was the nitrogen effect (j = 0N, 100N, 200N), and e was the unexplained variance.

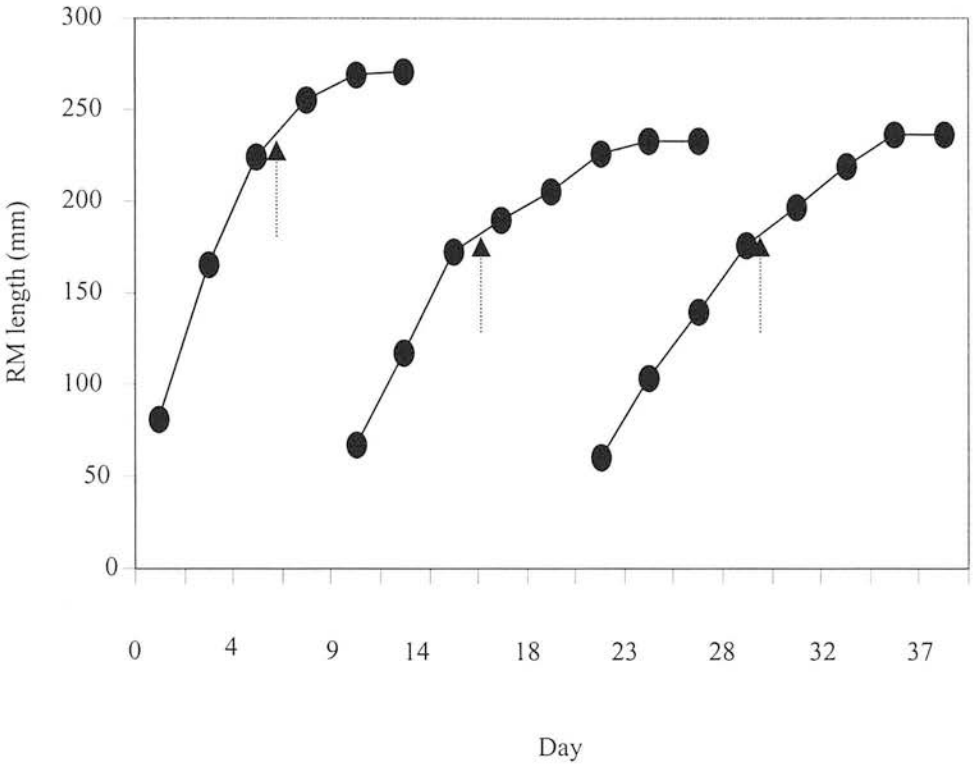

Figure 3.2. RM leaf length measurements (from the tip of a leaf to the ligule of two previous leaves) for a series of three consecutive leaves in a tiller. Arrows indicate the point at which the leaf (blade + sheath) reaches its maximum length. Points that are after the arrow correspond to internode elongation associated to that leaf.

Within each microsward 3 tillers of the initial 8-10 were chosen for the statistical analyses in order to attain a balanced design of the data. These 3 tillers were chosen as those with the closest to the average final tiller weight and appeared at the beginning of the period from basal nodes. Tiller values of swards under 0N regime, which were replicated three times for each temperature, were averaged in order to obtain for all the combinations of nitrogen and temperature levels 3 final observations. In this way a balanced analysis of variance was performed considering the experiment with a factorial design.

LER values for the fertilised microswards showed a trend to decrease along the regrowth period (figure 3.3), indicating soil nitrogen depletion. Analyses of variance of the morphogenetic variables were performed using only leaf observations from the first half of the regrowth period.

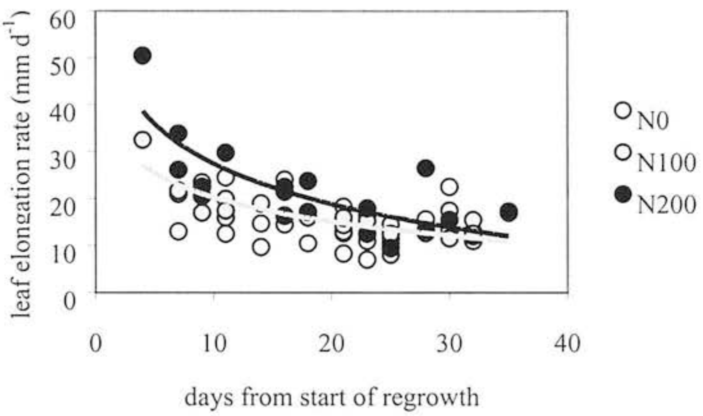

Figure 3.3. Dynamics of leaf elongation rate along the regrowth period (period 3) for plants under different nitrogen fertilisation regimes. The lines represent the logarithmic regressions (R^2=0.69 for N200 and R^2= 0.77 for N100). The regression for N0 was not significant.

Regulation of leaf length

LED was calculated dividing the values of the length of mature leaves (*FLL*) obtained destructively at the end of the regrowth period by *LER*. The value of *LED* thus obtained corresponded to that of rapid *LER* and did not consider the initial stage of establishment of the cell division zone (Skinner and Nelson, 1995).

Regulation of leaf length was initially considered analysing the relationships between the variables defining *FLL*: *LER* and *LED*. In a second stage the relationships between *LED* and phyllochron values were studied to prove the hypothesis of an association between leaf growth initiation and some stage in the development of the previous leaf (Skinner and Nelson, 1994a), and thus the existence of a regulation in the number of simultaneously growing leaves in the tiller. The effects of temperature and nitrogen upon these relationships were analysed.

Internode elongation

Internode elongation rate (*IER*) for single internodes proved to be fairly constant until one measure before reaching the final internode length (see points after the arrows in Figure 3.2). The duration of internode elongation (*IED*) was therefore calculated dividing the final internode length (*FIL*) by the initial *IER* for each internode.

A graphic representation of the morphogenetic and structural characteristics of successive phytomers of a tiller is presented in Figure 3.4.

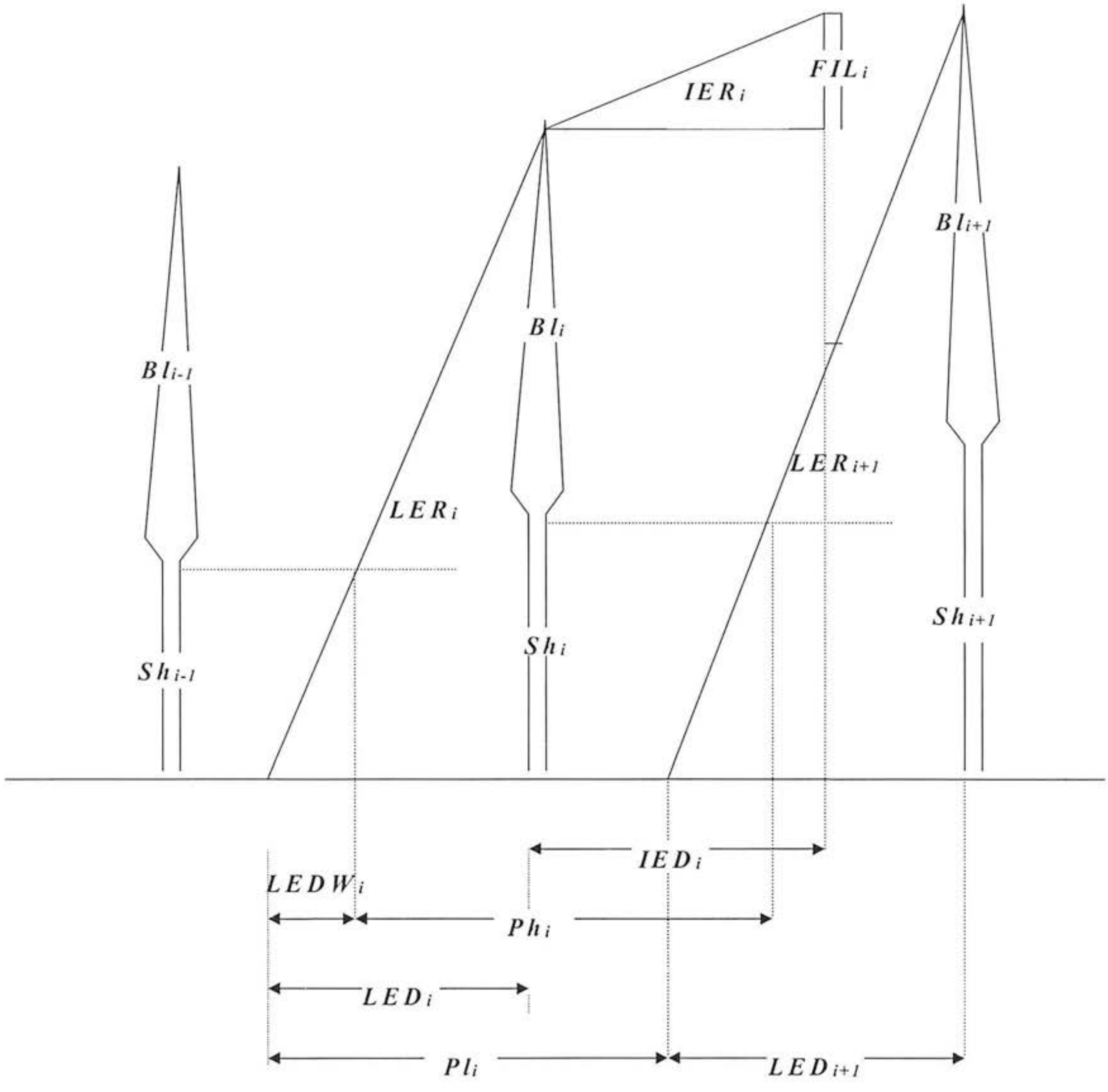

Figure 3.4. Intervals, rates and length relations in the growth of three consecutive leaves of a tiller. *LER*, leaf elongation rate; *LED*, duration of leaf elongation; *LEDW*, elongation period inside the whorl; *Ph*, phyllochron; *Pl*, interval between the initiation of two consecutive leaves (plastochron). *IER*, internode elongation rate; *IED*, duration of internode elongation; *Bl*, length of leaf blade; *Sh*, length of leaf sheath; *FIL* length of internode. Subscripts *i-1*, *i* and *i+1* correspond to consecutive leaves in order of appearance.

3.3 Results

Morphogenetic characteristics

LER was the leaf growth morphogenetic variable more sensible to temperature and nitrogen fertilisation, while *LAR* was less affected (Table 3.1). *LAR* responses to nitrogen and temperature were more significant ($p<0.1$) when *LER* was used as covariate in the analysis of variance, with mean values similar to those when no covariate was used (0.10, 0.15 and 0.17 for increasing levels of N; 0.12, 0.17 and 0.12 for increasing levels of temperature; s.e.=0.014). *IER* had a much higher variability than any other morphogenetic characteristic (see coefficient of variation in Table 3.1). The duration of leaf and internode elongations were not significatively affected by the treatments.

The highest values in all the growth rates (*LER*, *LAR* and *IER*) were obtained at 30°C and at the highest level of nitrogen fertilisation (Table 3.1). Both *LER* and *IER* responded more than linearly with the level of nitrogen fertilisation, although in the case of *IER* the response was not statistically significant. *LER* and *IER* were significantly higher at 30°C, while they showed similar lower values at 25°C and 35°C. The interaction between nitrogen and temperature was never important.

Structural characteristics

Both the effects of temperature and nitrogen fertilisation affected very significantly *FLL* and blade length, with a similar response to that seen for *LER*. The responses of sheath length to changes in the treatment levels followed similar trends to those of *FLL* and blade length, though they were not so acute. Similar trends in the responses to nitrogen and temperature were also found for *IER* and internode length (Table 3.1).

When leaf insertion number was considered in the analysis of variance (only done for structural characteristics) it always proved significant. Its interaction with temperature was also significant for blade and sheath length ($p<0.05$), but not for internode length. Blades at 30°C maintained a constant length after reaching a certain leaf insertion number, while at the other temperatures they showed a progressive slight decrease in length. Sheath lengths at 25°C and 30°C were stable from leaf insertion number 3 onwards, while at 35°C they showed a decreasing trend (Figure 3.5).

Table 3.1. Means of morphogenetic and structural characteristics of phytomers of signal grass for different levels of nitrogen and temperature. Level of significance and variance explained by each effect, standard error (s.e.), coefficient of determination of the models (R^2) and coefficient of variation (CV%) of the response variables.

	Morphogenetic characteristics					Structural characteristics			
	LER	LAR	LED	IER	IED	FLL	Bl	Sh	FIL
Nitrogen	**	NS	NS	NS	NS	***	***	**	NS
0	15.4	0.12	10.5	5.7	7.9	161.0	112.2	48.7	36.1
100	18.9	0.15	10.0	6.5	7.9	184.5	130.1	54.4	41.4
200	22.7	0.15	9.3	8.1	8.0	205.6	147.1	58.5	44.8
Variance explained	0.44	0.14	0.12	0.13	0.01	0.40	0.41	0.29	0.05
Temperature	*	NS	NS	*	NS	***	***	**	***
25	17.4	0.13	10.5	4.9	8.9	176.9	122.1	54.8	32.7
30	21.6	0.16	10.1	9.5	8.5	210.6	151.2	59.5	59.5
35	17.9	0.13	9.3	6.0	6.4	163.5	116.2	47.3	30.2
Variance explained	0.17	0.15	0.11	0.50	0.39	0.47	0.47	0.45	0.72
s.e.	1.10	0.012	0.56	0.94	0.83	4.49	3.50	1.74	3.86
Effect NxT	NS	NS	NS	NS	--	NS	NS	NS	NS
R^2 of model	0.82	0.56	0.60	0.65	0.40	0.93	0.93	0.84	0.82
CV (%)	24.2	24.1	15.8	42.3	22.1	16.2	17.7	14.3	39.3

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels respectively. NS: not significant.

LER: leaf elongation rate (mm d^{-1}); LAR: leaf appearance rate (leaves d^{-1}); LED: leaf elongation duration (d); IER: internode elongation rate (mm d^{-1}); IED: internode elongation duration (d); FLL: final leaf length (mm); Bl: final blade length (mm); Sh: final sheath length (mm); FIL: final internode length (mm).

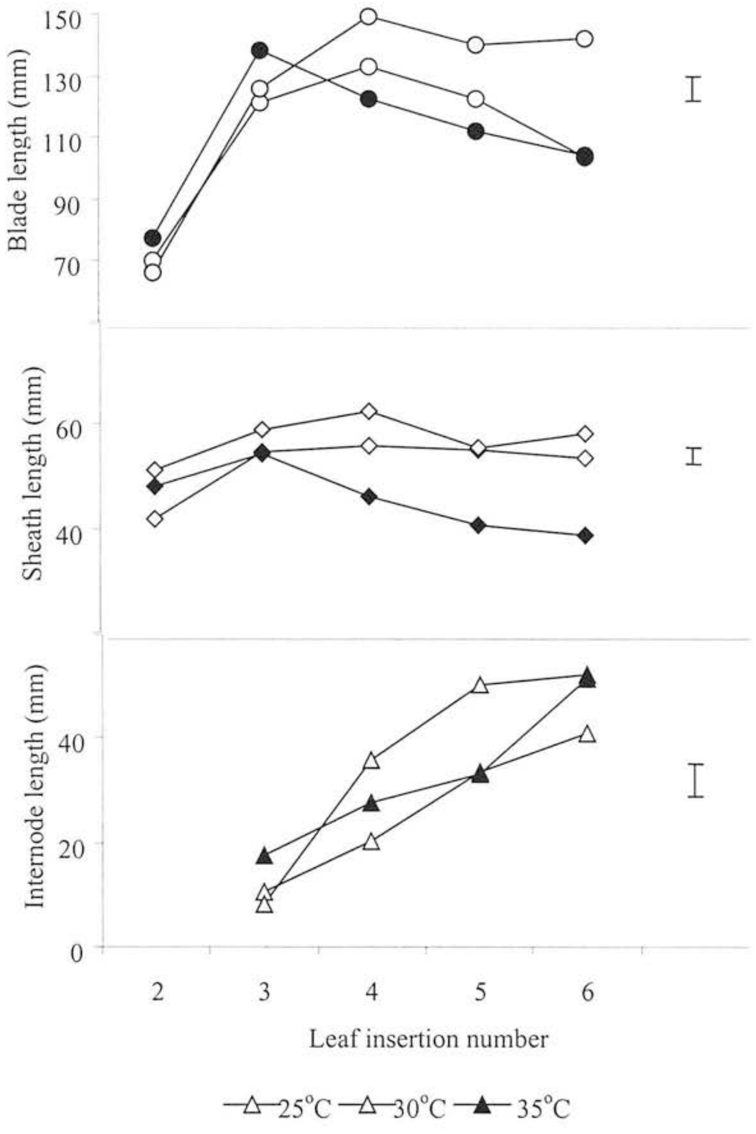

Figure 3.5. Effect of temperature and leaf insertion number on the lengths of blades, sheaths and internodes. Vertical bars indicate the magnitude of the standard errors at the mean values of each variable.

Regulation of final leaf length

FLL is the result of multiplying *LER* and *LED*. Table 3.2 shows a high negative correlation between these two morphogenetic variables, while *FLL* was mainly determined by *LER*. Although *LED* and the previous sheath (Sh_{i-1}) did not show any significant bivariate correlation, their partial correlation when *LER* was controlled was strong (partial r=0.77). A multiple regression with *LED* as response variable and *LER* and Sh_{i-1} as explanatory variables explained much more variance than a single regression between *LED* and *LER* (R^2=0.53):

$$LED = 12.3(\pm 0.67 s.e.) - 0.53(\pm 0.045) LER + 0.13(\pm 0.019) Sh_{i-1}$$

$$R^2=0.80 \quad p<0.001 \quad n=37 \qquad \text{(equation 3.3)}$$

Neither temperature nor nitrogen produced significant different relationships or added more explained variance to the above model.

Table 3.2. Correlations between variables involved in the regulation of leaf length.

	LER	LED	Ph	FLL	LED/Ph	Sh_{i-1}
LER	1	-0.74	-0.72	0.76	NS	0.73
LED	--	1	0.74	NS	NS	NS
Ph	--	--	1	-0.35	-0.70	-0.38
FLL	--	--	--	1	NS	0.90
LED/Ph	--	--	--	--	1	0.33

LER: leaf elongation rate (mm d^{-1}); LED: leaf elongation duration (d); Ph: phyllochron (d); FLL: final leaf length (mm). Sh_{i-1}: length of previous sheath (mm). Number of observations: 37.

The relationship between the phyllochron and *LED* fitted significatively a line passing through the origin and with a slope equal to 1 (Figure 3.6):

$$ph = 1.00(\pm 0.021 s.e.) LED \quad R^2=0.55 \quad p<0.001 \quad n=37 \qquad \text{(equation 3.4)}$$

Although the prediction of the phyllochron was not improved adding the temperature or nitrogen effects (Figure 3.6), it was highly improved when the difference in length of the two previous sheaths (*difsh*) was included in the regression:

$$ph = 2.5(\pm 1.45 s.e.) + 0.77(\pm 0.135) LED + 0.10(\pm 0.025) difsh$$

$$R^2=0.67 \quad p<0.001 \quad n=37 \qquad \text{(equation 3.5)}$$

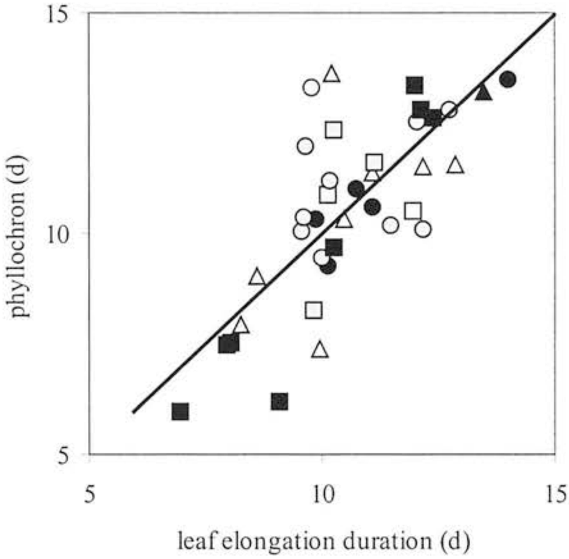

Figure 3.6. Relationship between the phyllochron and the duration of leaf elongation. Dot shapes correspond to the different nitrogen levels (●: 0N; ▲: 100N; ■: 200N). Dot colours correspond to temperatures (white: 25°C; grey: 30°C; black: 35°C).

Regulation of blade length

Blade length (Bl_i) was best explained considering the lengths of the previous sheath (Sh_{i-1}) and internode (FIL_{i-1}) by the multiple linear regression:

$$Bl_i = 25.4(\pm 3.24 s.e.) + 1.64(\pm 0.069 s.e.) Sh_{i-1} + 0.34(\pm 0.046 s.e.) FIL_{i-1}$$

$$R^2=0.81 \quad p<0.001 \quad n=164 \quad \text{(equation 3.6)}$$

This equation increased R^2 in 4 points with respect to a simple linear regression relating Bl_i and Sh_{i-1}.

The treatments explained additional variance to the regression model of equation 3.6. A model including nitrogen as fixed effect and its interaction with Sh_{i-1} and FIL_{i-1} increased R^2 to 0.85. The 200N level increased the coefficient of Sh_{i-1} ($p<0.001$) and decreased the coefficient of FIL_{i-1} ($p<0.01$). Intercepts were not affected. Temperature only affected the value of the original intercept of equation 3.6 ($p<0.01$), increasing it in a similar amount for 30°C and 35°C with respect to 25°C. The variance explained in this case corresponded to $R^2=0.82$.

Regulation of internode length

IER was mainly related with *LER* ($r=0.75$). It was also related to *IED*, but only when *LER* was controlled (partial $r=-0.52$). A stepwise regression with *IER* as response variable chose *LER* as the first explanatory variable, absorbing 55% of the variance, and *IED* as the second explanatory variable, adding 11 units to the former R^2. The equation obtained was:

$$IER = 2.3(\pm 1.41 s.e.) + 0.50(\pm 0.063 s.e.)LER - 0.54(\pm 0.156 s.e.)IED$$

$$R^2=0.66 \quad p<0.001 \quad n=35 \quad \text{(equation 3.7)}$$

Nitrogen did not affect this relationship, while temperature affected the intercept ($p<0.01$; $R^2=0.74$), with a significative increase at 30°C ($p<0.001$).

Internode elongation of phytomer_i overlapped with leaf elongation of phytomer_{i+1}, continued growing usually after the appearance of the leaf of phytomer_{i+1} and finished before leaf initiation of phytomer_{i+2} (Figure 3.4). Apart from that order of events, no relationships were found between IED_i and the duration from initiation to appearance of the leaf of phytomer_{i+1}, or between *IED* and phyllochron or *LED*.

IER and *IED* explained 80% and 17% of the variance in *FIL* respectively.

3.4 Discussion

Morphogenetic characteristics

LER in grasses can be considered mainly a function of the external conditions of the sward (Lemaire and Chapman, 1996). This seemed to apply in this experiment, as *LER* was the only morphogenetic variable that changed significantly with nitrogen and temperature (Table 3.1). Supporting the sensitivity of *LER* to the level of external resources was also its decreasing trend with time from the moment nitrogen was applied for microswards under the 100N and 200N levels (Figure 3.3).

The more than positive linear response of *LER* to nitrogen during the first half of the regrowth period (Table 3.1) suggests that plants were under the critical plant N% (Lemaire and Gastal, 1997) even for the 200N level. This is supported by the responses of *LER* to nitrogen in another C_4 perennial grass species (*Paspalum notatum*), linear up to 400 kg N $ha^{-1}yr^{-1}$ under field conditions (Hirata, 2000). Another experiment supporting indirectly the probable low nitrogen nutrition index of the plants of our study refers to the more than linear increase observed in dry matter yield of signal grass in field conditions from 100 to 300 kg N ha^{-1} for the first 120 days after cutting (Okeagu *et al.*, 1989).

The optimum temperature for *LER* (30°C) was similar to that reported for *Pennisetum clandestinum* (Murtagh *et al.*, 1987) and higher than those of several temperate grasses (Lemaire and Agnusdei, 2000; Parsons and Chapman, 2000).

Though not as sensible as *LER*, *LAR* responded to nitrogen and temperature, especially if *LER* was used as covariate in the analysis. *LAR* responses to external factors are usually contradictory in studies of different grasses or of the same grass in different ontogenetic states, mainly because of its high dependence on other associated structural and morphogenetic variables (Cruz and Boval, 2000; Duru and Ducrocq, 2000a). These relationships are discussed in the next section. A similar LAR increase with nitrogen has been observed in signal grass plots grown in glasshouse in Brazil (Ferragine *et al.*, 2001). *LAR* also increased with low nitrogen applications in a temperate grass (*Phleum pratense*) mainly in Spring, when tillers were small and increase in consecutive sheath lengths was presumably not important (Belanger, 1998). Other results (Hirata, 2000, Duru and Ducrocq, 2000b) do not show clear responses of *LAR* to nitrogen. In these cases changes in *LER*, internode and sheath length values with nitrogen may have compensated to produce stability in *LAR*.

The optimum temperature found for *LAR* in signal grass (30°C) was similar to that found in maize (Warrington and Kanemasu, 1983) and several rice varieties (Yin and Kropff, 1996). In comparison to other forage grasses, it was similar to that of *Panicum maximum* var. trichoglume and *Panicum coloratum* var. *makarikariense* and higher than the optimal temperature for *Cenchrus ciliaris*, *Chloris gayana* and *Pennisetum clandestinum* (Ivory and Whiteman, 1978) and all temperate grasses (Parsons and Chapman, 2000).

Regulation of final leaf length.

The high correlations observed between the variables *LER*, *LED* and phyllochron (Table 3.2) suggest the existence of a regulation in the growth of a leaf and a co-ordination between the end and initiation of growth in consecutive leaves of signal grass tillers.

The regulation of leaf growth and the co-ordination between the growth of successive leaves is related to the existence of contrasted growth stages at the cell level (Skinner and Nelson, 1994a). Although our data refer only to measurements at the leaf level, it is possible to interpret some of the mechanisms occurring at the cell level for signal grass under the external conditions used in the experiment. This interpretation is useful to rule out certain models of leaf growth regulation, but cannot be used with accuracy to understand certain processes only approachable when studying explicitly the cell level of detail.

The model obtained relating *LED* negatively with *LER* and positively with the length of the previous sheath (equation 3.3) explained most of the *LED* variance. These relationships can be explained considering two known processes in leaf development: the positive relationship between *LER* and cell production rate (Skinner and Nelson, 1995) and the end of leaf cell division regulated by the length of the previous sheath

(Wilson and Laidlaw, 1985; Casey *et al.*, 1999). Although *LER* is the result of the product of rate of cell production, rate of cell expansion and duration of cell expansion (Skinner and Nelson, 1995; Schnyder *et al.*, 2000), in most situations it is mainly defined by the rate of cell production at the initial stages of leaf development. Thus, increasing *LER* appears to coincide with increased cell production, with more elongating cells (Skinner and Nelson, 1995) and a higher leaf length at a given time. On the other hand, the decline in leaf cell division coincides with leaf tip emergence from the previous sheath (Casey *et al.*, 1999). The combined result is that the higher the *LER* the sooner the tip of the leaf will emerge from the whorl and so the sooner cell division will finish. For constant duration of cell expansion, usual when water is readily available (Schnyder *et al.*, 2000), *LED* will be a function of the number of cells produced and not yet fully elongated. This number will be lower for higher *LER* as a higher proportion of produced cells would have elongated at the same time.

The low absolute value of the slope between *LED* and *LER* (equation 3.3) resulted in a higher sensibility of *FLL* to *LER* than to *LED* (Table 3.2). This low slope is a consequence of *LED* being mainly a function of the number of cells produced but not fully elongated at the time of tip emergence, while *LER* is a function of the total number of cells produced and also of the cell extension rate. While *LED* would not be affected by variation in cell expansion rate, *LER* would increase the higher the cell expansion rate. Changes in cell expansion rate are mainly caused by changes in temperature (Schnyder *et al.*, 2000). The higher *LER* values were obtained at 30°C, while *LED* was not much affected by temperature (Table 3.1). The reason why temperature did not add explained variance to the regression involving *LED* and *LER* (equation 3.3) was probably a consequence of the previous sheath length absorbing the effect that temperature should have produced. The residuals of *LER*, after regressing against *LED* and previous sheath length were used as indicators of *LER* linked to cell expansion rate. They showed a significant effect of temperature ($p<0.05$) and not of nitrogen or the interaction of both treatments. The mean values of the residuals for the different temperatures were –1.01, 0.34 and 0.58 for 25°C, 30°C and 35°C respectively (s.e= 0.41), indicating that cell expansion rate was probably higher at the two higher temperatures. The lack of a significant effect of nitrogen on the *LER* residuals was probably a consequence of its little effect upon cell expansion rate, as compared to its effect on cell division (Gastal and Nelson, 1994).

Cruz and Boval (2000) considered an added mechanism of *LER-LED* negative regulation in C_4 tropical grass species linked to their capacity to elongate their internodes. They suggested that internode elongation shortens the duration of leaf elongation inside the whorl. This mechanism implies simultaneous leaf and internode elongation of the same phytomer, a fact that was not observed in signal grass, at least for the vegetative tillers of our experiment (see Figure 3.2).

The phyllochron was mainly associated with *LED* (equation 3.4 and Figure 3.6), suggesting that tip appearance and end of cell division in one leaf was linked with the initial stage of initiation (i.e. cell division) in the next leaf. The significative effect that the difference in length of the two previous sheaths had on phyllochron values (equation 3.5) suggests that *LED* was better related to the plastochron (i.e. interval between leaf initiations) than to the phyllochron. This fact and the co-ordination between plastochron and phyllochron could be established transforming the values of phyllochron to those of estimated plastochron, considering equal *LER* values inside and outside the whorl, and relating the estimated plastochron to *LED*. The regression obtained showed a R^2=0.62, higher than the simple linear regression between phyllochron and *LED* (equation 3.4). Plastochron and phyllochron in grasses are considered to be co-ordinated except in early vegetative growth and under water-stress conditions, when the shoot apex builds up a supply of developed primordia for future compensation when the source of stress is released (Nelson, 2000).

Co-ordination between phyllochron and *LED* showed that, on average, there was only one leaf growing at a time, irrespective of temperature or nitrogen level. Duru and Ducrocq (2000a) found that, for *Dactylis glomerata* in the field with a range of 10°C to 21°C, the number of simultaneous growing laminae increased with temperature as a result of higher decreases in phyllochron than in *LED*. On the contrary, Lemaire and Agnusdei (2000a) demonstrated the constancy of the relationship between *LED* and phyllochron for different C_3 and C_4 grass species (*Lolium multiflorum*, *Paspalum* *dilatatum* and *Cynodon dactylon*) cohabiting in the same grazed community of a flooded pampa in Argentina at different seasons of the year. Cruz and Boval (2000) found contrasting results in C_4 tropical grasses of the same growth habit under different nitrogen nutrition index. They found that the number of simultaneous growing leaves did not change with increasing nitrogen level for *Dichanthium aristatum* because of the parallel reduction of *LED* and *LAR*. However, *LED* was more reduced than *LAR* for *Digitaria decumbens*, thus decreasing the number of simultaneous growing leaves with nitrogen level. These authors attributed this different behaviour to a contrasted effect of internode elongation in both species, though they stressed the need for more detailed analyses of the morphogenetic characters *LER*, *LAR* and *LED* for a full understanding of leaf length regulation in stoloniferous grasses. Finally, Nelson (2000) recognises the variability in the number of simultaneous growing leaves, especially for C_4 grasses and identifies it as an added source of phenotypic plasticity at a longer time scale to that of *LER*. For vegetative temperate grasses (*Festuca pratensis*, *Lolium perenne*), this author indicates the general pattern of two simultaneous growing leaves, with the appearance of a leaf linked to the end of elongation in the next older leaf and the beginning of rapid elongation in the next younger leaf.

Regulation of blade length.

Apart from the well-known blade length regulation by the previous sheath length (Sh_{i-1}) (Begg and Wright, 1962; Grant *et al.*, 1981; Wilson and Laidlaw, 1985), a positive association was also observed between Bl_i and previous internode length (equation 3.6). On the other hand, FIL_{i-1} was not significatively related to the length of the new sheath (Sh_i) or to LED_i. This suggests that FIL_{i-1} was probably more associated with the final length of blade cells of $leaf_i$ rather than with its final number. This mechanism contradicts that observed in temperate grasses where internode elongation, characteristic of the reproductive phase, produces progressively shorter blades and longer sheaths (Skinner and Nelson, 1995). It also contradicts the suggestion of Cruz and Boval (2000) relating negatively internode elongation with leaf elongation duration and final blade lengths. Contrary to what occurred in signal grass (Figure 3.2), these two cases consider the occurrence of simultaneous leaf and internode elongation of the same phytomer, thus reducing the length of the tube the leaf has to travel through.

The effect of nitrogen on the regression between Bl_i and Sh_{i-1} and FIL_{i-1} (equation 3.6) was to minimise the effect of FIL_{i-1} and increase the effect of Sh_{i-1}. This could be explained considering that nitrogen did not produce significative changes in internode length while it affected positively blade and sheath length (Table 3.1). On the other hand, the change in the intercept with temperature could be caused by a change in cell expansion rate.

Finally, the multiple linear regression observed between Bl_i and Sh_{i-1} and FIL_{i-1} was similar and not statistically different to that obtained for leaves of maximum lengths (equivalent to those numbered 3 to 5 in Figure 3.5) in tillers from grazed paddocks in different seasons in the tropics of Bolivia (unpublished data):

$$Bl_{i-field} = 27.4(\pm 3.86 s.e.) + 1.73(\pm 0.079 s.e.)Sh_{i-1} + 0.31(\pm 0.068 s.e.)FIL_{i-1}$$

$R^2=0.73$ $p<0.001$ $n=287$ (equation 3.8)

This similarity suggests that blade length in signal grass is mainly regulated intrinsically by the tiller structure, and only minor modifications are due to external environmental conditions. It also proves that the use of growth chambers is a valuable mean in the study of the morphology and development of signal grass without incurring in major deviations from field conditions with realistic grazing management.

Regulation of internode length

No references were found on the nature of internode elongation of vegetative tillers at the cell level. The results obtained in this study are not thus directly related to processes occurring at the cell level.

Internode elongation occurred mainly after leaf elongation, as was empirically observed from destructive observations and supported by marked different growth slopes in the development of the phytomer (Figure 3.2). This behaviour in stem growth is different from other morphogenetic types of tropical perennial forage grasses: tufted species without internode elongation in the vegetative stage and stoloniferous species with internode elongation simultaneous to leaf elongation (Cruz and Boval, 2000). The resulting dynamics of leaf size with leaf number in signal grass (Figure 3.5) was more similar to that of tufted grasses like *Setaria anceps*, with increasing leaf length up to a certain leaf number and stable leaf length afterwards. Instead in more stoloniferous grasses like *Dichantium aristatum* or *Digitaria decumbens*, leaf length decreased markedly after reaching a maximum value at low leaf insertion numbers (Cruz and Boval, 2000). The leaf insertion number at which constant leaf length was reached seemed to be lower in signal grass than in tufted grasses, coinciding with the early increase in internode elongation, as occurs in stoloniferous grass species.

Internodes did probably act as a mechanism to overcome light limitations imposed by a limit in the increase of successive sheath lengths (Figure 3.5). In order to clarify this function of the internode, different potential relationships between sheath and internode of successive phytomers were tested. The relationship that produced the stronger linear association was that relating the sum of internode and sheath lengths of successive phytomers:

$$(FIL + Sh)_{i+1} = 17.6(\pm 3.53 s.e.) + 0.85(\pm 0.04 s.e.) \times (FIL + Sh)_i$$

$$R^2=0.72 \quad p<0.001 \quad n=155 \quad \text{(equation 3.9)}$$

This relationship shows that the limited plasticity in sheath elongation in signal grass could be overcome by its capacity to elongate more the subsequent internodes. It also shows that both organ sizes were well co-ordinated and should be studied together in the analysis of the structure of the tiller.

Two important relationships were observed with regards to the regulation of internode lengths (equation 3.7). First the positive relationship between *IER* and *LER* indicates the close association between elongation of different organs of the phytomer, presumably a result of the number of cells produced during the initial cell division stages. The fact that IER_i-LER_i relationship (r=0.75) was stronger than IER_i-LER_{i+1} (r=0.58), even though

internode_i and leaf_{i+1} elongated simultaneously for some time, adds evidence to the existence of the same cause explaining the elongation rate in the two organs of the same phytomer. The second relationship regulating the internode length refers to the negative association between *IER* and *IED*. This association was of similar nature to that found in the leaf between *LER* and *LED*, although the importance of the duration of the elongation in defining the internode final length was much smaller that in the case of the final leaf length. *IED* was not linked to any other morphogenetic time related variable (i.e. phyllochron or *LED* of that or of the next leaf), suggesting the lack of a regulation mechanism for the end of internode elongation apart from that intrinsically defined by the rate at which it elongated. Finally, the significative effect that temperature had on *IER*, increasing it by a fixed value at 30°C may indicate a higher effect of temperature on cell expansion rates in cells of internodes than in cells of leaves.

3.5 Conclusions

This study has shown that signal grass has a well-established pattern of vegetative growth. This pattern is the consequence of regulation and co-ordination mechanisms within and between the growth of consecutive phytomers. The phenotypic plasticity of the genotype studied was different depending on the morphogenetic or structural characteristic analysed. In this way leaf elongation rate proved to be the most plastic variable studied, as common for all grasses, with very high sensitivity to the external conditions prevailing. Other variables like the duration of leaf elongation or the phyllochron were tightly associated with leaf elongation rate and with structural characteristics of the tiller, thus much less affected directly by their environment. As a result, the number of simultaneous growing leaves presented a low phenotypic plasticity, characteristic that may be important in separating grasses in different morphogenetic types. The occurrence of internode elongation from early stages of the tiller development and not simultaneously with leaf elongation also differentiated signal grass from other stoloniferous and tufted grasses. This type of internode elongation proved to have important implications in tiller development and structure through the regulation of the size of leaf blades and its compensation with sheath sizes.

The association of temperature with cell expansion rates and of nitrogen fertilisation with cell production rates allowed simple interpretations of the effect of these external factors in the plasticity of some of the relationships observed between morphogenetic or structural variables.

The similarity found between the observed in the growth rooms and what occurs under field conditions suggest the universality of some of the relationships investigated and the appropriateness of experimenting in growth chambers. Further studies in signal grass should focus in investigating the same processes at the cell level in order to understand better the mechanisms of regulation. It is also felt important to perform comparative studies to distinguish the different grass morphogenetic types, especially concerning the plasticity in number of leaves growing simultaneously and patterns of internode elongation.

Chapter 4 Structure of signal grass pastures. Seasonal variation and grazing intensity effects [*]

Abstract

Structural characteristics of signal grass (*Brachiaria decumbens* Stapf.) pastures at different levels of organization were studied for different periods of the year and grazing intensities in Santa Cruz, Bolivia during 1998/99. Intact plants were sampled throughout the year extracting cores previous to the introduction of cattle in rotationally grazed pastures under three different stocking rates (1, 2 and 3 livestock units ha^{-1} yr^{-1}). Variables measured for each core were litter weight, number and weight of plants, number and type of tillers per plant and number and weight of other species. Detailed analyses of tillers (lamina, sheath and internode lengths, lamina width and live proportion) and plants (dry weights of 8 different components) were performed in a sub-sample of plants.

The size of the components of successive phytomers in a tiller followed a well-defined pattern modified slightly by environmental conditions. Grazing affected this pattern indirectly through its effect on the probability of defoliation of the last emerged sheath of the tiller, this affecting the morphogenesis and size of the next appearing phytomer.

The proportion of young tillers in the sward was high and independent of grazing intensity. It only decreased significantly in winter, after a longer resting period and less favourable climatic conditions. At the plant level, the presence of flowering tillers was accompanied by lower proportions of young tillers.

Germination was an important source of plant recruitment, while plant fragmentation during the flowering period decreased the size of the plants towards the following winter.

Though not significantly changing the sward biomass, grazing intensity affected its potential productivity measured by tiller size-density values. The joint use of demographic and biomass measurements is considered to enhance the evaluation of the productivity and degradation state of this type of pastures.

---

[*] Based on Busqué, J. and Herrero, M. Seasonal variation in the structure of signal grass pastures under different cattle grazing intensities. *Journal of Agricultural Science (Cambridge)*. (submitted).

4.1 Introduction

The structure of a monospecific sward can be expressed as the integration of structural characteristics at different hierarchical levels of organisation: phytomer, tiller, plant and population (Brock and Hay, 1993; Briske and Silvertown, 1993; Figure 2.1). Each of these state variables are related to those of contiguous levels of organisation, and finally are regulated by morphogenetic characteristics controlling their demography through appearance, senescence and growth of their units (Chapman and Lemaire, 1993).

In order to predict the responses of pastures to a wide range of different grazing managements and environmental conditions, it is necessary to know the mechanisms involved in pasture growth at their different hierarchical levels, their relationships and their responses and plasticity to variation in their environment. Variables at the sward level used in grazing management (e.g. forage allowance) allow the sustainability of the pasture if the capacity of regrowth of the forage plant after defoliation can be maintained. Under this situation, components of the lower organisational levels of the pasture do vary in their net growth rates but compensate to produce similar productivities at a higher level, as observed for example in the relation between tiller density and tiller weight (Bircham and Hodgson, 1983; Grant *et al.*, 1983). Each grass species or variety shows a different degree of phenotypic plasticity that determines its capacity to reach equilibrium after increasing levels of disturbance (Chapman and Lemaire, 1993). If disturbance levels are greater than the capacity of reaction of the plant, some lower levels of the pasture organisation are not capable of compensating for the decrease of others, and an overall decrease in pasture production occurs (Mears and Humphreys, 1974).

The collection of data on structural and morphogenetic characteristics of sward species usually requires the use of different approaches (Davies, 1993; Grant and Marriott, 1994; Hay *et al.*, 2000). Some structural characteristics of caespitose or stoloniferous species, such as those relating to the plant level of organisation are difficult to obtain unless destructive methods are used (Brock *et al.*, 1988, 1996; Brock and Fletcher, 1993). On the other hand, morphogenetic characteristics are best analyzed following non-destructively the fate of the units to study (Vine, 1983; Simoes and Baruch, 1991; Duru and Ducrocq, 2000a,b; Hirata, 2000). This study deals strictly with destructive measurements, complementing non-destructive field (chapters 5 and 6) and laboratory experiments (chapter 3) that considered the fate and growth of different sward units.

Signal grass was chosen for three main reasons: its wide distribution in many tropical and subtropical areas of the world (Hacker and Jank, 1998), its decline in productivity in many grazing systems only a few years from establishment (Macedo, 1997), and the scarcity of information on population structure and dynamics of tropical forage grasses (Humphreys, 1991; McIvor *et al.*, 1993).

The objectives of this work were (i) to describe the structure and population of signal grass at different levels of organization of the pasture; (ii) to establish the relations between variables at contiguous levels using the general rule that the size of a unit at a higher level is equal to the number of units at a lower level multiplied by the size of those units; (iii) to establish the relations existing within components at the same level of organization, and (iv) to establish the sensitivity of the above variables and relationships to season and grazing intensity.

4.2 Materials and methods

Site

The experiment was conducted at the Estación Experimental Agrícola de Saavedra, Santa Cruz, Bolivia (lat. 17° 14' S; long. 63° 10' W; alt.: 320m a.s.l.). The soils at the experimental site were Alfisols Ustalf, not seriously limited by any macro-nutrient, and characterised by pH levels of 5.5 and low water retention due to their high proportions of sand (loamy sand textural class) and low soil organic matter percentages (1.6%±0.65 s.d.). Climatic data was collected from a meteorological station, 1 km from the experimental site. The climate of the area is tropical with two marked seasons of approximately similar duration: hot rainy summer from October to March, and cool dry winter from April to September. Means of the last 47 years showed that the coolest months were June and July (average daily temperature of 20.2°C) and the hottest were December and January (25.8°C). The wettest month was January (224 mm) and the driest was July (42 mm). Figure 4.1 shows temperature and precipitation values at regular intervals, while Table 4.1 shows average values of some climatic variables for each of the regrowth periods of the study.

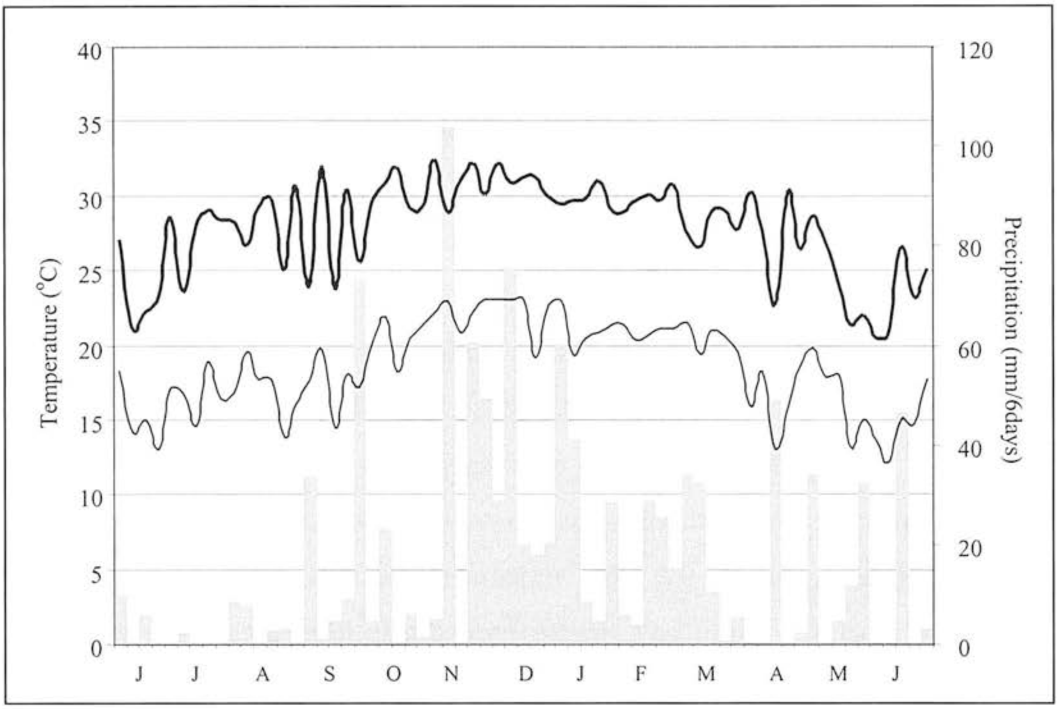

Figure 4.1. Mean minimum and maximum temperatures (lines and left vertical axis) and precipitation for six-day periods (columns and right vertical axis) in the experiment site, from July 1998 to June 1999.

Table 4.1. Climatic characteristics of the regrowth periods.

Period	Average regrowth days (sd)	Period of sampling		Average daily temperature (°C)	Average daily radiation (MJ m^{-2})	Average daily real/potential evaporation ratio†	Average accumulated rainfall (mm)
August-October	101	05-Oct	15-Oct	22.6	11.0	0.25	151.1
November	25 (3.1)	03-Nov	18-Nov	25.6	14.1	0.26	12.5
December	27.3 (1.8)	01-Dec	21-Dec	26.7	14.6	0.95	214.2
January	31.7 (1.8)	06-Jan	25-Jan	25.9	14.8	0.92	159.5
February	28 (3.1)	02-Feb	24-Feb	25.4	15.4	0.84	56.3
March	27.7 (1.2)	08-Mar	25-Mar	24.8	12.8	0.90	131.4
April-July	72.5 (3.6)	05-Jul	23-Jul	19.8*	10.2*	0.61*	133.2*

† Calculated according to Rowell (1994; p. 252)

*Average values from 2/May/99 to 30/June/99, as no data was available for July 1999.

Pastures and grazing management design

Signal grass swards established 10 years before were used in the experiment (Figure 4.2). The management of the pastures prior to the experiment consisted of rotational grazing with Criollo dual-purpose cattle at low stocking rates (1 livestock unit ha^{-1} yr^{-1}), slashed once a year at the end of summer and never receiving any type of inorganic fertilization.

Six plots of 400 m^2 were set up in each of two paddocks and rotational grazing was performed in all the plots with cycles of one month from October to April and of 2-4 months the rest of the year (Table 4.1). The duration of grazing was always of one day per plot. Three different grazing intensities were employed, equivalent to 1, 2 and 3 livestock units per hectare and year. These corresponded to instantaneous stocking rates of 2, 4 and 6 Criollo cattle (dry cows of approximately 450 Kg of liveweight) per day and plot respectively. The same level of grazing intensity was applied in each plot for each of the grazing periods, except in the first period (October), when the treatment grazing intensity was not yet implemented. This period was also different in that sward samples were collected from different plots within each paddock to those plots used in the rest of the periods. From November onwards, two replicates of the same grazing treatment were performed in each paddock.

Plant sampling

Sampling was done immediately before the grazing date in each plot. Monthly sampling started in October of 1998 and was followed regularly until March of 1999. A last sampling was done in July of 1999 (Table 4.1).

Each plot sampling for each period consisted in the extraction of 3 20x25cm turf cores located randomly within the plot. Turves were removed intact using a steel edged quadrat introduced to a depth of 15-20 cm into the soil. Once the quadrat was randomly positioned in the plot, it was slightly moved in order to avoid dissecting signal grass plants at the edges. Before washing the turves litter was collected (Figure 4.3). After washing the soil, the number of plants and the type of tillers within each plant were recorded (Figure 4.4). Tillers were either classified as born from aerial axilar nodes, from basal nodes, or germinated from seed. The number of reproductive tillers per plant was also recorded. Litter and individual plant dry weight were obtained after drying the samples in an oven at 60°C for 48 hours.

Figure 4.2. One of the signal grass paddocks of the experiment and one of the plots being grazed by Criollo cattle. November, 1998.

Figure 4.3. Pasture core during the washing process. Litter had been already removed. March, 1999.

Figure 4.4. Signal grass plants from one core corresponding to March of 1999. Each aluminium pot holds one plant.

Figure 4.5. Signal grass plant components. From left to right, and from top to bottom: live stem, new hidden leaf, sheath, blade, basal nodes, dead stem and dead leaf. March, 1999.

From each core a sub-sample of approximately 20% of the total number of plants and tillers was randomly chosen for further analysis. Each tiller of these plants was classified as before, and axilary borne tillers were further identified by the number of the node of the mother tiller from where they were born. Phytomers of all the tillers of the sub-sampled plants were enumerated starting from the oldest one. For each phytomer the following data was collected: blade length, visual proportion of live tissue and occurrence of defoliation, sheath length and occurrence of defoliation and internode length. Once the phytomers were measured, tillers were dissected and plants were separated in eight different parts: blade, sheath, live stem, dead stem, dead attached leaves, basal internodes, inflorescences and new hidden leaf material (Figure 4.5). Each plant component was dried and weighed in the same way as for the pooled samples of plants and litter.

Over the whole experiment 237 turves were analysed, corresponding to 3151 plants (9923 tillers), of which 515 plants (1924 tillers) were further sub-sampled. Growing point numbers and total dry weight per core of other species apart from signal grass were also recorded.

Statistical analyses

Statistical analyses mainly consisted of analyses of variance considering the effects of period of sampling (7 levels) and grazing intensity (3 levels).

The effect of period was first analysed separately to that of grazing in order to include the values of the first period (October), when grazing treatments were not yet established and different plots were used to the rest of the periods in the study. The use of different plots in this first period required averaging the values of the response variables for each paddock and period in order to obtain a balanced design of the data. In this way the analyses were performed for 7 periods with 2 repetitions (paddocks) per period.

The effect of grazing was analysed for the 6 sampling periods going from November to July. The design of the experiment fitted a split-plot design in which grazing was the main factor and period was the split factor. Since different plants from different locations in each plot were used at each sampling period, the inclusion of time in the analysis was valid and no account of repeated measurements had to be considered (Brock and Fletcher, 1993).

4.3 Results

Characteristics of the regrowth

The months before and after summer (August to November and April to July) were characterised by low average values of all the climatic variables (Table 4.1). Water stress, measured by the ratio of daily real to potential evaporation, was especially acute for most of the August-October and November regrowth periods, representing a major limitation for pasture growth. Nevertheless, rains at the end of each of the two periods (Figure 4.1) provided abundant water for a few days in each case. The best environmental conditions for pasture growth occurred from December to February. In March temperatures and radiation started to decrease, and in the July regrowth period the lowest temperature and radiation values were reached.

Structure of leaves within a tiller

The blade length increased linearly from the first leaves up to leaves of insertion number 6, remaining constant or decreasing afterwards. For leaves with insertion numbers 2 to 7, the blade length was highly correlated with sheath length of the previous leaf (enclosing sheath): r=0.91, this correlation being higher than that with its corresponding sheath (r=0.73). Previous internode length was also related significatively to blade length. Sheath length increased up to leaf number 5 and remained constant or decreased afterwards. Similar trends were observed for internode lengths, with highest values for leaf number 6 (Figure 4.6).

Blade, sheath and internode lengths were affected significatively by the interaction between period and leaf insertion number, mainly due to the different trends observed at high leaf insertion numbers (Figure 4.6). Blade length showed its lowest values in January and highest values in March and July. This trend remained unchanged when the analysis of variance was performed using the length of the previous sheath as covariate. The steep decrease in blade length observed in January and February for the two highest leaves was a consequence of the structural changes characteristic of flowering tillers. Highest sheath length values were reached at the end and after the flowering season (March to July), while internode lengths were highest at the end of the flowering season and lowest before the start of the flowering season.

No significative interactions were observed between grazing intensity and leaf insertion number for any of the leaf structural characteristics. The negative effect of grazing on blade length (Table 4.2) was caused by its effect on the reduction of previous sheath lengths, as was demonstrated by the lack of response of blade length to grazing intensity when the previous sheath length was used as covariate in the analysis. Low stocking rates produced longer sheaths and internodes than the rest of grazing intensities (Table 4.2).

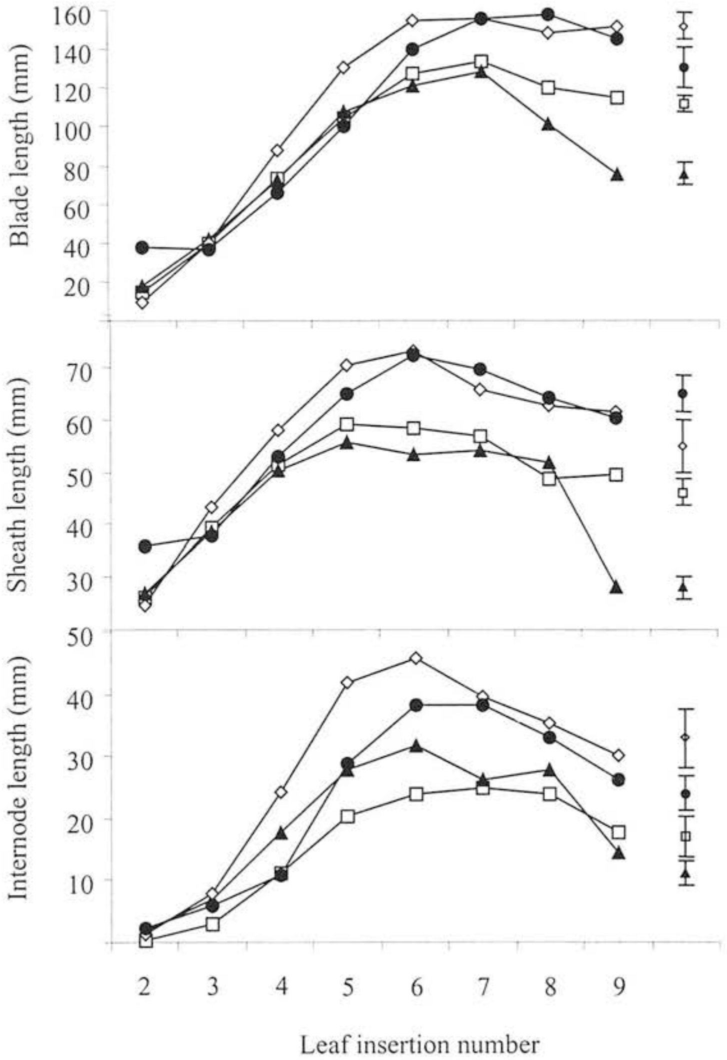

Figure 4.6. **Variation in mean signal grass leaf characteristics depending on leaf insertion number in the tiller (leaf 2 is the oldest leaf in the tiller with internode) and season of the year (□ October-December; △▲ January-February; ◊ March; ● July). Vertical bars are s.e.d. for each season.**

Structural characteristics of the tiller population.

Tillers were classified according to their age and size. The variable used to measure tiller age was the total number of leaves, while tiller size was measured using total live leaf and stem lengths (Figure 4.7).

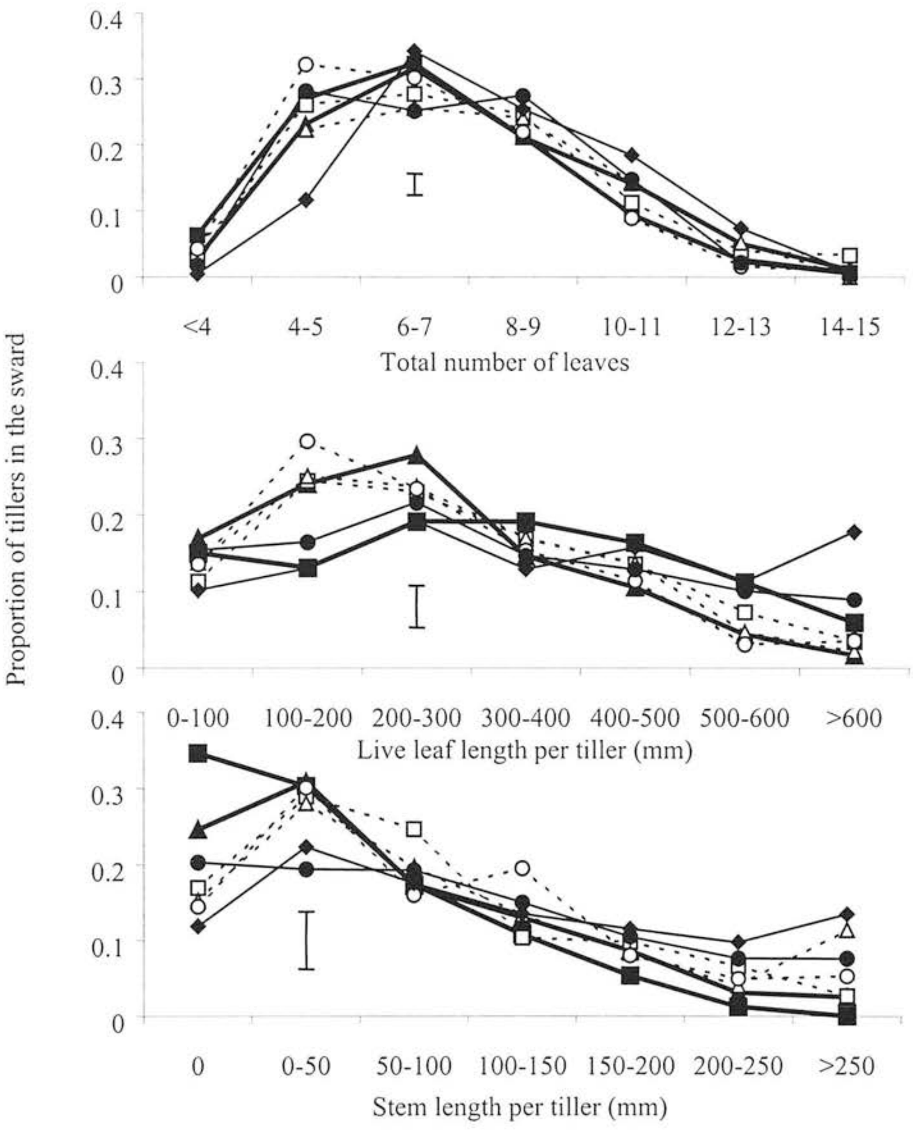

Figure 4.7. **Proportion of sward tillers in each of the categories of tiller age (number of leaves per tiller) and tiller size (live leaf and stem length per tiller) for the different periods (■ October; ▲ November; □ December; ○ January; Δ February; ● March; ♦ July). Vertical bars are the s.e.d. of the interaction between categories and periods.**

The distribution of tillers in the sward according to their age showed that most tillers in the sward at the end of the regrowth periods had between 4 and 9 leaves (Figure 4.7). The main difference among periods was the lower number of tillers with 4-5 leaves in July. This age category mainly corresponded to tillers appearing at the beginning of that regrowth period, after the last grazing event (new tillers). Grazing intensity did not affect the tiller age distribution in general, though a significative interaction with period ($p<0.05$) was observed in the proportion of new tillers (Figure 4.8). This proportion was similar for the different grazing intensities, except in November, when grazing intensity was linearly associated with the proportion of new tillers.

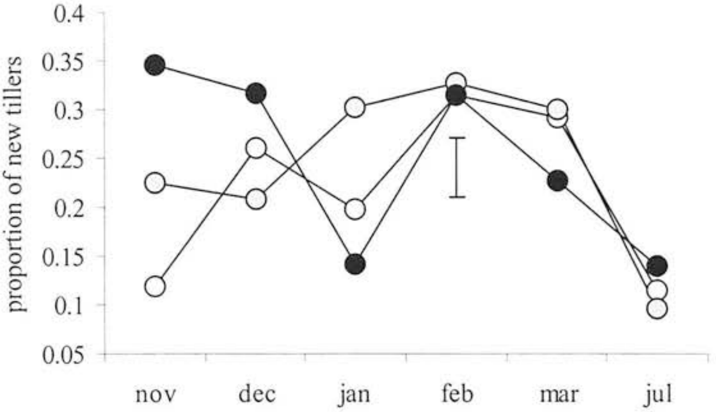

Figure 4.8. Proportion of new tillers (tillers with 4-5 leaves) for the different grazing intensities (white: low; grey: medium and black: high) and periods. Vertical bar is the s.e.d. of the interaction between grazing intensity and period.

The distribution of the amount of green leaf per tiller in the sward from the initiation of growth in October up to the flowering season, showed a trend towards higher proportion of tillers with low live leaf length values and lower proportions with high values (Figure 4.7). Post-flowering periods (March and July) reverted this trend, resulting in a more heterogeneous green leaf per tiller distribution, although a higher proportion of tillers of the highest live leaf length category were present. Neither the general distribution of live leaf length showed in Figure 4.7, nor any specific green leaf category was significatively affected by grazing intensity.

The tiller stem length distribution changed with the period of the year, being progressively more heterogeneous from the initiation of fast growth (October) towards the end of the growing season (March and July). While October showed the highest proportion of culmless tillers and the lowest proportion of tillers with high stem lengths, July showed the opposite distribution (Figure 4.7). Grazing intensity affected tiller distribution of stem lengths independently of the period of the year ($p<0.01$), with the low grazing intensity showing a lower proportion of tillers with short stems and a higher proportion with long stems (Figure 4.9).

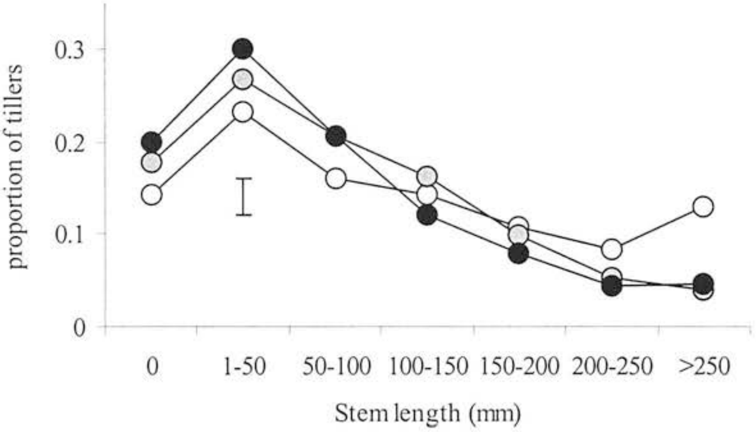

Figure 4.9. Tiller stem length distribution in the sward for the different grazing intensities (white: low; grey: medium and black: high). Vertical bar is the s.e.d. for the interaction between stem length category and grazing intensity.

In order to study the tiller density of the sward, tillers were classified according to their birth type (germinated or born vegetatively), tiller connection in the plant for vegetatively born tillers (aerial or basal nodes) and phenological stage (vegetative or reproductive). Vegetative basal and aerial tillers were finally classified depending on whether they belonged to plants with any reproductive tiller or not. Figure 4.10 represents the seasonal changes in the density of each of those tiller categories. Germinated tillers were especially important in October, when they accounted for 12% of the total density of tillers in the sward. Reproductive tillers started to appear in December, and from January to March they accounted for 4-7% of the tiller population, without significant differences in their numbers among those 3 periods. Similar trends to those of reproductive tillers were observed for vegetative basal and aerial tillers belonging to plants with reproductive tillers. Changes in signal grass tiller densities with period were mainly caused by changes in the density of vegetative tillers from plants without reproductive tillers (Figure 4.10).

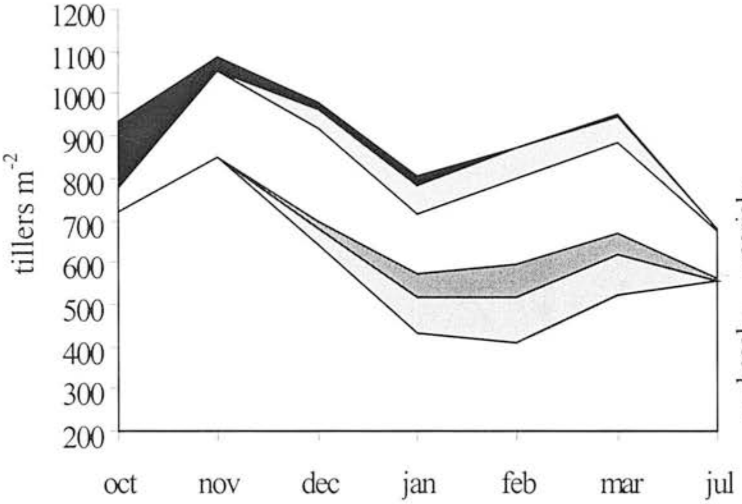

Figure 4.10. Seasonal variation in tiller density for different tiller types. Bottom:: basal tillers; top: aerial tillers; white: from plants with only vegetative tillers; bright grey: vegetative but from plants with reproductive tillers; dark grey: reproductive tillers; black: germinated tillers.

Increasing grazing intensity resulted in an increase in the density of aerial tillers of vegetative plants and a decrease in the density of germinated tillers (Table 4.2). On the contrary, the density of aerial vegetative tillers from reproductive plants was highest at the low grazing intensity in January (117.5, 55 and 24.2 tillers m^{-2} for low, medium and high grazing intensity respectively; s.e.d.=14.74), but was not affected by grazing in the other flowering periods.

For basal or aerial vegetative tillers, there were no significant differences in average structural values (number of leaves, live leaf length and stem length) for tillers corresponding to vegetative or reproductive plants. On the other hand, reproductive tillers were always older and had higher stem length values than both vegetative basal or aerial tillers. Aerial vegetative tillers always showed the lowest structural values (Figure 4.11).

Only for stem lengths there was a clear seasonal trend for all the tiller types, with increasing values for vegetative tillers and decreasing for reproductive ones.

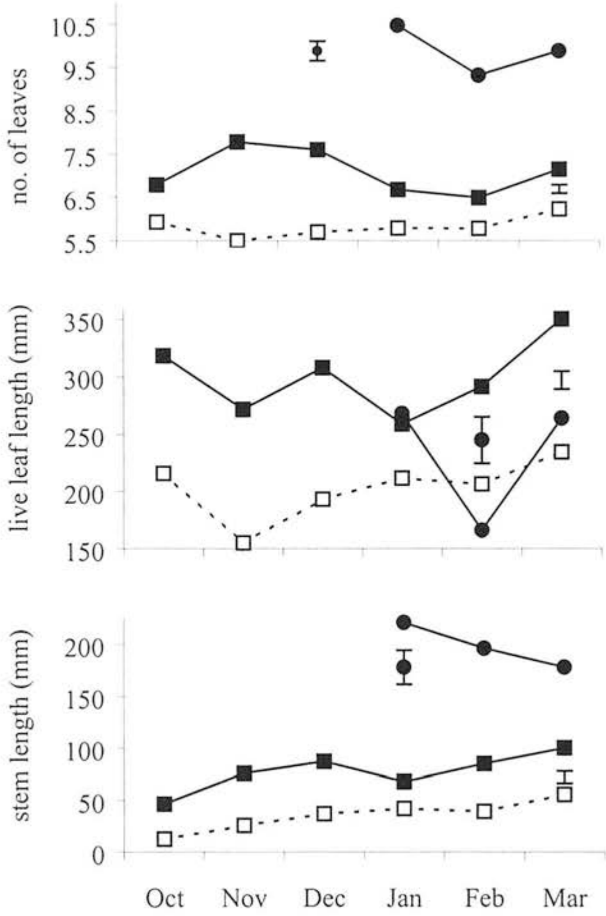

Figure 4.11. Seasonal variation in mean structural characteristics of different types of signal grass tillers (□ vegetative aerial; ■ vegetative basal; ● reproductive). Vertical bars are s.e.d. of the interactions between periods and types of vegetative tiller. Vertical bar with a circle is the s.e.d. between periods for reproductive tillers.

Grazing intensity affected similarly the structural characteristics of vegetative basal and aerial tillers. For both types of tillers grazing intensity produced a decrease in their average size, both in terms of live leaf and stem length (Table 4.2), while average tiller age was not affected. With respect to reproductive tillers, the low grazing intensity resulted in tillers with longer stems than for higher grazing intensities (Table 4.2).

Table 4.2. Effect of grazing intensity on variables at different levels of sward organisation.

	Grazing intensity				
	Low	Medium	High	s.e.d.	P
Leaf level					
Blade length (mm)	108.3	103.6	99.0	4.5	0.176
Sheath length (mm)	59.4	54.5	52.7	2.5	0.071
Internode length (mm)	28.6	22.8	20.9	2.2	0.020
Tiller level					
Live leaf length vegetative tiller (mm)	313.9	293.2	270.7	11.8	0.020
Stem length vegetative tiller (mm)	(9.93) 98.6	(8.54) 72.9	(8.03) 64.5	(0.86)	0.131
Stem length per reprod. tiller (mm)[1]	288	147	159	30.2	0.003
Aerial tiller density (m^{-2})	130	203	222	39.2	0.082
Germinated tiller density (m^{-2}) [2]	49.1	18.3	10.0	14.11	0.102
Sward level					
Inflorescence weight ($g\ m^{-2}$) [1]	15.2	13.0	11.4	2.22	0.026

Values between brackets correspond to means and s.e.d. from transforming the original data to squared roots to attain normality.

Total D.F.=70; Paddock stratum error D.F.=1; Paddock. Plot stratum error D.F.=8; Paddock. Plot. Period stratum error D.F.=44

[1] Only for the months January to March. Total D.F.=34; Paddock. Plot. Period error D.F.=17

[2] Only for the months November to December. Total D.F.=35; Paddock. Plot. Period error D.F.=18

Plant structure

Plant density varied between 230 and 310 plants m^{-2}. Minimum values, not considering newly germinated plants, were obtained at the beginning of the growing season (October) and in winter (July), mainly due to the low number of large plants (Figure 4.12). The number of large plants (>4 tillers) increased after the first regrowth period (November) and decreased significantly at the start of the flowering season, maintaining low numbers until the end of the growing season and winter period. The other categories of plant size did not change with the period, except for plants with 3 tillers, which showed lower numbers than average during December. Grazing intensity did not alter the total plant density or the density of any of the plant size categories.

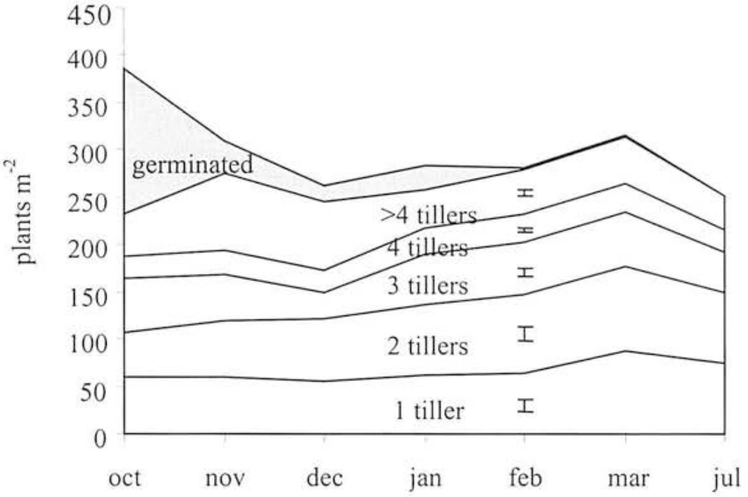

Figure 4.12. Seasonal dynamics of plant density as a whole and for different plants according to their size. Vertical bars are s.e.d. for each of the plant sizes.

During the flowering season (January-March), 14% (st.dev.=8.6) of plants had at least one reproductive tiller. This percentage did not change with period or grazing intensity. The size distribution of plants with reproductive tillers during the flowering season was significantly different to that of plants with only vegetative tillers, with a higher proportion of larger plants and a lower proportion of smaller ones (Figure 4.13).

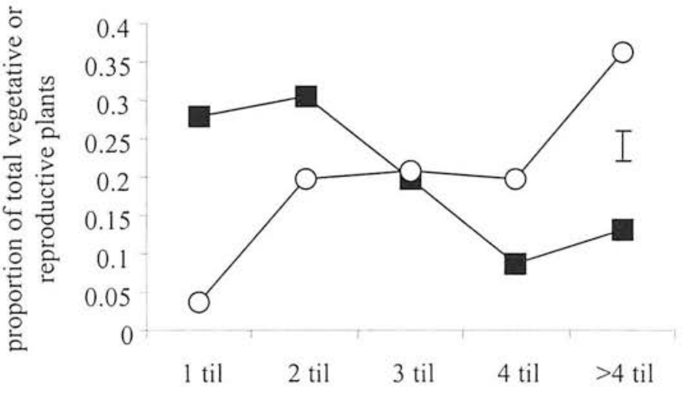

Figure 4.13. Proportional distribution of plant sizes with (○) or without (■) reproductive tillers during the flowering season (January-March). Vertical bar is the s.e.d of the interactions between plant size and plant types.

At least at the beginning of the flowering season, plants with reproductive tillers showed a significant lower proportion of young tillers, irrespective of the grazing intensity (Figure 4.14).

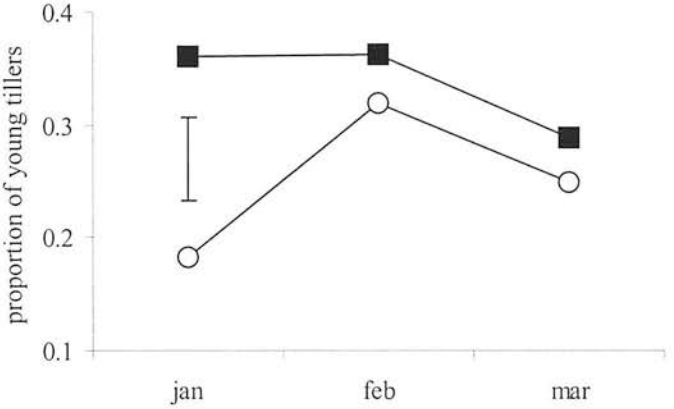

Figure 4.14. Proportion of young tillers (with less than 6 leaves) in plants with (○) and without (■) reproductive tillers during the flowering season. The vertical bar indicates the s.e.d. for comparisons between plant types at each period.

The sward structure

Litter was always the major component of the sward aboveground biomass and it showed a slight tendency to decrease along the wet season, from October to March. Signal grass standing biomass did not change significantly with the season of the year, though lowest values were observed in January. All of the signal grass standing biomass components, except the inflorescences, showed minimum values in January, especially significant in the case of dead stems and dead leaves. Standing biomass of other species apart from signal grass was always a low proportion of the total aboveground biomass (Figure 4.15).

Grazing intensity as a main effect only affected significatively the inflorescence weight, with lower weights for higher grazing intensities (Table 4.2). All the other sward components were not significatively affected by grazing intensity. Only for the case of pseudostem weight a significant effect (p=0.045) of the interaction period x grazing intensity was found, but lacking a reasonable pattern.

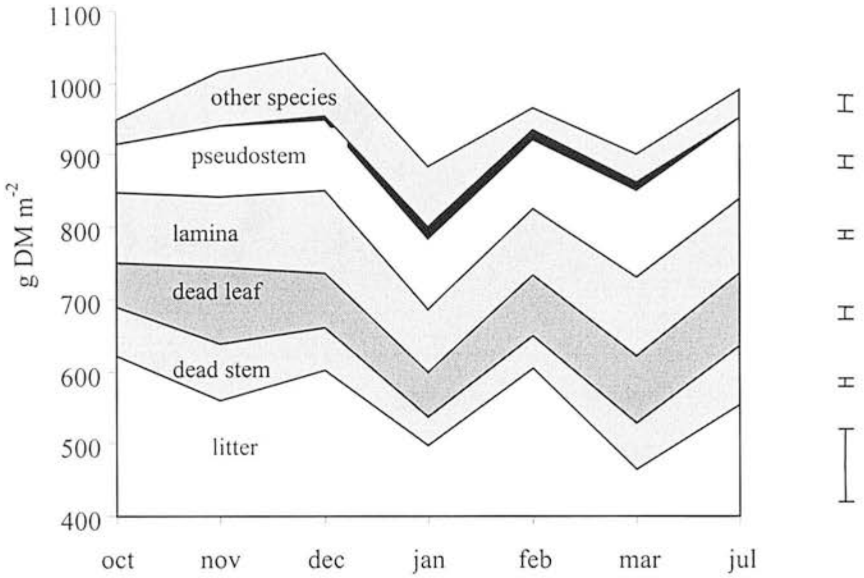

Figure 4.15. Seasonal variation in dry matter of the different components of the sward (black: inflorescence). Vertical bars are the s.e.d. for the periods within each sward component.

Considering the minimum distance of sward samples to a hypothetical threshold line relating sward tiller density and tiller weight at ceiling leaf are index (Matthew *et al.*, 1995), a productivity index (*prind*) can be calculated, with values negatively related to that distance (Hernández Garay *et al.*,1999). The productivity index values obtained fitted well (R^2= 0.96; $p<0.001$; n=236; Figure 4.16) a linear function with the logarithm of signal grass standing aboveground biomass (*dw*):

$$prind = -0.97(\pm 0.024) + 0.72(\pm 0.009)\log dw$$

Highest values of the index (the 15% highest of the curve of Figure 4.16) corresponded to values of standing signal grass dry matter of above 600g m^{-2}.

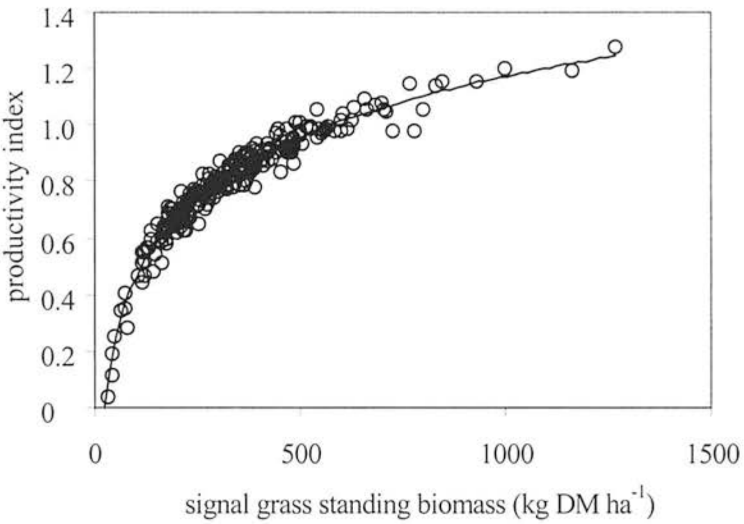

Figure 4.16. Relationship between signal grass standing biomass and the proposed productivity index of Hernández-Garay et al. (1999).

Neither grazing nor season affected significantly the productivity index, but they affected ($p<0.01$ and $p<0.001$ respectively) the intercept of the above regression. The medium and high grazing intensities increased the intercept 0.01 and 0.02 with respect to the low grazing intensity. Periods 2 and 3 (November and December) increased the intercept 0.03 and 0.02 respectively, while period 8 (July) decreased it by 0.03. Similar results were obtained when the productivity index was calculated considering only average tiller lamina dry weight instead of average tiller dry weight and regressing it against signal grass lamina dry weight.

From the table of correlations between signal grass standing biomass and its components (Table 4.3), it could be seen that variations in standing biomass were more related to changes in weight when considering the plant level (except in October and March), and to changes in the number of units when considering the tiller level (except in October, January and for the low grazing intensity). Of both correlations, that between standing biomass and tiller density was higher than the one between standing biomass and plant weight for certain periods (November, February and March), and for the medium grazing intensity. On the other hand, the correlation between standing biomass and plant weight was higher for December and January, also corresponding to high correlations between standing biomass and tiller weight. Densities and weights of plants were always more negatively related than densities and weights of tillers, though in any case correlations were especially high. Plant and tiller densities were normally highly correlated, except in December. Correlation values for October should be considered with care, as plant weights for each turf were only measured in a sub-sample of plants.

Table 4.3. Linear correlations between signal grass standing biomass and its components at the plant and at the tiller level for the different periods and grazing intensities. NS: non significant. Original values were transformed to their logarithms.

	standing biomass			standing biomass			tiller vs.
	plant density	plant weight	density - weight	tiller density	tiller weight	density - weight	plant densities
All periods	0.51	0.59	-0.30	0.62	0.58	-0.28	0.69
October	0.63	0.55	NS	0.55	0.80	NS	0.76
November	0.53	0.56	-0.40	0.74	0.55	NS	0.75
December	0.55	0.85	NS	0.74	0.78	NS	NS
January	NS	0.64	-0.57	NS	0.73	-0.33	0.82
February	0.54	0.68	NS	0.80	0.36	NS	0.78
March	0.55	0.46	-0.46	0.71	0.59	NS	0.67
July	0.42	0.59	-0.47	0.56	0.62	NS	0.81
Low	0.40	0.59	-0.48	0.51	0.60	-0.39	0.68
Medium	0.53	0.55	-0.39	0.78	0.35	-0.31	0.76
High	0.57	0.70	NS	0.62	0.52	-0.34	0.63

4.4 Discussion

Structure of leaves within a tiller

The change in length with leaf insertion number of the different signal grass phytomer components (Figure 4.6) did not fit clearly to any of the patterns shown in other tropical tufted or stoloniferous perennial grasses (Cruz and Boval, 2000). Blade and sheath lengths were progressively longer up to a certain leaf insertion number, after which they usually remained stable. This pattern was similar to that of tufted grasses in their vegetative state (Chapman and Lemaire, 1993). However, unlike tufted grasses, signal grass showed internode elongation from early stages of its vegetative growth. Successively increasing internode lengths in signal grass did not affect negatively the corresponding blade and sheath lengths, contrary to what has been observed in stoloniferous grasses (Cruz and Boval, 2000). These authors concluded that the negative relationship between internode length and leaf length was linked to the simultaneous leaf and internode development of the same phytomer, a process not occurring in signal grass (see chapter 3). A positive relationship, already detected under controlled environments (see chapter 3), was observed between the internode and the sheath length of a leaf and the blade length of the next leaf.

While maximum blade, sheath and internode length values were similar in field and controlled environment conditions, differences were observed in their patterns of change with leaf insertion number (Figure 4.6 and see figure 3.5). Maximum blade and sheath lengths were achieved 2 to 3 leaves earlier under controlled environments, with a higher increase in blade length detected from the first to the second leaf measured (see figure 3.5) in comparison to the equivalent leaves under field conditions (Figure 4.6). In the case of internode lengths under controlled environment, they continued increasing in leaves that had already reached the maximum blade and sheath length, while under field conditions internode lengths followed a similar pattern to that of blade lengths. The different light conditions existing in the controlled environment experiment, both in quality and photosynthetic photon flux density, was considered to be the main cause producing these contrasted patterns, as they have seen to modify markedly elongation rates and durations of different phytomer organs (Lemaire, 2001).

The period of the year in which tillers were growing affected markedly the structure of their phytomers. These differences were clear for leaves of high insertion numbers (higher than 3 in Figure 4.6). It was in March and in July when the highest values for blade, sheath and internode lengths were obtained. Higher blade and sheath length in these periods could be explained by the values of leaf elongation and leaf appearance rates observed in a parallel non destructive experiment during that time (see tables 6.8 and 6.9), assuming a close relationship between phyllochron and leaf elongation duration (see chapter 3). The significative effect of period of the year upon blade length when previous sheath length was used as covariate indicated the existence of plasticity in the determination of blade length, normally associated with processes related to cell expansion, usually more independent of tiller structure (Nelson, 2000). On the other hand, grazing intensity did not affect blade length, especially when the previous sheath length was used as covariate in the analysis. Although defoliation has immediate negative effects on leaf elongation rate by reducing the rate of cell production and the duration of cell expansion (Schnyder *et al.*, 1999), this effect occurs only shortly after the defoliation (Richards, 1993), and was probably minimised by the long regrowth periods used. In any case, the structure of the phytomers was very sensitive to grazing intensity. Defoliation directly affected sheath and internode length, while blade length reduction with increasing grazing intensity was mainly a consequence of the reduction in sheath length. The general negative effect of grazing intensity on sheath length is a consequence of its positive effect on the frequency and severity of the defoliation of the youngest sheath, and of the co-ordination in the growth of successive leaves (Skinner and Nelson, 1995; see chapter 3). The cutting of the last developed sheath in the tiller, if not accompanied by tiller decapitation, results in a readjustment in the growth of the next phytomer, as the amount of cells produced for future elongation depends on the length of the tube in which the growing phytomer is included initially (Casey *et al.*, 1999). The result is shorter future blades, sheaths and internodes.

Structure of the tiller

The average structure of the tiller population did not change markedly along the growing season when three different types of tillers were considered: reproductive, vegetative basal and vegetative aerial (Figure 4.11). The total number of leaves was always smaller for aerial vegetative tillers than for basal vegetative tillers, this in accordance with the shorter lifespan of aerial tillers (see figure 6.6).

The proportion of new tillers in the sward (those with less than 6 leaves; Figure 4.7) at the end of each regrowth period was a function of the tiller recruitment shortly after the previous grazing event, but also of their surviving rate during the regrowth period. The low value of new tillers obtained in July was mainly a consequence of the decrease in the number of aerial tillers (Figure 4.10), due to their lower rates of appearance and higher death rates (see Table 6.8). Grazing intensity affected positively and linearly the proportion of new tillers only in November (Figure 4.8) when their survivorship was much higher than in subsequent periods (see tillers born in October in Figure 6.8). The overall lack of response of effective tiller recruitment to grazing intensity was not caused by a similar long-term tillering pattern as observed in rangeland grass species (Briske and Silvertown, 1993). On the contrary, defoliation intensity promoted tillering throughout the experiment (see Table 6.7), and this agrees with the responses of grass species of more mesic environments (Grant *et al.* 1981). However survivorship of born tillers was negatively related to grazing intensity, finally resulting in the lack of response of grazing to net tiller recruitment.

The amount of green leaf per tiller was the result of the balance between leaf growth and leaf senescence. After the appearance of a tiller there is an initial period without leaf senescence, but once this starts, its importance increases up to a point in the tiller's development when leaf senescence equals or exceeds leaf growth (Chapman and Lemaire, 1993). The dynamics in the length of successive leaves has a great importance in determining the point of maximum green leaf per tiller (Cruz and Boval, 2000). In this experiment leaf senescence equalled leaf growth when tillers attained 7 to 9 leaves, depending on the season and the amount of leaf defoliated in the tiller in the previous grazing event. With higher number of leaves tillers maintained or decreased slightly its live leaf length, in accordance to the dynamics in blade length (Figure 4.6). The average live leaf length values obtained for vegetative basal tillers (Figure 4.11) were always lower than the potential maximum values, as a high proportion of tillers (40-60%) had less than 8 leaves (Figure 4.7), irrespective of season or grazing intensity. Although not affecting the average tiller age or its distribution, grazing intensity changed negatively and linearly the average vegetative tiller live leaf length. This change was mainly a consequence of the negative effect of grazing intensity on leaf blade length (Table 4.2), and could not be counteracted by the negative effect of defoliation on senescence rates of live tillers (see Table 6.7). Added to the leaf senescence from living tillers, it is important to consider the important pool of leaf senescence coming from decapitated tillers, strongly affected by grazing intensity (see Table 5.2), and that from tillers dying of causes not directly related to grazing. These pools will be considered in chapter 6.

Average stem length per tiller (Table 4.2) and its distribution in the sward (Figure 4.9) were significatively affected by grazing intensity. In this case this was solely the consequence of the effect upon internode length (Table 4.2), as grazing intensity did not affect tiller age or its distribution. While internode elongation has been demonstrated to be linked with lack of light in temperate species (Matthew *et al.*, 2001), this was not corresponded in our case with differences in the amount of standing biomass in the sward or any of its components.

Finally, the variance in the distribution of the tiller structural characteristics was always high (Figure 4.7), indicating a large heterogeneity in the structure of the swards. Rotational grazing might promote tiller heterogeneity when compared to other grazing management systems (Brock and Hay, 1993). Defoliation frequency should apparently affects the degree of structural heterogeneity of signal grass swards more than defoliation intensity, which seems to be very stable at the tiller level with stocking rate (Wade *et al,* 1989; Lemaire and Agnusdei, 2000). Although defoliation frequency was affected by grazing intensity (see table 5.2), grazing intensity did not affect the relative tiller structural heterogeneity of the sward probably because of the short lifespan of tillers in all cases, with high rates of tiller death not related directly to grazing (see figure 6.10). Tiller structural heterogeneity increased along the growing season, producing highest values in the last period (July). This explains the extended use of mechanical slashing at the end of summer in swards under the same type of management in order to regain homogeneity in tiller structure, accelerate dead material recycling and avoid competition from other species.

Plant structure

The seasonal dynamics in the distribution of the plant population according to different plant sizes (Figure 4.12) was the result of the processes of plant birth, death and fragmentation. Plant birth corresponded to germinated tillers and was only important in the first growing period, where it accounted for 39% of the total number of plants.

Assuming that from October to November death and appearance from fragmentation of established plants was negligible, the survival percentage of germinated tillers from October to November would have been 28%. Although not directly measured, it is reasonable to assume low numbers of both plant deaths and fragmentations from October to November, as climatic conditions were favourable to growth and main plant tillers were in a vegetative ontogeny, less prone to die and subsequently fragment or kill their plants. This seedling survival value was at the edge of the 28 to 93% values found after one month from germination by Andrew and Mott (1983) for the annual grass *Sorghum intrans* in north-west Australia (similar climatic conditions to those of our experiment). However, these authors remark that seedling mortality would have been much higher if individuals were grazed by large herbivores. On the other hand, seedling survivorship values for annual grasses in a much drier tropical ecosystem (Sahel of southern Mauritania: 400 mm of rainfall concentrated in 3-4 months; Carriere, 1989) were lower than in our case (20% in *Panicum laetum*, 17% in *Schoenefeldia gracilis* and 13% in *Aristida mutabilis*), being the degree of soil water constrain the main factor differentiating both sites. Finally, it is also important to consider that survival of seedlings that emerge from sowing into a seedbed is often far higher than that of seedlings emerging in an established pasture, as competition from established plants is removed and in many cases stored soil water and mineral nitrogen is more available (Hay *et al*, 2000).

Plant fragmentation showed the highest frequency at the start of the flowering season. From December to January a significant decrease in the density of plants with more than 4 tillers was not accompanied by a decrease in the total plant density of the sward, this remaining more or less stable (Figure 4.12). Instead the density of plants with 3 tillers increased, pointing out to the occurrence of plant fragmentation. The larger sizes of plants with reproductive tillers in comparison with plants with only vegetative tillers during the flowering season (Figure 4.13) was also probably related to plant fragmentation. Flowering tillers need higher support from common plant reserves (Colvill and Marshall, 1984), this explaining their higher frequency in larger plants. They normally correspond with main tillers in the plant with more than one inter-tiller connections (Brock and Fletcher, 1993). Following the death of the flowering tiller more chances will exist for one of its inter-tiller connections to break and fragment the plant.

Another important difference between plants with and without reproductive tillers referred to their proportion of young tillers at the beginning of the flowering season (Figure 4.14). This supports the hypothesis of reproductive tillers of a certain stage inhibiting plant vegetative growth, either through inhibition of tiller appearances or survival of new tillers (Colvill and Marshall, 1984). The phenological stage of reproductive tillers was measured by their proportion of live leaf length with respect to the average maximum live leaf length, considering only the last 3 leaves of each tiller. Values of 0.60 (49%), 0.50 (74%) and 0.65 (63%) were found for January, February and March respectively (coefficients of variation between brackets). Reproductive tillers in January were more grouped into a similar phenological stage, which appeared to be near a plant physiological state producing inhibition of new tillers (Figure 4.14).

Sward structure

The very low or non-significant correlations existing between tiller density and tiller weight (Table 4.3) indicates that these swards were generally far from reaching the size-density compensation (SDC) line common to swards near their ceiling leaf area index (Hernández Garay et al. 1999). However, the use of a productivity index based on the minimum distance of the sward tiller density-size logarithmic co-ordinates to a reference line with a -3/2 slope, proved to be an excellent indicator of sward standing and green leaf biomass (Figure 4.16). Increases in sward standing or green biomass of a monospecific sward can be the result of one or a combination of the following processes: (i) the sward progressing towards the -3/2 SDC line (i.e. increase in the productivity index); (ii) the sward moving towards the left side of the SDC line without necessarily changing its productivity index; and (iii) the sward increasing its SDC line, again without necessarily affecting the productivity index. Figure 4.17 represents the processes (ii) and (iii), which correspond to some changes occurring in the swards of this experiment with grazing intensity and period, and reflected in the significant different intercepts detected in the regression between signal grass standing biomass and productivity index.

An increase in sward standing biomass at constant productivity index (i.e. decrease in the intercept of the regression of Figure 4.16) is represented by a change between the extreme processes of moving from point 1 to point 2 or from point 1 to point 3 (Figure 4.17). A change in the sward tiller size-density co-ordinates from point 1 to point 2 at constant productivity index implies that the SDC line remains constant. This change is characteristic of swards maintained at the same environmental conditions and disturbed at levels within their plasticity range (Chapman and Lemaire, 1993). However, when the sward changes from point 1 to point 3, keeping its productivity index constant, the increase in standing biomass is caused by an increase in the productivity of the sward. This is due, in many cases, to changes in the leaf architecture of the individual tillers (Lonsdale and Watkinson, 1983; Sackville Hamilton *et al*, 1995), but also could merely be the response to improvements from supra-optimal environmental conditions (Westoby, 1984).

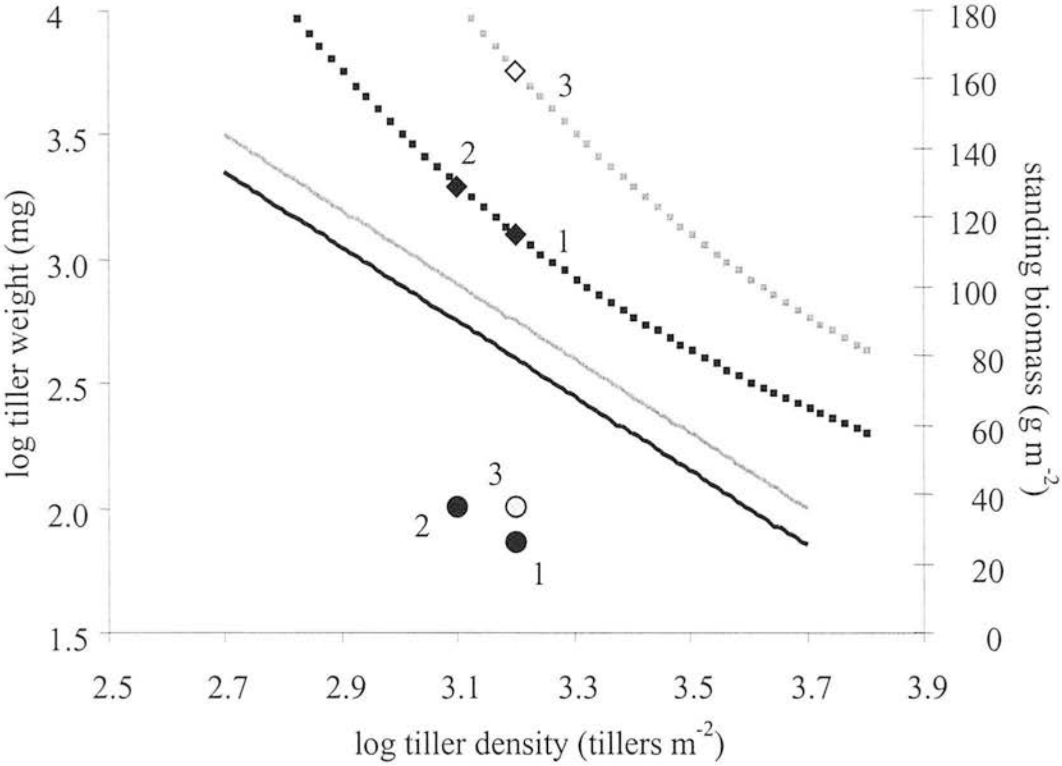

Figure 4.17. Alternative hypotheses explaining a change in the intercept of the regression between signal grass standing biomass and the productivity index (see Figure 4.16). Circles are the tiller weight-density co-ordinates of 3 swards with the same productivity index (the same distance to the -3/2 size-density compensation lines: the full straight lines). Rhomboids are the sward standing biomass (right axis) corresponding to each circle. Dashed lines represent standing biomass for swards with equal productivity index. Grey points and lines correspond to high productivity swards, while black points and lines correspond to low productivity swards.

The most contrasted values of the intercept in the productivity index - standing biomass relationship among periods were observed in November and July. While in November it implied low standing biomass values for the same productivity index, in July the contrary occurred. This behaviour was mostly explained by the significant differences in tiller weights (330 and 554 mg; s.e.=57.3) and densities (1127 and 739 tillers m^{-2}; s.e.=130.1) between November and July respectively. The value close to -3/2 of the ratio between the differences in tiller weight and the differences in tiller densities for these two periods (-

1.22), suggests that the movement of the tiller size-density co-ordinates among periods occurred relative to a similar SDC line, as is the case of the movement from point 1 to point 2 in Figure 4.17.

In the case of the increase of the productivity index - standing biomass intercept (i.e. decrease in sward standing biomass for the same productivity index) at medium and high grazing intensities with respect to low grazing intensity, it was accompanied by a significant reduction in tiller size values (Table 4.2) but not by a significative increase in tiller density. Thus, a slight downward parallel movement of the SDC line might have occurred with increasing grazing intensity, similar to that corresponding to the change from points 3 to 1 in Figure 4.17. Although sward standing biomass was not significantly reduced with stocking density, the subtle changes observed in the productivity of the sward using the relationship between the productivity index and the standing biomass might uncover an initial state of sward degradation due to increases in grazing intensity.

These results may prove the usefulness of using demographic measurements along with standing biomass measurements to produce a sward productivity index. Apart from giving the productivity status of a sward it may also be capable of indicating early stages of pasture degradation not detected when only using biomass or coverage measurements.

4.5 Conclusions

The structural pattern of the successive phytomers in signal grass tillers resembles that of typical tufted grasses, except for the particularity of the presence of internodes from very early stages in the tiller's development.

Although growth regulation among different phytomer components of successive leaves were similar in the field to what observed previously under controlled environments, the timing in the development of largest blade, sheath and internode lengths was markedly different. The contrasted light conditions of both experiments was the factor considered as causing these differences.

While period of the year affected the length of the different phytomer components because of the change in morphogenetic characteristics (e.g. leaf appearance and elongation rates), grazing intensity affected these values mainly due to its direct effect on tiller defoliation and structure modification.

The age and size structure of the tiller population increased in heterogeneity along the growing season. In general grazing intensity did not affect the tiller age structure distribution because of the existing compensation between tiller births and deaths. However, grazing intensity affected negatively the average amount of green leaf per tiller, although it meant the rejuvenation of defoliated tillers towards their high net growth phase with increasing successive blade lengths and low senescence rates.

The plant population structure showed marked changes at the start of the rains due to germination of new plants, and during the flowering season due to plant fragmentation and tiller recruitment inhibition linked to the phenology of reproductive tillers.

The combined use of tiller size-density values and of their product, the sward standing biomass, proved to be a very sensitive indicator of structural changes and productivity at the sward level. Although similar standing biomass values were observed for the different periods and grazing intensities, sward structural changes occurred when considering the position occupied by tiller size-density co-ordinates. In this way, reversible sward changes with season were identified with reference to a unique potential productivity level. On the other hand, increasing grazing intensity produced a slight decrease in sward productivity potential that could indicate an initial process of pasture degradation.

Chapter 5

Patterns of defoliation of signal grass pastures. Effects of grazing intensity and effects on pasture structure [*]

Abstract

The effects of three different stocking rates (1, 2 and 3 cows/ha/yr) on sward structures and the amount and patterns of live leaf defoliation were studied at different levels of organisation of signal grass (*Brachiaria decumbens* Stapf.) pastures. Permanently marked plants and tillers were followed non-destructively during eight consecutive grazing periods for each stocking rate treatment. Leaf and stem lengths were measured before and after the introduction of cattle for all the tillers marked. The vertical distribution of leaves and stems within the sward was quantified. The fate of tillers after grazing was also considered.

Vertical live leaf weight distributions in the canopy followed those of their apical meristem heights. The proportion of tillers defoliated per plant mainly explained the proportion of live leaf defoliated per plant at different stocking rates. The proportion of tillers decapitated after grazing was related to the proportion of tillers defoliated per plant. Apical meristem height was the main physical barrier to leaf defoliation upon a minimum leaf height of 2-4 cm, under which leaves were inaccessible to cattle. Different stocking rates created different tiller heights and canopy structures in the medium term, but did not affect the pattern of defoliation relative to the height of the apical meristem.

---

[*] Based on Busqué, J. and Herrero, M. Sward structure and patterns of defoliation of signal grass pastures under different cattle grazing intensities. *Tropical Grasslands.* (in press)

5.1 Introduction

The growth of signal grass and many other tropical grass species is characterised by the elongation of their stem internodes from early stages of their vegetative ontogeny (Cowan and Lowe 1998). This fact has major consequences in sward structure, grass organs composition and livestock grazing behaviour.

As grazers mainly select for green leaf blade (Stobbs 1975; Hodgson 1985b), tropical sward canopies maintained at herbage allowances meeting animal needs do usually have higher herbage masses than temperate pastures due to the existence of a much higher proportion of stem weight (Stobbs 1973). The existence of stems and of a variety of tillers at different developmental stages (see chapter 4) result in sward canopies with a wide vertical distribution of green leaves. Indirect measurements such as sward surface height fail to predict herbage or green leaf weight accurately in this type of swards (Murtagh 1980; Cowan *et al.,* 1986), and have little use in predicting the amount of live leaf defoliated after grazing, opposite to what occurs in temperate swards (Wade *et al.,* 1989).

Defoliation and the structure of a sward have to be studied at different levels of its organisation in order to determine the level at which the pattern of defoliation is more sensible to grazing intensity.

The objective of this study was to determine the patterns of defoliation and structures of signal grass swards subjected to different stocking rates under eight rotational grazing periods comprising both dry and wet seasons.

5.2 Materials and methods

Experimental site

The location, climate and soil type of the experimental site are described in chapter 4. Figure 4.1 shows temperature and precipitation dynamics during the duration of the experiment.

Pastures and grazing management

Signal grass swards established 10 years before were used for the experiment. Pastures had never received any type of inorganic fertilization and were slashed once a year at the end of the summer. Grazing management before the trial consisted of rotational grazing of Criollo dual-purpose cattle at low stocking rates (1 livestock unit/ha/yr). Fourteen plots of 400 m^2 each were set up with electric fences replicated in two different paddocks (Figure 4.2). These were managed under rotational grazing with rotation cycles of one month from October to March and of 2-4 months for the rest of the year. The duration of the grazing period was always of one day per plot. A total of eight rotation cycles were carried out. Three different grazing intensities were used, equivalent to 1, 2 and 3 livestock units/ha/yr (low, medium and high stocking rates, respectively, from now on). These corresponded to instantaneous stocking rates of 2, 4 and 6 Criollo dry cows (approximately 450 Kg of liveweight) per plot and day, respectively. The experiment was designed as a non-balanced factorial of grazing intensity and nitrogen fertilisation, though the latter factor was not considered in this particular study (see chapter 6). The low stocking rate (control) was repeated in two plots per paddock, while the medium and high stocking rates were replicated in six plots per paddock each.

Figure 5.1. Identified tillers of a plant within a permanent quadrat.

Figure 5.2. Normal plant measurement procedure. One person measures tillers lying on a portable bed for minimal pasture disturbance, while the other person annotates measurements. July 1998

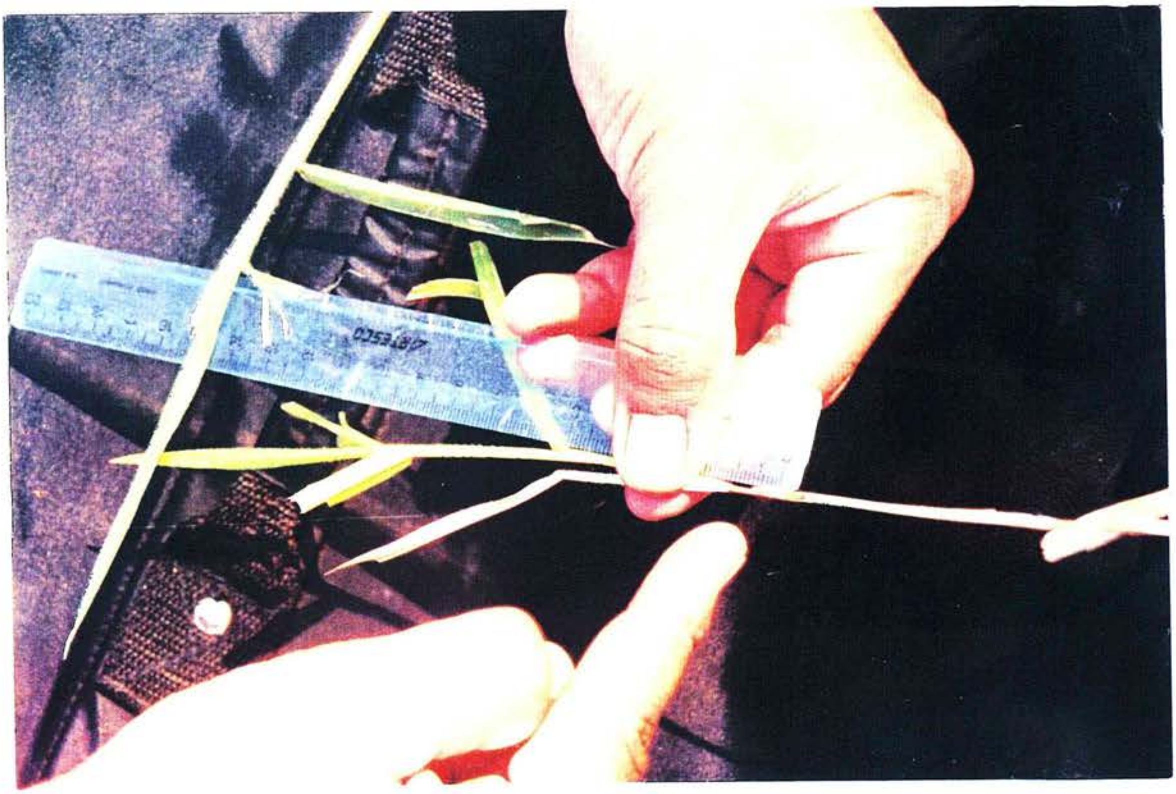

Figure 5.3. Tiller measurement with a ruler.

In each plot a permanent quadrat of 50 x 50 cm was set using a ground-level grid dividing the quadrat in 25 squares of 10 x 10 cm each made of telephone wires. Within the quadrat all the tillers of a selected plant were identified by tagging with numbered wire loops at their base if they were born from basal nodes (basal tillers) or by their parental tiller if they were born from axilar aerial nodes (aerial tillers). These tillers were followed throughout the whole experiment or until they died (Figure 5.1). New tillers were identified as they appeared in the plant and a new plant within the permanent quadrat was marked if all the tillers of a plant were found dead.

Sward measurements and data analysis

Analyses were performed at different scales of the sward: canopy, plant, tiller and phytomer. These were based on data collected on length of all the leaf blades (referred as leaves from now on), leaf sheaths and internodes of the marked tillers in each plot before and after the introduction of cattle (Figures 5.2 and 5.3). The number of days between pre and post-grazing measurements was less than five days for all the plots, though in most cases data were collected one day before and one day after grazing.

Canopy level. Live leaf biomass

At the canopy level the data collection and analysis dealt with the vertical distribution of live leaf biomass weight before and after grazing for the different grazing intensities and seasons. Seasons were defined according to soil water availability and temperatures: wet (December to March) and dry season (October, November and April and July). Live leaf weight per plant (Y, g) was predicted from the values of cumulative plant live extended lamina length (LLLp, m) of observations of a parallel destructive experiment (chapter 4). The regression equation obtained was:

$$Y = -0.0585\ (0.0264) + 0.53\ (0.032)\ \text{LLLp} \quad R^2=0.79 \quad P<0.001 \quad n=78 \qquad \text{(eq. 5.1)}$$

Estimates of leaf weight per plant were transformed to values per unit area considering the number of tillers per plant and the density of tillers in the permanent plots where the plants were located. Estimated pasture live leaf biomass before and after each grazing event is shown in Figure 5.4.

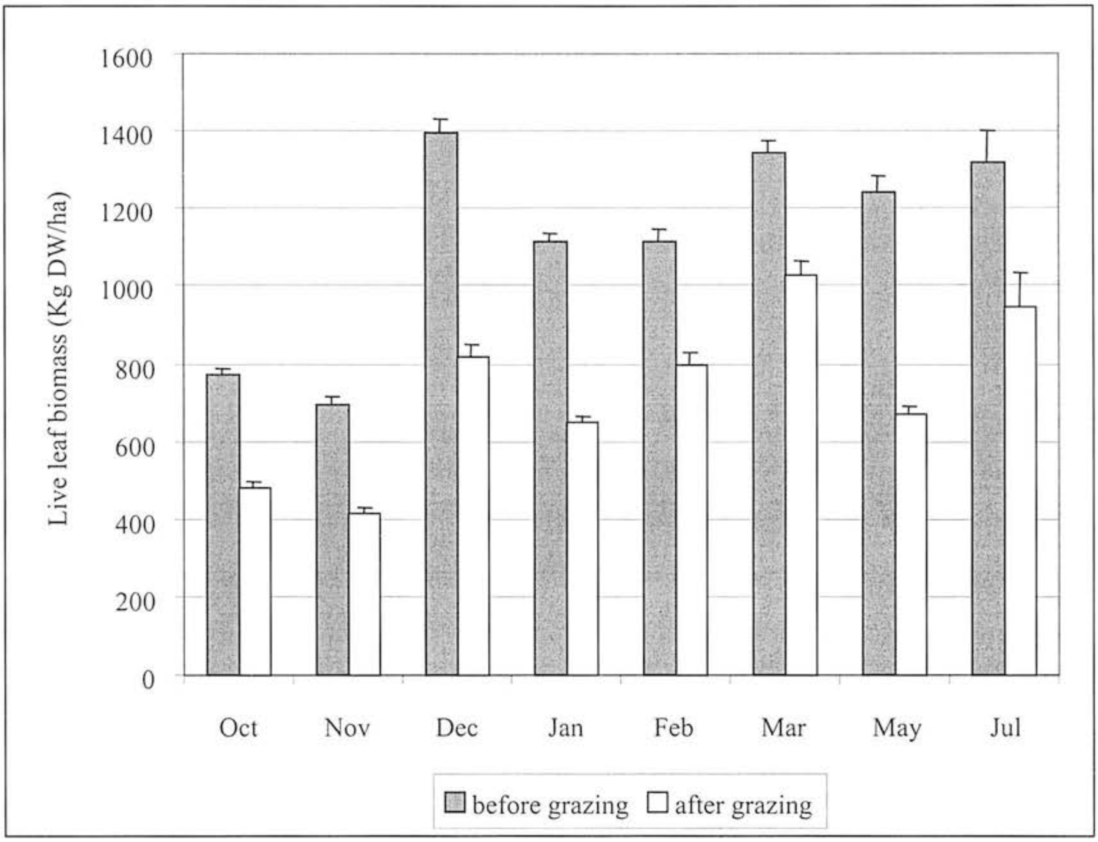

Figure 5.4. Average live leaf lamina biomass per grazing period, before and after grazing. Vertical bars above the columns represent the standard errors of the means.

Vertical distribution of leaf biomass

The vertical distribution of live leaf biomass before and after grazing was obtained averaging the values for each season and stocking rate. Leaf heights were assigned to fixed height strata of 4 cm each. The height of a leaf was estimated adding up the lengths of its tiller internodes and its sheath up to its ligule (Figure 3.1). Leaf height calculated in this way did not correspond to real height, as tillers did usually present a certain angle from verticality and leaf blades also showed a certain angle above horizontality. These sources of variation were assumed fairly constant for all tillers and leaves in each plant.

Once calculated the proportions of leaf in each stratum, they were transformed to leaf biomass per plant using equation 5.1 and leaf biomass weight per area considering tiller densities and number of tillers per plant. A gamma function was fitted by non-linear least squares (Marquardt-Levenberg algorithm) to the vertical distribution data (Illius *et al.*, 1995). These functions explained a high proportion of the variance in canopy structure (R^2=0.87-0.99), and enabled a continuous and accurate description of the vertical accumulation of leaf biomass and density within the sward. The average height of the apical meristems was also recorded. These data were compared before and after grazing for each season and stocking rate.

Plants

At the plant level the total amount of live leaf defoliated, the proportion of tillers defoliated and decapitated per plant and the proportion of leaf defoliated per defoliated tiller were studied. Tillers were classified and analysed separately according to their ontogeny and position of birth (vegetative basal, vegetative aerial and reproductive tillers), since they differed markedly in their leaf arrangement and height (Table 5.1).

Live leaf length (LLL) was calculated as the product of the blade length and its visual proportion of green area. When LLL measurements were not taken immediately before or after grazing, the values obtained were corrected according to the number of days between the measurement and the day of grazing.

Table 5.1. Tiller morphological characteristics and fate after grazing according to their ontogeny and position of birth.

	Basal vegetative	Aerial vegetative	Reproductive
Number of tillers sampled	1047	323	44
Lamina length before grazing (mm)	241.5 (167.2)	180.8 (125.7)	193.9 (126)
Apical meristem height (mm)	61.6 (67.6)	98.3 (72.7)	553 (169.1)
Height of highest extended leaf (mm)	119.1 (91.9)	147.1 (87.4)	461.9 (156.0)
Height of lowest extended leaf (mm)	63.9 (56.8)	108.8 (71.9)	258.1 (99.4)
% tillers defoliated	57.2	60.1	63.6
% tillers decapitated	17.7	13.6	59.1
% tillers disappeared	3.1	11.1	0.0
% tillers alive after grazing	76.9	68.4	39.0

Standard deviations are given between brackets.

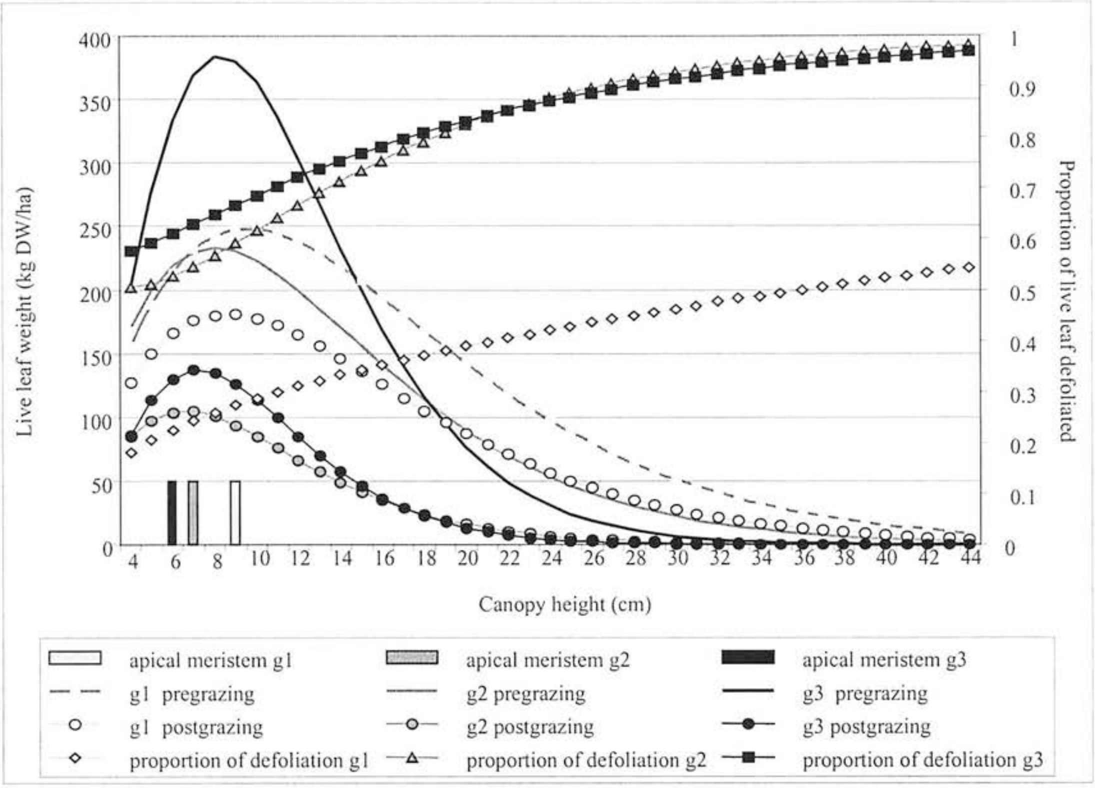

Figure 5.5. Effect of grazing intensity on the average tiller apical meristem height, live leaf weight distribution in the canopy before and after grazing, and proportion of live leaf defoliated. g1, g2 and g3 refer to the low, medium and high grazing intensities respectively.

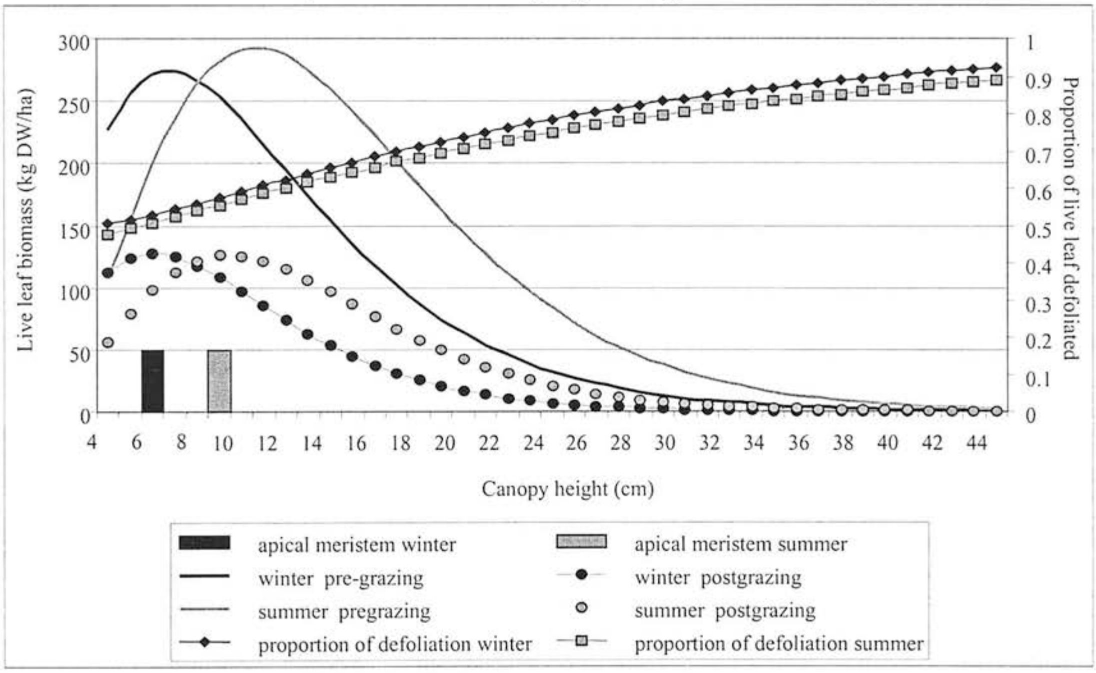

Figure 5.6. Effect of season on the average tiller apical meristem height, live leaf weight distribution in the canopy before and after grazing, and proportion of live leaf defoliated.

Tillers.

To quantify leaf defoliation at the tiller level, a different approach to that used at the plant level was considered. Only totally extended leaves with live proportion greater or equal to 0.5 were chosen for each tiller at the dates before grazing. The completion of elongation in these leaves simplified the analysis of the data, as no correction was needed to account for the small but variable length of time between measurement dates and the grazing day. It was assumed that not considering growing leaves did not produce large errors, as they accounted only for an average of 25% of the total tiller leaf length before grazing (s.e. = 2% of the mean), and their weights per unit length were smaller than for extended live leaves. Therefore their contribution to the total green leaf mass was small.

Regressions and general linear models were the main statistical analyses performed on all variables. All analyses were performed using GENSTAT statistical software (Genstat 5 Committee, 1989).

5.3 Results

Canopy structure

Figures 5.5 and 5.6 represent the vertical distribution of live leaf weight per unit area along the pasture canopy for different grazing intensities and seasons. All the distributions observed before grazing had their maximum amount of live leaf close to the average height of the apical meristems. Leaf distributions after grazing were similar to those before grazing, though their maximum biomass accumulation always moved to lower heights, even nearer to the average apical meristem height.

Figure 5.5 shows that stocking rates and skewness of live biomass distribution were positively related. This was also accompanied by a positive relation between stocking rate and skewness in the distribution of the apical meristem heights (skewness values of 0.76, 1.19 and 1.32 for low, medium and high stocking rates respectively). The proportion of live leaf defoliated per stratum was similar or higher than 0.8 in the top 24 cm of the sward (20-44 cm) for the high and medium stocking rates. From 20 cm towards the bottom of the sward, the proportion of live leaf defoliated decreased more slowly at the high stocking rate than at the medium stocking rate. The proportion of live leaf defoliated at the low stocking rate was much lower than at higher stocking rates. Regarding the season (Figure 5.6), the distribution of live leaf in winter was more skewed towards the bottom layers of the canopy than in summer. This again corresponded with average lower and more skewed apical meristem heights (skewness values of 1.68 vs. 0.93 for winter and summer respectively). The pattern of defoliation was very similar for both seasons, though the proportion of live leaf defoliated was an average of 3 units lower in summer than in winter for all the strata.

Defoliation at the plant level

Proportion of leaf defoliated per plant

Low stocking rates produced a lower proportion of live leaf defoliated per plant than medium and high stocking rates (Table 5.2). Medium and high stocking rates had similar proportions of defoliation. The individual regressions between live leaf biomass before grazing (X_i) and after grazing (Y_i), where i corresponded to the stocking rate, produced the equations:

$$Y_1 = 0.36\ (0.043)\ X_1 \qquad R^2 = 0.37 \qquad P<0.001$$
$$Y_2 = 0.58\ (0.030)\ X_2 \qquad R^2 = 0.62 \qquad P<0.001$$
$$Y_3 = 0.66\ (0.017)\ X_3 \qquad R^2 = 0.89 \qquad P<0.001$$

The slopes of the regressions ratified the proportions of leaf defoliated at each stocking rate (Table 5.2). The coefficients of determination showed that the lower the stocking rate the more variable the process of defoliation was. In any case, neither the periods, their interaction with grazing intensity nor the pre-grazing live leaf weight per plant affected the proportion of live leaf defoliated per plant.

Table 5.2. Effect of stocking rate on percentages of live leaf defoliated per plant (estimated live weight and tillers defoliated), per tiller (estimated live weight), and on percentages of tillers decapitated after grazing and on percentages of aerial and reproductive tillers before grazing.

	Stocking rate ($LU\ ha^{-1}yr^{-1}$)			s.e.d.
	1	2	3	
Plants defoliated (%)	57	84	93	
Live leaf defoliated per plant (%)	35	58	62	4.9
Basal vegetative tillers defoliated (%)	25	52	59	9.3
Aerial vegetative tillers defoliated (%)	27	62	61	13.4
Reproductive tillers defoliated (%)	52	55	75	26.1
Leaf defoliated/basal defoliated tiller (%)	61	64	69	5.2
Leaf defoliated/aerial defoliated tiller (%)	63	72	70	11.0
Leaf defoliated/reprod. defoliated tiller (%)	77	87	90	11.6
Tillers decapitated (%)	9	14	21	4.4
Aerial tillers (%)	13	16	17	5.8
Reproductive tillers (%)	13	6	4	2.4

The proportions of basal and aerial vegetative tillers experimenting defoliation per plant (i.e. with proportions of defoliation per tiller equal or greater than 10% of the total length of their live leaf before grazing) were significantly affected ($P=0.008$ and $P=0.075$, respectively) by grazing intensity (Table 5.2). They followed a similar trend to the proportion of live leaf defoliated per plant as a whole. Stocking rate did not affect the proportions of defoliated reproductive tillers (Table 5.2).

Considering only those tillers classified above as defoliated for each plant and period, their average proportion of live leaf defoliated did not change significantly with grazing intensity, regardless of tiller type (Table 5.2).

Decapitation

Decapitation of apical meristems was the main cause of death for tillers defoliated by cattle: 31% of basal defoliated tillers and 80% of basal defoliated tillers that died after grazing. Although the proportion of decapitated tillers per plant (Y) seemed to be significantly affected by grazing intensity (Table 5.2), it appeared to be a fairly constant fraction of the proportion of the total amount of tillers defoliated (X):

$$Y = 0.31\ (0.02)\ X \quad R^2 = 0.64 \quad P<0.001 \quad n = 24$$

Different grazing intensities did not display different regressions to the one above.

The proportion of decapitated tillers per plant after the previous grazing (X) provided the best explanation for the proportion of aerial tillers per plant before grazing in the next period (Y):

$$Y = 0.05\ (0.026) + 0.71\ (0.151)\ X \quad R^2=0.54 \quad P<0.001 \quad n=21$$

Grazing intensity did not affect the proportion of aerial tillers per plant (Table 5.2) and did not modify the above relation.

Reproductive tillers

Reproductive tillers started to appear in November and had their potential peak proportion per plant in December and January. The proportion of reproductive tillers per plant was mainly explained by the grazing intensity ($P<0.011$), where the low stocking rate doubled the proportion of the two other levels (Table 5.2).

Defoliation at the tiller level

Tiller heights before grazing

The low grazing intensity produced higher (height of the highest extended live leaf of the tiller) basal vegetative tillers before grazing ($P<0.001$; Table 5.3). This effect was not obvious until December, but continued unchanged until March (Figure 5.7). Finally the effect was lost in May and July, when the resting periods were longer. In the case of aerial vegetative tillers, tiller height before grazing was not affected by grazing intensity (Table 5.3).

Defoliated basal vegetative tillers were higher before grazing than not defoliated ones ($P<0.001$; Table 5.4), and that trend did not change with the stocking rate. Tiller heights were lowest (60 mm) for non-defoliated tillers subjected to medium and high stocking rates. Defoliated aerial tillers were lower than undefoliated ones only in the case of high stocking rates ($P<0.01$; Table 5.4).

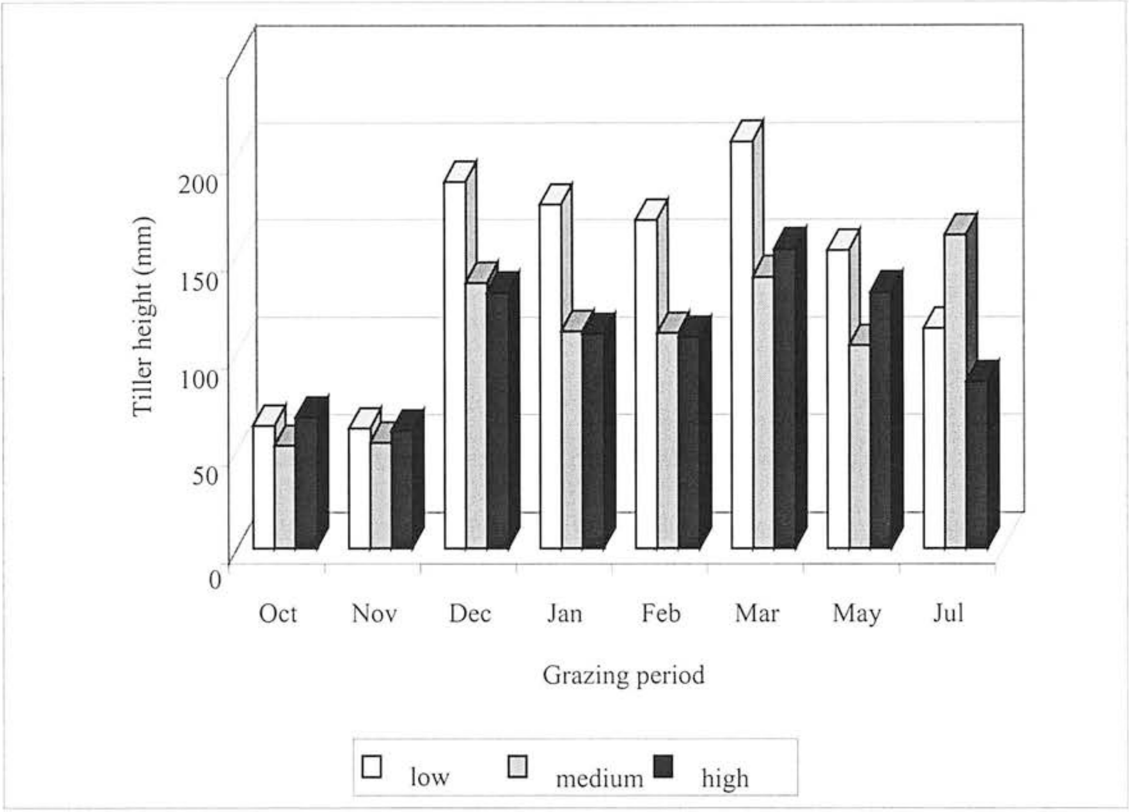

Figure 5.7. Effect of grazing intensity and period of grazing on the average tiller height before grazing. The pooled standard error of the difference of means is 0.9715, corresponding to the square root of the tiller height values. Tiller height was measured as the accumulated length of all the internodes of the tiller plus the sheath length of the highest extended leaf.

Considering together basal and aerial vegetative tillers, apical meristems of decapitated tillers were higher than tillers defoliated but not decapitated, and these showed higher apical meristems than those of non-defoliated tillers ($P<0.001$; Table 5.3). Grazing intensity affected negatively apical meristem heights in a similar manner for not defoliated, decapitated and defoliated tillers. Height of apical meristems and height of tillers as considered in the previous paragraph were highly correlated ($r = 0.94$).

Table 5.3. Effect of stocking rate on tiller heights (top leaves and apical meristems) before grazing.

	Stocking rate (L.U. $ha^{-1}yr^{-1}$)			s.e.d.
	1	2	3	
Top leaf height of basal tillers (mm)	134	104	102	
	(11.57)	(10.20)	(10.10)	(0.35)
Top leaf height of aerial tillers (mm)	124	124	113	
	(11.14)	(11.14)	(10.63)	(0.62)
Apical meristem height of vegetative tillers not defoliated (mm)	81	37	31	11.7
Apical meristem height of defoliated, but not decapitated vegetative tillers (mm)	91	72	54	17.9
Apical meristem height of decapitated vegetative tillers (mm)	175	98	97	27.1

Values between brackets refer to means and s.e.d. from squared root transformed data.

Table 5.4. Characteristics of defoliated and non-defoliated tillers and leaves before grazing.

	Defoliated	Non-defoliated	s.e.d.
Top leaf height of basal tillers (mm)	132 (11.49)	73 (8.54)	(0.24)
Top leaf height of aerial tillers at high SR (mm)	139 (11.79)	89 (9.43)	(0.88)
Leaf height of basal tillers relative to apical meristem position (mm)	47.0	-0.7	2.29
Leaf blade length of basal tillers (mm)	102.6	70.3	2.49
Leaf height of aerial tillers relative to apical meristem position (mm)	19.9	-1.4	8.8

Values between brackets refer to means and s.e.d. from squared root transformed data.

Lower limits of tiller defoliation

The height of the lowest defoliated leaf per defoliated vegetative basal tiller (Y_{dl}, mm) was mainly a function of the apical meristem of the tiller (X, mm):

$$Y_{dl} = 22.30\ (2.66) + 0.82\ (0.026)X \quad P<0.001 \quad R^2=0.76 \quad n=306$$

The height of the lowest non-defoliated leaf per defoliated basal tiller (Y_{ndl}, mm) was also a function of the apical meristem (X, mm):

$$Y_{ndl} = 19.28\ (2.23) + 0.58\ (0.021)X \quad P<0.001 \quad R^2=0.73 \quad n=289$$

Grazing intensity did not affect these relations. Both regressions were different in their intercepts and in their regression coefficients ($P<0.001$). The high coefficients of determination remarked the pattern of distribution of live leaves around apical meristems. The similar intercepts for defoliated and non-defoliated leaves delimited clearly the lower strata that was never defoliated by cattle: the first 2 cm approximately, corresponding to young tillers which had not yet developed their internodes. The regressions indicated that defoliated leaves were mostly above the apical meristem, while non defoliated leaves were above the apical meristems only when they were positioned low in the canopy, up to around 4 cm of height, and later they were located below the apical meristems.

For aerial tillers, considering only those tillers not disappearing after grazing, a similar pattern was observed between height of lower leaves and apical meristems. In this case defoliated (Y_{dl}) and non-defoliated (Y_{ndl}) lower leaves shared the same coefficient of regression, but maintained different intercepts ($P<0.001$):

$$Y_{dl} = 29.1\ (4.31) + 0.86\ (0.03)\ X$$

$$R^2 = 0.84 \qquad P<0.001$$

$$Y_{ndl} = 9.6\ (4.36) + 0.86\ (0.03)\ X$$

Again, grazing intensity did not add any further explanation to these models.

Defoliation at the leaf level

Analyses of leaf defoliation were performed on leaves belonging to defoliated vegetative basal tillers (n=1944). A comparison was made between leaves completely defoliated after grazing (n=876) and those not defoliated at all (n=545). Most of the totally defoliated leaves (61%) belonged to tillers experiencing decapitation or disappearing as a consequence of grazing, while the rest belonged to tillers that remained alive after grazing. In the case of non-defoliated leaves, they belonged mainly to tillers remaining alive after defoliation (81%). Defoliated leaves were significantly larger than undefoliated ones and were positioned higher with respect to the apical meristem of the tiller (Table 5.4). This relative position was not affected by grazing intensity.

5.4 Discussion

The vertical distribution of sward mass and the general structures of the swards before grazing showed the typical skewed distribution pattern found for most tropical and temperate pastures species, with a progressively higher accumulation of biomass from the top towards the bottom of the sward (Chacón and Stobbs, 1976; Ludlow *et al.,* 1982; Milne *et al.*, 1982; Hendricksen and Minson, 1985: Hodgson, 1985a).

Only the upper horizons of the sward were grazed and the effect of increasing stocking rates was mainly mediated through an increase in the number of defoliated tillers, rather than through an increase in the proportion defoliated per tiller. Hence, the proportion of live leaf defoliated per plant was also a function of the number of defoliated tillers. Curll and Wilkins (1982) found increases in both the proportion of defoliated tillers and the proportion of defoliated leaf per tiller in ryegrass swards with increasing sheep stocking rates. For similar stocking rates to those of this study, they found a proportion of defoliated tillers 6 times higher to that of our experiment, while their proportion of defoliated leaf per tiller was an average of 10 points lower. The different structure of short grazed temperate swards, with higher proportions of leaf biomass, higher tiller density, lower variance in tiller size and absence of real stem, can explain their higher frequency of tiller defoliation and sensitivity of the amount of leaf defoliated per tiller to changes in stocking rate.

The post grazing sward vertical distributions were largely dependent on the height of the apical meristem, which acted as a barrier to grazing. This is in agreement with Barthram and Grant (1984), L'Huillier *et al.* (1984), Arias *et al.* (1990), Dougherty *et al.* (1992), and Flores *et al.* (1993), who found that the height of the apical meristems or pseudostems were a deterrent to grazing. In this study, animals also defoliated at low sward horizons, but this was mainly a result of the sward heterogeneity and patchiness commonly associated with tropical pastures (Stobbs 1975). This suggests that sward horizons *per se* were not being grazed uniformly, and the barrier imposed by the apical meristem height demonstrated that the constraint to grazing was imposed at the tiller level rather than at the canopy or plant level. This provides evidence of why sward height is not a good predictor of grazing processes in tropical pastures (Stobbs 1975; Humphreys 1991). Although sward bulk density also increases from the top to the bottom of the sward, and this has been found to be an important regulator of grazing in homogeneous experimental tropical swards (Black and Kenney 1984), this effect was not found in the present study due to the patchiness of the experimental pastures.

The slopes of the regressions investigating the effect of stocking rate on the proportion of live leaf mass defoliated per plant showed good agreement with the coefficients found in table 2. The low coefficient of regression of equation Y_1 (low stocking rate) demonstrates that defoliation at low stocking rates was variable. Under these circumstances, diet selection would have played an important role, since animals had a higher tiller availability for selecting those leaves of a higher nutritive value/lower tensile strength (Hendricksen and Minson 1980). As stocking rate increased, and consequently tiller allowance decreased, the process became more uniform. This was confirmed by the higher predictive ability of the regressions with higher stocking rates. Similar results were obtained by t'Mannetje and Ebersohn (1980). Although animals at the high stocking rates increased tiller defoliation per plant, they were limited by the height of the apical meristems of the tillers. They preferred not to graze stem possibly due to its higher physical strength in comparison with the leaves (Hendricksen and Minson 1980; Hodgson *et al.*, 1997) and also probably as a mechanism of weighting the energy costs and benefits obtained by grazing it (Illius *et al.*, 1995).

The proportion of leaf defoliated per basal or aerial tiller was relatively constant (around 60-70% of the leaf mass) irrespective of stocking rate. This result ratifies that leaf defoliation in a tiller was a function of the apical meristem height, since the remaining leaf mass was below the apical meristems (see table 5.4). Leafiness seems to have played an important role in defoliation, since the analyses demonstrated that defoliated leaves were usually larger than non-defoliated ones. These results are in agreement with the early observations of Stobbs (1975) on the importance of achieving high leaf masses in tropical pastures for increasing animal productivity.

The decrease in the proportion of reproductive tillers with increasing stocking rates was associated with higher tiller defoliation proportions, and especially with higher incidence of decapitated tillers. Thus, it is suggested that under high stocking rates the lifespan of the tillers was not long enough to reach the flowering stage.

5.5 Conclusions

The proportion of live leaf defoliated at different stocking rates was mainly explained by an increased proportion of tillers defoliated per plant as stocking rate increased, and not by an increased defoliation of the tillers *per se*.

The apical meristem acted as a physical barrier to grazing at the tiller level. As a consequence, the proportion of leaf defoliated per basal or aerial tiller was relatively constant irrespective of stocking rate.

Different stocking rates created different canopy structures but did not affect the pattern of tiller defoliation relative to the height of the apical meristem.

In heterogeneous tropical pastures describing grazing processes by horizons is useful if horizons are defined at the plant and tiller level but not at the canopy level.

Chapter 6 Morphogenesis of signal grass pastures. Seasonal variation and effects of nitrogen and grazing intensity

Abstract

Morphogenesis, structure, longevity and ontogenesis of signal grass (*Brachiaria decumbens* Stapf.) tillers were studied in established pastures during one year (1998/99) and under different grazing intensity and fertilization regimes. Data collected consisted of tiller and growing point densities in permanent quadrats, appearance and death of tillers from selected plants, and state and length of phytomers of the selected tillers.

At the community level, soil organic matter affected positively signal grass and legumes densities and negatively density of weed species.

At the population level, signal grass tiller density was lowest at the end of winter and highest in mid-summer. The highest tiller densities were achieved through increases in the proportion of aerial tillers. Neither grazing intensity nor nitrogen fertilization affected the size of signal grass tiller population.

At the tiller level, tiller birth and death rates were equally affected by defoliation. Tiller birth rate was higher than tiller death rate at the end of winter due to sporadic rains and open sward canopies. Nitrogen affected positively tiller birth rate only during that period. Tiller death rate was higher than tiller birth rate in the second half of the summer, and especially at the end of winter, when water availability had been low for a prolonged time. Aerial tillers lived less than basal tillers, and their deaths were less related to grazing. Parental tillers of aerial tillers were mostly recently decapitated at high grazing intensities, and flowering tillers at low grazing intensities.

A minimum of 8-10 leaves were needed for tillers to reach flowering, though some environmental signal was also needed to activate floral initiation at the tiller apex.

Leaf elongation and appearance rates were more sensible to environmental conditions, while leaf senescence and internode elongation rates were more dependent on tiller structural characteristics.

Tiller turnover rates were as important as leaf turnover rates in defining the efficiency of utilisation of the pastures.

6.1 Introduction

Leaf morphogenetic variables (leaf appearance, elongation and senescence rate, and internode elongation rate) define structural characteristics of the tiller and its population in a sward (Chapman and Lemaire, 1993). These structural characteristics (yield per green leaf, number of green leaves, number of nodes and stem length per tiller, and tiller density) conform, in terms, the sward leaf yield and leaf distribution in the canopy. Though leaf morphogenetic characteristics are mainly affected by environmental variables such as temperature, nitrogen and water availability (Wilson and t'Mannetje, 1978; Vine, 1983; Jones, 1988; Duru and Ducrocq, 2000a,b), tiller ontogeny and structural characteristics also affect them significantly (Wilson 1976; Parsons & Robson, 1980; Duru and Ducrocq, 2000a).

Monospecific grass swards are usually formed by a population of tillers of different ages and sizes (Harper, 1977). This cohort structure is quite stable in temperate swards under normal management regimes and environmental conditions (Brock et al, 1996), but can change under higher degrees of external disturbance (Brock & Hay, 1993) or during phases of phenological change (Matthew et al. 1989). Changes in sward cohort structure can lead to pasture degradation through the opportunity offered to undesirable species to appear and compete with the original grass species for nutrients and light (Torsell and Nicholls, 1978).

In tropical regions with a marked dry season, changes in pasture structure can be acute at certain periods of the year (Humphreys, 1991), though little information exists on grass species. Tiller regeneration at the beginning of the wet season through germination and vegetatively, also during flowering, are important processes in the persistence of the sward (see chapter 4), and they balance tiller deaths due to grazing, pests, flowering and competition for resources. The achievement of an adequate sward structure at the beginning of the dry season is important in order to prepare the pasture for the oncoming extreme climatic conditions. The reduction of the potentially high temperatures at the soil surface through a well covered canopy is especially important to limit tiller and bud deaths in the dry season to values that allow the sward to recover in the following wet period (Brock & Hay, 1993).

The present work followed non-destructively the dynamics of signal grass plants during three consecutive seasons (dry-wet-dry), covering one year. Each season was characterised by different climatic conditions with contrasted temperatures and soil water availabilities. The objectives of the study were: (i) to analyse the effects of season, grazing intensity, nitrogen fertilisation and tiller and sward structure on leaf morphogenetic characteristics at the tiller level; (ii) to analyse the dynamics of tiller appearance and death rates under the different environments and management regimes of the experiment, and their relationships with leaf morphogenetic variables; (iii) to define the life history of tillers (lifespan, phenology, causes of death) according to their origin, period of birth and sward management; and (iv) to characterise the sward structure (tiller density, species composition, aerial biomass) through the aggregation of structural and morphogenetic characteristics at lower levels of organisation, and the sensitivity of these relations to changes in environmental and management conditions.

6.2 Materials and methods

Experimental site and type of pastures

The location, climate and soil type of the experimental site were described in chapter 4. Temperature, precipitation and radiation averages and standard deviations according to the periods established in the experiment are presented in Table 6.1. The type of pasture, its management before the experiment and the rotational cycles performed during the experiment are partially described in chapter 5.

Design of the experiment

Three different designs were used depending on the time of the year. From July 1998 until October 1998 (Winter98) the pasture was not grazed, as grass growth was minimal from July to the beginning of September. During that period four plots per paddock were fertilized with urea at equivalent doses of 25 kg N ha^{-1} divided in two applications in August separated by 11 days (N25). The rest of the plots, 10 per paddock, remained unfertilized (N0).

From October 1998 to April 1999 (Summer) the experimental design was an incomplete factorial with three grazing intensities (1, 2 and 3 cows ha^{-1} yr^{-1}) and three urea fertilization regimes, 0, 50 and 100 kg N ha^{-1} (N0, N50 and N100), distributed in 5 occasions after each grazing event (10 and 20 kg N ha^{-1} in each application for the N50 and N100 levels respectively). The design was incomplete in the sense that plots under the low grazing intensity did not receive any fertilization, while those under medium and high grazing intensities could be under one of the three fertilization regimes. Each combination of treatments was replicated in two plots in each of the two paddocks used in the experiment.

From May 1999 to July 1999 (Winter99) pastures were not fertilized and only those plots that previously were under no nitrogen fertilization, six per paddock, were measured. The three grazing intensity levels were maintained, replicated twice in each of the two paddocks.

The marking and monitoring of tillers within permanent quadrats was explained in chapter 5.

Measurements in the permanent quadrats

Two different types of measurements were performed in the permanent quadrats of the sward: tiller and growing point densities and measurements of marked tillers of selected plants.

The first type of measurements involved the counting of signal grass tiller and other species growing point numbers in the permanent quadrats of all the plots before each grazing event, and also before the experiment started, in May 1998. Counting was done in each of the 10 x 10 cm. cells in which the permanent quadrats were divided. Signal grass tillers were distinguished as basal, aerial or seedlings, except in May 1998. Occurrence of dung, mole mounds, ants or spittlebugs were annotated for each 10 x 10 cm cell when detected. A total of 233 density observations were performed.

The second type of measurements involved the identification, measurement and tracking of all visible phytomers of the marked tillers (see chapter 5) throughout their whole life. Phytomers were identified by a number representing their level of insertion in the tiller. Measurements involved length of the phytomer components (blade, sheath and internode; see Figure 5.3), stage of blade development (extending folded, extending unfolding and completely extended), state of blade (whole, cut from the tip, bitten or disappeared), visual proportion of live blade, stage of sheath development (totally green, senescing, dead attached to stem and dead disattached), and state of sheath (whole, cut or disappeared). Number of spikelets and number of flowers per spikelet were also annotated when observed. Each time a new tiller was detected as coming from the selected plant, it was identified by a new number and by the number of its nearest tiller in the plant if it was basal (Figure 5.1). If the new tiller was aerial, it was identified by the number of the parental tiller and by the phytomer number from where it was born. Tiller measuring frequencies varied depending on the season (Table 6.1), mainly because of the number of tillers existing per plant in each season. In Winter98 an average of 15 measurements were taken per plant, with 6 days between measurements. In Summer only two measurements were usually taken between grazing events, separated an average of 27 days (40 days in April). In Winter99 (May-July) the number of measurements increased to 9 with a mean of 7.5 days between measurements.

A total of 1152 tillers (9027 phytomers) belonging to 44 apparent plants were monitored and measured. At the end of the experiment sward cores of 20 x 25 x 15 cm. centred on the selected plants within the permanent quadrats were extracted and analysed as in chapter 4. In most cases what was considered to be tillers physiologically integrated in the same plant corresponded to more than one plant.

Soil from each sward core was collected and physical and chemical characteristics were analysed in laboratory. Soil variables used in some of the analyses as covariates were organic mater (%), phosphorus (Olsen's method; ppm); potassium (exchangeable; cmol kg^{-1}) and acidity (exchangeable; cmol kg^{-1}). These variables were chosen for their importance in defining physical and chemical characteristics of the soils and because of their mutual low correlations and high correlations with other soil variables.

Daily climatic variables used were minimum, maximum and average temperatures (^{o}C), radiation (MJ m^{-2}), precipitation (mm) and potential evaporation (mm). Actual daily evaporation was calculated using the Bucket model for soil water budget (Rowell, 1994, p. 252). The ratio between daily actual and potential evaporation (Eap) was used as indicator of water availability for plants.

Table 6.1. Dates and average climatic characteristics of the growth periods for the whole year analyses.

Rotational cycle	Season	Periods	Growth days	1st date (Stddev: days)	2nd date (Stddev: days)	Daily temperature (°C)	Daily real/potential evaporation (Eap)	Daily radiation (MJ m^{-2})	Total rainfall (mm)
1	Win98	July	26.0	16-Jul (4.3)	11-Aug (0.5)	23.1	0.11	9.9	16.3
1		Aug	34.0	11-Aug (0.5)	14-Sep (0.5)	23.0	0.23	11.1	40.7
1		Sept	21.0	14-Sep (0.5)	05-Oct (0.5)	22.2	0.39	12.0	87.1
2	Summer*	Oct	25.0	16-Oct (5.7)	10-Nov (5.7)	25.6	0.22	13.6	90.8
3		Nov	33.0	11-Nov (5.7)	14-Dec (4.7)	26.8	0.96	14.7	229.3
4		Dec	34.0	15-Dec (4.7)	18-Jan (5.1)	25.8	0.93	14.9	162.2
5		Jan	28.0	19-Jan (5.1)	16-Feb (6.3)	25.3	0.76	15.4	47.0
6		Feb	30.0	17-Feb (6.3)	19-Mar (5.8)	24.8	0.95	12.8	137.1
7		Apr	43.0	20-Mar (5.8)	2-May (6.8)	22.9	0.51	12.8	56.3
8	Win99	May	25.0	03-May (5.7)	28-May (0.0)	20.9	0.52	10.4	81.2
8		June	34.0	28-May (0.0)	01-Jul (0.5)	18.8	0.71	9.9	52.0

* Summer measurement intervals were common for the whole year analyses and the summer season analyses.

Table 6.2. Dates and average climatic characteristics of the measuring intervals in the winter 98 and winter 99 periods.

Rotational cycle	Season	Measuring interval	Growth days	1st date (Stddev: days)	2nd date (Stddev: days)	Daily temperature (°C)	Daily real/potential evaporation (Eap)	Daily radiation (MJ m^{-2})	Total rainfall (mm)
1	Win 98	2	8.0	3-Aug (0.5)	11-Aug (0.5)	23.2	0.17	9.8	14.9
		3	3.9	11-Aug (0.5)	15-Aug (1.5)	22.3	0.19	11.6	0.0
		4	4.1	15-Aug (1.5)	19-Aug (0.5)	25.1	0.17	11.5	0.3
		5	6.2	19-Aug (0.5)	25-Aug (1.7)	22.6	0.13	11.2	2.5
		6	5.9	25-Aug (1.7)	31-Aug (0.5)	21.2	0.12	10.7	3.0
		7	3.0	31-Aug (0.5)	3-Sep (0.5)	25.1	0.03	11.3	0.0
		8	5.6	3-Sep (0.5)	9-Sep (0.5)	22.0	0.34	9.0	33.6
		9	5.3	9-Sep (0.6)	14-Sep (0.5)	25.2	0.59	13.4	1.3
		10	3.7	14-Sep (0.5)	18-Sep (1.6)	23.7	0.19	11.7	0.0
		11	6.3	18-Sep (1.6)	24-Sep (2.0)	19.4	0.14	11.7	4.5
		12	10.9	24-Sep (2.0)	5-Oct (0.8)	23.3	0.65	13.1	82.6
		13	5.3	5-Oct (0.9)	10-Oct (1.6)	24.8	0.91	13.9	4.7
8	Win 99	1	9.0	19-May (0.5)	28-May (0.0)	17.9	0.29	9.1	42.4
		2	12.0	28-May (0.0)	9-Jun (0.5)	17.4	0.86	9.8	1.9
		3	7.0	9-Jun (0.5)	16-Jun (0.5)	18.0	0.27	9.3	0.0
		4	5.0	16-Jun (0.5)	21-Jun (0.5)	18.3	0.40	9.8	46.8
		5	10.0	21-Jun (0.5)	1-Jul (0.5)	21.5	0.91	10.3	3.3
		6	6.0	1-Jul (0.5)	7-Jul (1.2)				

Data analysis

The variables analysed referred to sward morphogenetic and structural characteristics at the community, population and tiller levels, and to tiller life history characteristics.

At the population and community levels the structural characteristics analysed were signal grass tiller and other species growing point densities. Species other than signal grass were grouped as legumes, other graminoids and other broad-leaved species. Four species of legumes accounted for 99% of the total number of legumes (7 legumes in total). Two species of grasses and two cyperacea formed the group of graminoids, with *Paspalum notatum* comprising 80% of the total number of observations in the group. The 'other broad-leaved species' group was made of a total of 18 species, with 7 species comprising 95% of the total number of observations in the group (two species of the *Malvaceae* family accounted for 75% of the total number). Most of other broad-leaved species were common crop weeds in the area.

Morphogenetic characteristics studied comprised leaf elongation (LER), senescence (LSR) and appearance rates (LAR), internode elongation rate (IER) and tiller appearance (TBR) and death rates (TDR). Leaf elongation and senescence were studied at the tiller level, considering the changes in absolute and live leaf length per tiller between consecutive measurement dates without any grazing event between them. The structural characteristics analysed at the tiller level were the total number of leaves (TNLt), live leaf (LLLt) and internode length (INTt). Plant structural characteristics were not considered strictly as defining the plant due to the uncertainty of considering the tillers interconnected during the whole duration of the experiment. However variables as live leaf length per plant (LLLp) were used to study their relationships with morphogenetic characteristics of the tiller.

Tiller life history characteristics analysed were tiller longevity and causes of death, separately for aerial and basal tillers. The pattern of appearance and location of aerial tillers with respect to the parent tillers was detailed. Flowering tillers were analysed separately for their structural, life history and inflorescence characteristics.

Morphogenetic and structural variables were tested for differences with period, grazing intensity and nitrogen fertilisation treatments. Analyses of variance were used considering a split-plot design with period as the split factor. For tiller variables, pooled values were taken for each plot and measurement interval in order to obtain balanced designs. The use of repeated measurements of the same individuals was considered in the analyses of variance calculating the Box's epsilon statistic and using it to increase the critical values for *F* (Bullock *et al.*, 1994b).

In addition to period, grazing and nitrogen effects, covariates referring to soil, defoliation intensity or tiller, plant or population structures were also used for some of the analyses of variance if they affected significantly their outcome.

Chi-square tests were used when comparing counts of individuals of different groups under different treatments, and when observations could not be transformed into normal distributions.

Due to the seasonal differences in the design of the experiment, analyses were performed at two levels: for the whole year and for each differentiated period separately (winter 98, summer and winter 99).

Whole year analyses

Whole year analyses were performed only for those plots that were not fertilised for the whole duration of the experiment (6 plots per paddock). These plots were subjected to the 3 levels of grazing intensity (2 plots per paddock with the same grazing intensity level), except during the first three months of the experiment (before October of 1998). The year was divided in 11 growth periods: 3 before the first grazing event, 6 between grazing events, and two between the last two grazing events (Table 6.1).

Defoliation was considered using the covariate proportion of live leaf defoliated per plant at the previous grazing event (PRDEF).

Winter 98 analyses

Tiller and growing point densities were analysed comparing the differences between May and October values, and testing for the effect of nitrogen fertilisation.

Twelve consecutive measurement intervals were considered in the analysis of morphogenetic and structural characteristics (Table 6.2). Initially, morphogenetic and structural characteristics were studied pooling for all the tillers in each plant for the whole period. Considering all the measurement intervals in an ANOVA could not be done due to the large amount of null values obtained in the response variables. Analyses were performed for individual measurement intervals (intervals 2, 8, 9 and 12; Table 6.2) with normal distribution of the observations.

Summer analyses

Measurement intervals coincided with resting periods between grazing events (Table 6.1). Either grazing intensity as an effect or PRDEF as a covariate were used to account for defoliation. When choosing grazing intensity, the low level was not used in the analyses in order to obtain a balanced structure of the data regarding the combination grazing intensity x nitrogen fertilisation levels.

Winter 99 analyses

Tiller morphogenetic and structural variables were analysed first pooled for the whole period, and later considering the six measurement intervals (Table 6.2).

6.3 Results

Whole year

Characteristics of the growth periods

The first four periods (July to October) showed very low Eap values, indicating low water availability (Table 6.1). Periods of Winter 99 experienced higher Eap but lower temperatures than those of Winter 98. Average daily temperatures, radiation and Eap were always much higher in the summer periods than in the rest of the year.

Tiller and growing point densities

Signal grass tiller density laid between values of 900 and 1100, except in October (Figure 6.1). The effect of period was significant ($p<0.05$), though if the first period was omitted the significance was lost. PRDEF did not affect signal grass tiller density. The density of broad-leaved species was negatively related ($p<0.05$) to the density of signal grass tillers with a coefficient of -0.93 (±0.32 s.e.).

Proportions of aerial and basal tillers were calculated considering signal grass tiller density in the same period as covariate, and using grazing intensity and periods as effects. The proportion of basal tillers was not significantly affected by grazing intensity or by period, while the proportion of aerial tillers was affected by period ($p<0.05$) but not by grazing intensity. The proportion of aerial tillers was lowest after the longest resting periods: measurements of October and July (Table 6.1a). For both proportions, signal grass tiller density in the same period was significant at the plot ($p<0.05$) and at the plot x period levels ($p<0.001$). In the case of proportion of basal tillers the relation with tiller density was negative (coefficient of -1.2×10^{-4}), while for aerial tillers it was positive (coefficient of 1.8×10^{-4}).

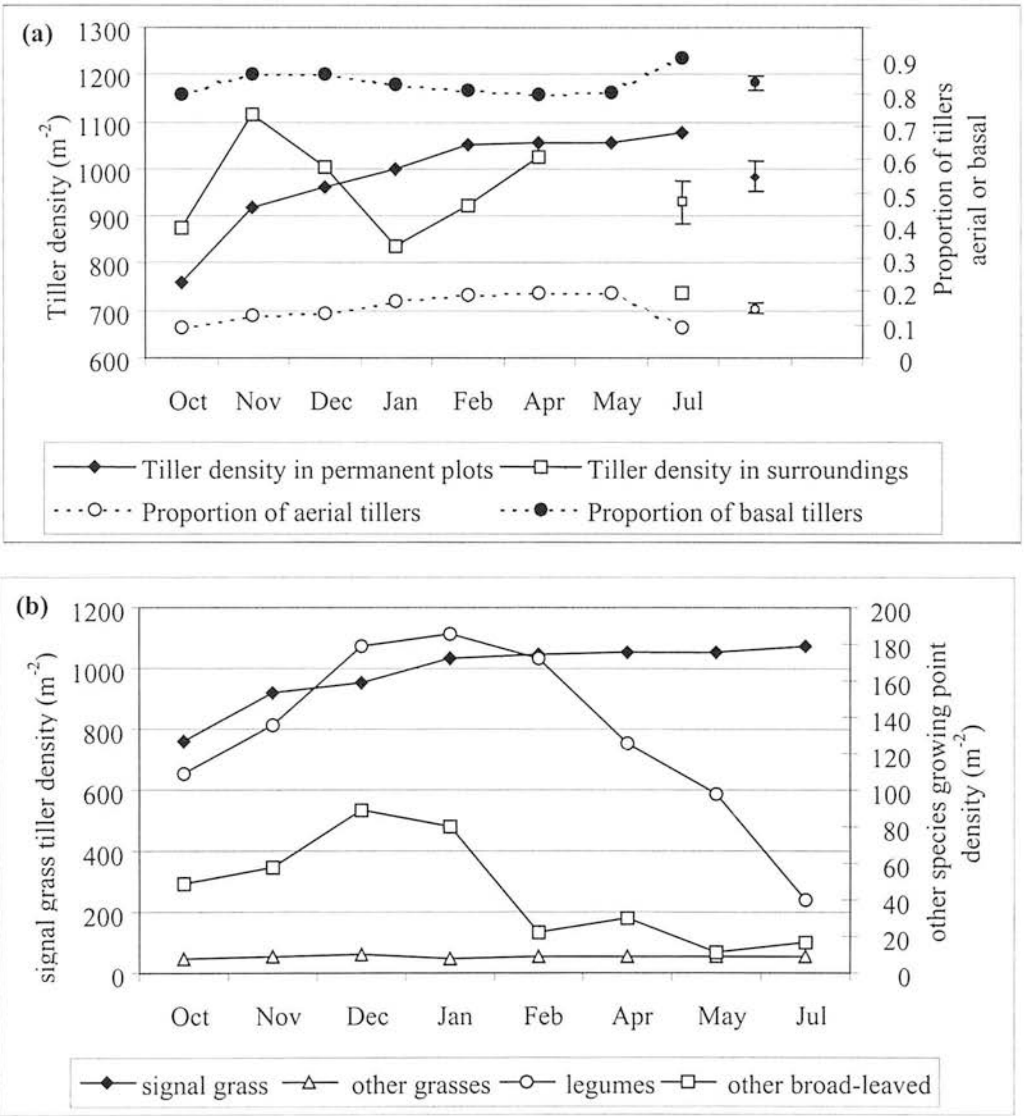

Figure 6.1. Seasonal variation in (a) signal grass tiller density and proportion of aerial and basal tillers, and (b) densities of the different groups of species in non-fertilized swards during 1998-99. Vertical bars in (a) correspond to s.e.d.

Signal grass seedlings were mainly present in October (average of 10.7% of the total tiller density in October, 1.3% in November, and less than 1% for the rest of the periods). For October, its proportion was negatively correlated with signal grass tiller density (r=-0.76) and with proportion of basal tillers (r=-0.95), and positively correlated with the density of non-legume broad-leaved species (r=0.76). The importance of seedling regeneration was much lower than regeneration through tillering in the previous two months, estimated to contribute an average of 60.4% of the total number of signal grass tillers in October.

Organic matter percentage was the soil variable better correlated to density variables, with correlation coefficients of 0.66 with signal grass tiller density, -0.40 with proportion of basal tillers, 0.50 with proportion of aerial tillers, and -0.43 with density of other broad-leaved species.

Signal grass maintained average tiller densities always over 70% of the total number of growing points in the sward. Values were lowest during December and January (74-77%) and highest from April to July of 1999 (87-91%). Legumes contributed on average over 10% of the total number of growing points from October to April, declining their proportion in the last two measurements. Other broad-leaved species were on average around 5% of the total number of growing points from October to January, declining afterwards to 1-2%. Other graminoids showed an average constant 4-5% proportion of the total growing point density (Figure 6.1b).

Chi-square tests for the average density of the four groups of growing points in each period showed that from October to January their relative proportions were not significantly different. These periods coincided with the highest density values for legume and other broad-leaved species (Figure 6.1b). Comparing signal grass and legume densities, relative proportions did not differ among periods from October to February. The later decrease in legume density (from April to July) made these periods significantly different from the first five. A similar result was found comparing signal grass and other broad-leaved species densities, though in this case relative proportions did not change from October to January. After January the density of other broad-leaved species decreased to very low average values. Relative proportions between signal grass and other graminoids did not change significantly with period.

The effect of defoliation on the species sward composition was studied comparing the proportional changes in growing points for each group of species between October, before the grazing treatment started, and April, after five consecutive rotational cycles for each of the three grazing levels (Table 6.3). Signal grass and legume densities changed similarly with grazing intensity, with higher increases under medium and high stocking rates. Other graminoids and broad-leaved species decreased their growing point densities, except under high stocking rates.

Table 6.3. Proportional change in growing point densities in the four groups of species between October and March for different grazing intensities.

Grazing intensity	Signal grass	Legumes	Graminoids	Other broad-leaved
Low	126	96 a	77 a	22 a
Medium	155	125 a	40 b	60 b
High	136	129 a	173 c	128 c

Reference density values in October were set to 100. Different letters within a column indicate significant differences ($p<0.05$; χ^2 test) in the change of that group compared to the change in signal grass density (second column).

Structural and morphogenetic characteristics at the tiller level

All the structural variables at the tiller level were significantly affected by the period (Table 6.4) and showed similar seasonal dynamics: lower values in winter and higher in summer (Figure 2a).

All the morphogenetic variables were also significantly affected by period (Table 6.5), but differently by initial structural variables. Leaf elongation rate was not affected by any structural characteristic of the tiller. Leaf senescence rate was highly affected by initial LLLt, irrespective of tiller age (i.e. TNLt). The initial TNLt affected negatively leaf appearance rate. Internode elongation rate was best explained by the initial LLLt.

Morphogenetic characteristics showed similar seasonal dynamics to the structural variables (Figure 2b), with higher values during summer and lower in winter. Internode elongation, leaf appearance and elongation rates were highly correlated among them ($r>0.60$).

Table 6.4. Results (*F*s) of split-plot ANOVA on average tiller structural variables for the end of each period.

Stratum	Factor (d.f.)	Live leaf length	Number of leaves	Stem length
-*Paddock* x *plot*	Covariate (1.9)	16.9** (1.19±0.29)	126.25***(0.976±0.087)	22.83***(1.24±0.26)
-*Paddock* x *plot* x *period*	Epsilon	0.2744	0.2661	0.279
	Period (10,90)	9.12***	18.10***	12.05***
	Covariate (1,9)	56.24*** (0.727±0.097)	425.51***(0.819±0.040)	110.73***(0.993±0.094)

* P<0.05; ** P<0.01; *** P<0.001

Covariates correspond to the values of the same variables at the start of that period. The coefficients of each covariate and their standard errors are given between brackets after the *F* values. Epsilon is the statistic considering repeated measurements and modifying the critical *F* value in the Paddock x Plot x Period stratum.

Table 6.5. Results (*F*s) of split-plot ANOVA on average tiller morphogenetic variables for the end of each period.

		Leaf elongation rate	Leaf senescence rate	Leaf appearance rate	Internode elongation rate
Covariate		---	live leaf length	number of leaves	live leaf length
PaddockxPlot	Covariate (1,9)	---	3.89*** (0.017±0.003)	0.00^{NS}	13.34** (0.0035±0.0010)
PaddockxPlotxPeriod	Epsilon	0.2608	0.2108	0.3232	0.3077
	Period(10,90)	11.77***	5.41*	8.31***	22.13***
	Covariate (1,9)	---	152.44*** (0.015±0.001)	24.26*** (-0.0077±0.0016)	253.4*** (0.0019±0.0004)

* P<0.05; ** P<0.01; *** P<0.001

The covariate for each response variable corresponds to a structural characteristic variable at the start of each period that significantly affected the response variable and produced the greatest reduction of the residual variance. The coefficients of each covariate and their standard errors are given between brackets after the *F* values. Epsilon is the statistic considering repeated measurements and modifying the critical *F* value in the Paddock x Plot x Period stratum.

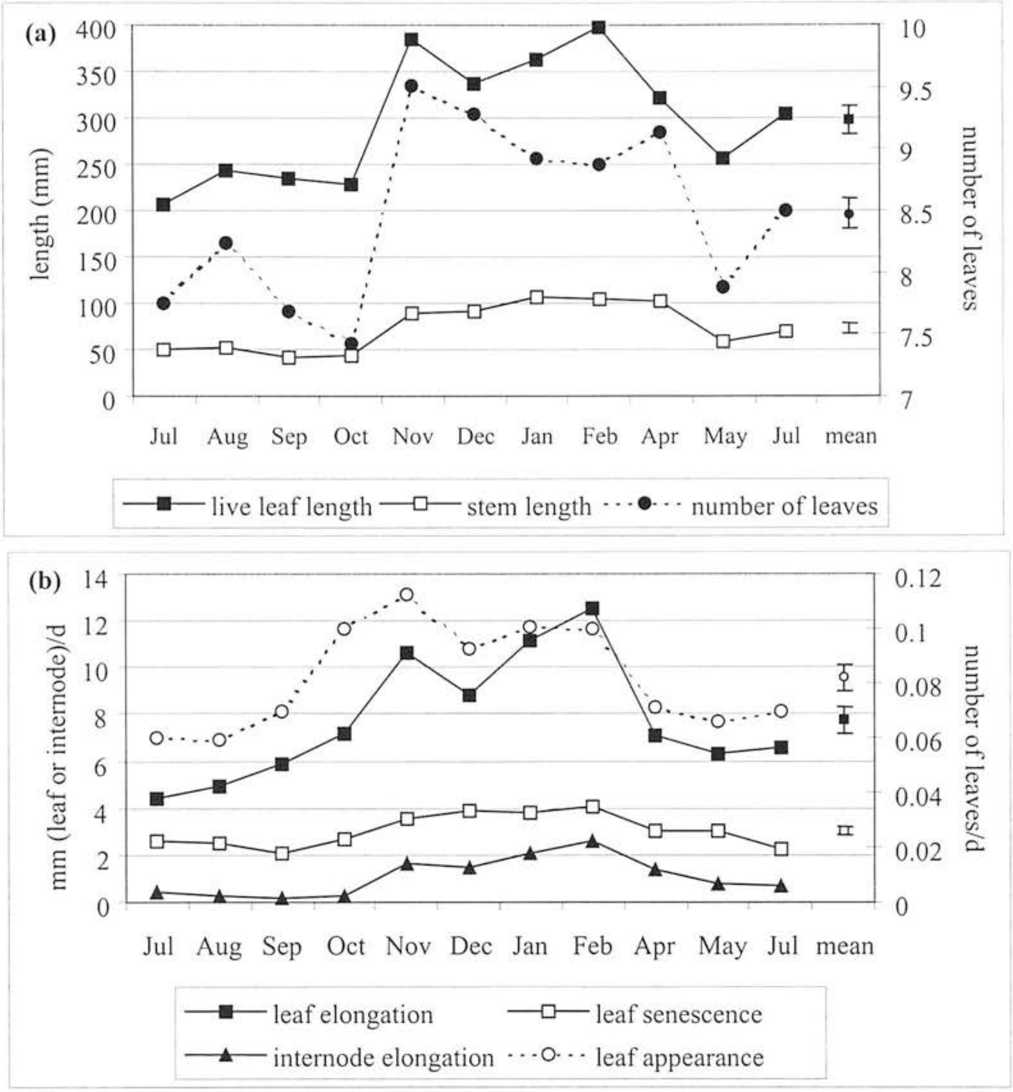

Figure 6.2. Pooled structural (a) and morphogenetic (b) characteristics at the end of each period for non-fertilised plots. Values of the structural characteristics were corrected for their initial values in each period. Values for the morphogenetic characteristics were corrected for the structural variables specified in Table 6.5. Vertical lines indicate s.e.d. centred at the means, without correcting for repeated measurements. Internode elongation in (b) was originally analysed transforming the observations to their square roots.

Tiller birth and death rates

Tiller appearance rate was lowest during winter, showed a peak after the first important rains (October) and maintained high values in the following summer.

Tiller death rates did usually follow a similar trend to that of leaf appearance rate, except during the first winter (Figure 6.3).

An analysis of variance performed for observations from November to April (range of observations with normal distribution) showed that no significant seasonal changes occurred in both tiller appearance and death rates. Tiller appearance rate was related to leaf appearance rate at the pad x plot stratum ($p<0.01$; coefficient: 0.122±0.035). Tiller death rate was only affected by the proportion of live leaf defoliated in the previous grazing event at the pad x plot x period stratum ($p<0.05$; coefficient: 0.010±0.003).

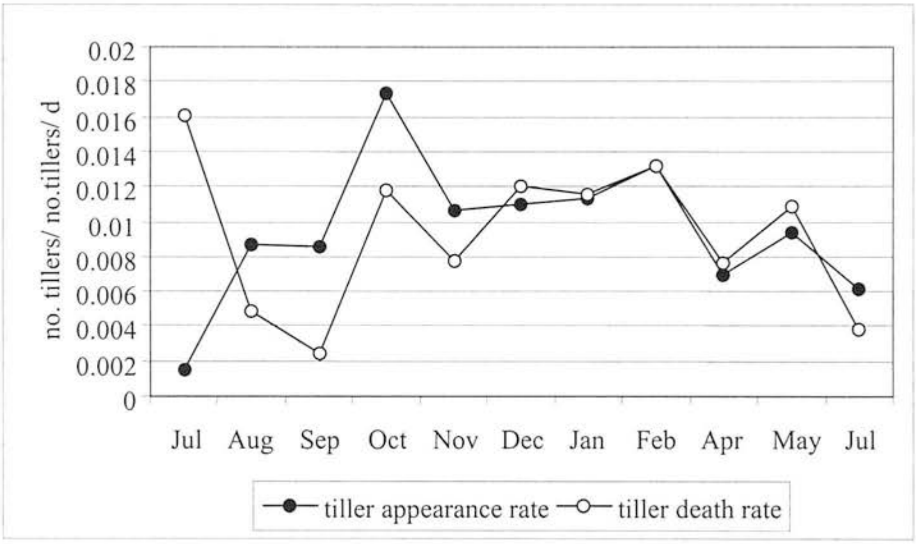

Figure 6.3. Seasonal dynamics in tiller birth and death rates. No statistical analyses were performed due to the skewed (large amount of null values) distribution of the observations.

Winter of 1998

Characteristics of the measurement intervals

This period was characterised by scarce rain, low radiation, water availability and average temperatures with respect to the following periods of the experiment (Table 6.1). Soil water availability was generally very low, as reflected by the values of Eap, except immediately after the rains that took place at the beginning of August and at the beginning and end of September (Table 6.2). Average daily temperatures remained fairly stable during the period, most of the time between 20 and 25°C. The same occurred with solar radiation, with average values between 10 and 12 $MJm^{-2}d^{-1}$.

Tiller and growing point density

The difference in signal grass tiller densities between May and October (*Difbd*) was correlated positively with the difference in legume growing point density (*Difleg*; r=0.40) and negatively with the difference in other species (other grasses plus other broad-leaved species) density (*Difoth*; r=-0.52). A multiple regression considering these two variables, low correlated between them (r=0.23), and their interaction with the effect of nitrogen fertilisation (*N*) was highly significant ($p<0.001$), and the coefficient of determination was relatively high ($R^2=0.65$; n=27).

$$\mathit{Difbd}=-3.07(\pm 0.61)\,\mathrm{x}\,\mathit{Difoth} + 2.46(\pm 0.49)\,\mathrm{x}\,\mathit{Difleg} + 2.141(\pm 0.78)\,\mathrm{x}\,\mathit{Difioth}\,\mathrm{x}\,N - 4.05(\pm 1.81)\,\mathrm{x}\,\mathit{Difleg}\,\mathrm{x}\,N$$

This function shows that without fertilization signal grass density increased with increases in legume and decreases in other species, while when nitrogen was applied increases in signal grass occurred simultaneously with increases in other species and decreases in legumes. Neither any of the soil variables nor nitrogen fertilisation alone affected the change in signal grass density significantly.

Morphogenetic and structural characteristics

Average values for the whole period showed that nitrogen fertilization increased leaf elongation and tiller appearance rates significantly. The rest of morphogenetic characteristics (LAR, LSR, IER and TDR) were not affected by nitrogen (Table 6.6). TBR was also positively related to LAR, while LSR and IER were positively affected by LLLt at the beginning of the period. Although nitrogen did not change any of the tiller structural characteristics, it affected markedly the structure at the plant level. The marked increase in the number of tillers per plant produced a similar increase in its amount of live leaf.

LAR, LER and TBR showed three distinctive peak values during winter of 1998 (Fig. 6.4). These peaks coincided with intervals of higher than average Eap. For the rest of the measurements LAR, LER and TBR values were very low, in many cases zero. LSR and IER remained with low values, 2-4 mm/d and less than 1 mm/d respectively, during all the winter.

Table 6.6. Effect of nitrogen on average values of morphogenetic and structural characteristics of leaves, tillers and plants of signal grass during winter 1998 (August-October).

	N0	N25	s.e.d.	P	Covariate	Coefficient
LEAF MORPHOGENETIC CHARACTERISTICS						
Leaf appearance rate (leaves d^{-1})	0.07	0.08	0.007	0.149	--	--
Leaf elongation rate (mm d^{-1})	5.1	6.0	0.48	0.055	--	--
Leaf senescence rate (mm d^{-1})	2.9	2.3	0.37	0.153	LLLt*	0.04±0.002
Internode elongation rate (mm d^{-1})	0.3	0.3	0.05	0.858	LLLt***	0.002±0.0004
TILLER MORPHOGENETIC CHARACTERISTICS						
Tiller appearance rate (til til^{-1} d^{-1})	0.014	0.022	0.0029	0.010	LAR***	0.375±0.0890
Tiller death rate (til til^{-1} d^{-1})	0.005	0.008	0.0021	0.114	--	--
TILLER STRUCTURAL CHARACTERISTICS						
Live leaf length at the end (mm)	216	210	36.7	0.868	NS	--
Total number of leaves at the end	7.8	7.6	0.95	0.818	NS	--
Stem length at the end (mm)	27.9 (3.33)	22.4 (3.11)	(0.418)	0.602	*	(0.40±0.154)
PLANT STRUCTURAL CHARACTERISTICS						
Number of live tillers at the end	6.0	10.1	1.14	0.002	***	1.21±0.185
Live leaf length at the end (mm)	1348	2136	245.9	0.004	***	1.52±0.210

Total d.f.: 23(4 missing values). * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS: $p>0.05$.
Covariates used for the structural characteristics corresponded to the values of the same variable at the beginning of the period.
N0: without nitrogen fertilisation; N25: with 25 kg N ha^{-1}; s.e.d.: standard error of the difference; P: probability that N0 and N25 observations come from the same population.
LLLt: Average live leaf length per tiller at the beginning of the period (mm); LAR: Leaf appearance rate.

Separate ANOVAS for leaf morphogenetic variables during the intervals of higher growth (intervals 2, 8, 9 and 12; Table 6.2) showed that the significant effect of nitrogen on LER (Table 6.6) was localised on the intervals 9 (LER of 12.4 and 17.2 mm d^{-1} for the N0 and N25 levels respectively; s.e.d.=2.26) and 12 (LER of 7.5 and 9.2 mm d^{-1} for the N0 and N25 levels respectively; s.e.d.=0.67). The effect of LLLt on LSR was only significant in the interval 9 (coefficient of 0.0232±0.0044), while IER was positively affected at least by one of the structural characteristics of the tiller (LLLt, TNLt and INTt) in each of the four intervals. LAR was significantly affected by nitrogen only in the interval 9 (LAR of 0.13 and 0.20 leaves d^{-1} for the N0 and N25 levels respectively; s.e.d.=0.03). TBR and TDR could not be analysed for this short intervals because of the skewed distributions of their observations.

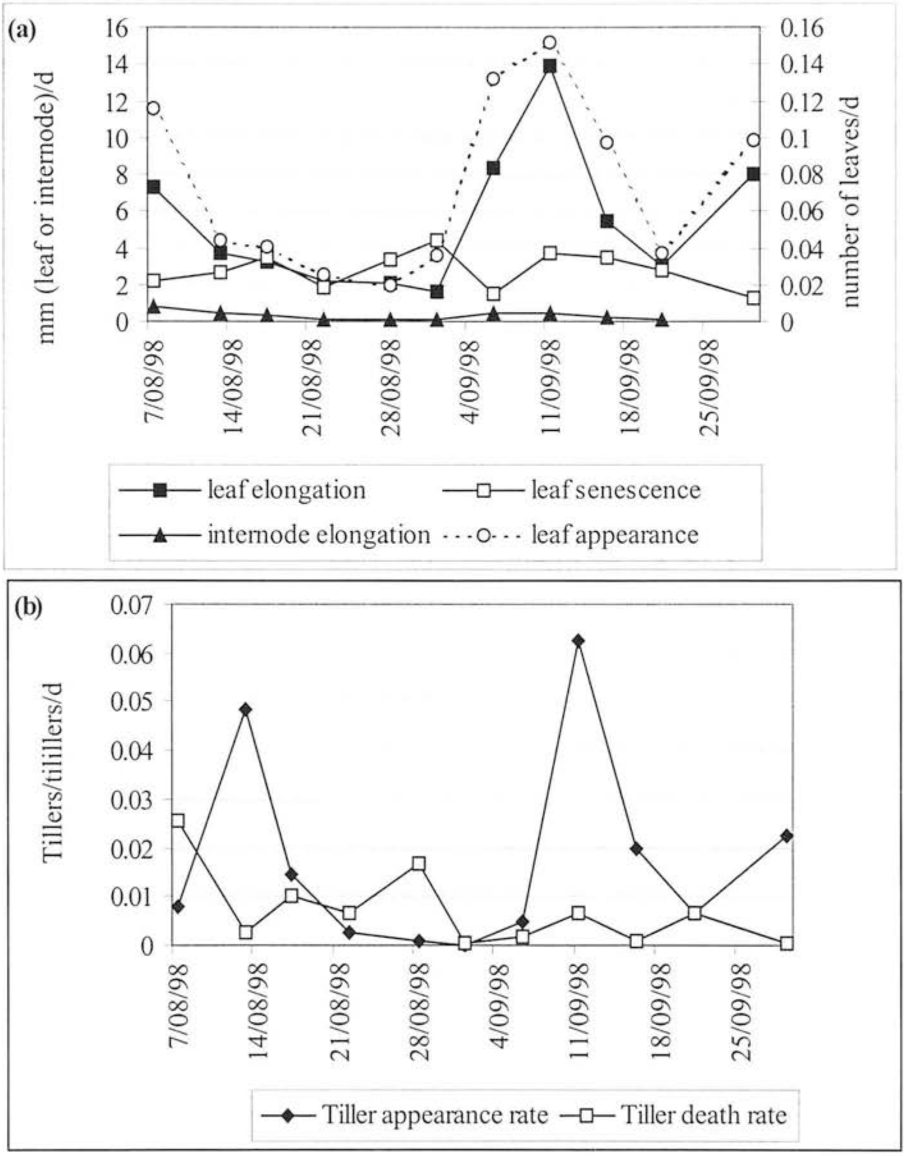

Figure 6.4. Seasonal dynamics of leaf (a) and tiller (b) morphogenetic characteristics of signal grass during the winter of 1998. Values are arithmetic means for basal tillers in (a) and for all the tillers in (b). No statistical analyses were made because of the skewed distribution of the data.

Table 6.10 shows that during winter 98, the changes in live leaf per tiller (LLLtR) were more determined by LER during the intervals of active tiller growth and by LSR in the rest of intervals. The balance for the whole period was a similar effect of LSR and LER. A similar pattern was observed at the tiller level, with TDR defining the change in the total number of leaves per plant (TNTR) for all the intervals but those of active growth. TBR defined clearly TNTR when the whole period was considered. At the higher level of organisation, LLLtR had similar importance that the change in tiller numbers (TNTR) in defining the change in live leaf per plant (LLLpR) for the whole period. Both LLLtR and TNTR were negatively correlated for most of the intervals, but not for the whole period. The shortness of the intervals was the probable cause for these negative correlations.

Summer

Characteristics of the measurement periods

Daily average temperatures were around or above 25°C for all the periods, except for the last one in April (Table 6.1). Soil water was still limiting for plant growth in October. After high Eap values from November to February indicating soil water surplus, this ratio decreased in April, but was still much higher than the values observed in October. Average daily radiation was highest from October to January, decreasing to medium values from February to April.

Tiller and growing point densities

In the analysis of the effects of nitrogen, grazing intensity and period on signal grass tiller density, soil organic matter and density of growing points of other broad-leaved species were used together as covariates, as both had significant correlation coefficients with signal grass tiller density ($r=0.58$ and $r=-0.58$ respectively). The considerable correlation between these two variables (-0.41) made difficult the interpretation of their individual effect on signal grass density.

As with the analysis for the whole year, in which only non-fertilised plots were used, tiller density was significantly affected by period because of the lower values found in October. The trend of signal grass density along the summer was similar to that seen in non-fertilized plots (Figure 6.1), with increasing values from October to December, and stable afterwards. These values agreed with the balance between rates of appearance and death in marked tillers (Table 6.8), except during January and February, when higher than expected values were obtained, probably due to consider flowering tillers as dead when calculating tiller death rates while been counted in the density measurements.

As with non-fertilised plots, grazing intensity or PRDEF did not affect signal grass tiller density. On the other hand, it showed a tendency ($p=0.108$) to increase with nitrogen application (954, 1146 and 1167 tillers m^{-2} for N0, N50 and N100 respectively; s.e.d.=110.4). None of the possible interactions between period, grazing and nitrogen were significant.

The proportion of aerial tillers was positively affected by PRDEF in the previous grazing event ($p<0.01$; coefficient: 0.104±0.0323) and by tiller density ($p<0.001$; coefficient: $2.36 \times 10^{-4} \pm 0.233 \times 10^{-4}$). These variables were not correlated between them ($r=0.01$). Opposite effects of PRDEF ($p<0.01$; coefficient: -0.127±0.0336) and tiller density ($p<0.001$; $-2.08 \times 10^{-4} \pm 0.374 \times 10^{-4}$) were observed for proportion of basal tillers. Neither nitrogen, period, grazing (as effect), nor any of their interactions affected the proportions of aerial or basal tillers.

Changes in growing point densities for species other than signal grass were analysed considering their densities at the beginning (October) and at the end of summer (April). The relative change in density between these two periods for each group of species was compared with those of signal grass for the different combination of treatments (grazing x nitrogen) using chi-square tests. Signal grass tiller density increased an average of 15%, while legume density decreased an average of 39% between the first and the last measurements of summer. Treatments did not significantly affect these proportions ($\chi^2=7.03$, 6 d.f.). The relative changes of signal grass and other graminoids were different at the high grazing intensity than at the two other grazing levels ($\chi^2=1.60$ 3 d.f.). In this case, graminoids increased 46% at high grazing intensity, and decreased 31% at the other grazing intensity levels. For the group of other broad-leaved species, densities decreased or remained stable except under the combination of high grazing intensity and N100 levels, when final densities were 9 times higher than initial ones.

Morphogenetic and structural characteristics

While nitrogen fertilization did not affect significantly any morphogenetic variable during this period, grazing, either used as effect or as covariate (PRDEF), affected significantly all the morphogenetic variables, except LER (Table 6.7). PRDEF affected positively TBR, TDR and LAR. On the contrary, it had a negative effect on IER and LSR. TBR and TDR behaved similarly ($r=0.60$), they were similarly affected by defoliation (Table 6.7), but tiller death was higher than tiller appearance during the last half of the summer (Table 6.8). LER was positively correlated with LAR (r=0.66) and IER (r=0.81), while LAR and IER were not clearly correlated (r=0.35).

Table 6.7. Result of split-plot ANOVA (*F*-values) on morphogenetic variables and tiller density during the summer season.

Stratum	Effect/Covariate	LAR	LER	LSR	IER(loge)	TBR(sqrt)	TDR(sqrt)	Density*
Paddock x Plot	Nitrogen (2,22)	0.44^{NS}	0.51^{NS}	0.71^{NS}	0.35^{NS}	0.15^{NS}	0.45^{NS}	0.90^{NS}
	Defoliation (1,22)	0.69^{NS}	1.99^{NS}	8.17**	6.76*	5.70*	6.40*	2.09^{NS}
Paddock x Plot	Epsilon	0.5398	0.3595	0.3513	0.5092	0.6663	0.5781	0.5618
x Period	Period (5,80)	11.30***	5.33*	2.07^{NS}	18.14***	1.57^{NS}	7.50**	5.13**
	PeriodxNitrogen(10,80)	0.77^{NS}	0.98^{NS}	1.89^{NS}	1.24^{NS}	1.72^{NS}	1.3^{NS}	1.87^{NS}
	Defoliation (1,80)	5.92*	0.98^{NS}	23.63***	5.50*	15.19***	18.66***	0.19^{NS}
	coefficient± s.e.	0.032± 0.0132		-4.33± 0.763	-0.45± 0.150	0.142± 0.0318	0.148± 0.0290	

The degrees of freedom are shown between brackets in the second column. The epsilon value modifies the critical *F*-value in the Paddock x Plot x period stratum. Defoliation is a covariate and refers to the proportion of live leaf length per plant defoliated in the previous grazing event.

Residual d.f. at the Paddock x Plot level =24; residual d.f. at the Paddock x Plot x period = 99.

LAR: leaf appearance rate (leaves d^{-1}); LER: leaf elongation rate (mm d^{-1}); LSR: leaf senescence rate (mm d^{-1}); IER: Internode elongation rate (mm d^{-1}); TBR: Tiller appearance rate (til til^{-1} d^{-1}); TDR: Tiller death rate (til til'1 d^{-1}).

* $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS: $p>0.05$.

Table 6.8. Seasonal dynamics of signal grass morphogenetic characteristics and tiller density during summer.

	Oct	Nov	Dec	Jan	Feb	Apr	s.e.d.
LAR	0.10	0.12	0.10	0.11	0.10	0.07	0.007
LER	8.3	11.2	10.1	11.7	12.6	8.7	1.02
LSR	4.1	3.4	4.1	4.3	3.4	3.4	0.43
IER	1.5 (0.44)	2.6 (0.97)	2.5 (0.92)	2.7 (0.99)	3.2 (1.15)	2.1 (0.74)	(0.081)
TBR	0.012 (0.110)	0.019 (0.137)	0.028 (0.166)	0.023 (0.153)	0.021 (0.144)	0.019 (0.138)	(0.0198)
TDR	0.012 (0.109)	0.013 (0.116)	0.027 (0.165)	0.027 (0.164)	0.037 (0.193)	0.024 (0.155)	(0.0166)
Densit*	950	1043	1117	1132	1116	1060	43.5

Values between brackets correspond to the original transformed data (natural logarithms for IER and square roots for TBR and TDR). s.e.d. corresponds to the split-plot design without considering repeated measurements.

* Tillers m^{-2}. Values at the end of each period.

LAR seemed to reach its maximum values earlier (October) than the maximum values of LER and IER (November) (Table 6.8 and Figure 6.2b). This agrees with the higher correlations of LER and IER with Eap (r=0.32 and 0.40 respectively) and the low Eap values in October (Table 6.1). On the other hand, LAR showed much higher correlations with average daily temperature (r=0.45) than any of the other leaf morphogenetic variables.

For all the periods during summer, LER was much better correlated with LLLtR than LSR, though in some periods LER and LSR were positively correlated (Table

6.10). LSR also showed lower correlations with LLLtR with higher grazing intensities. TBR and TDR were highly positively correlated in all of the periods and levels of treatments, except in January and February, and under medium grazing intensity. In all the periods and treatment levels TBR was better correlated with TNTR than TDR. Finally, the change in live leaf per plant was similarly caused by changes in tiller number and live leaf per tiller, except for November and December, when change in tiller number was more important than change in tiller live leaf.

Winter of 1999

Morphogenetic and structural characteristics

Comparing the results for the whole of this period (Table 6.9) with those for the N0 level of the previous winter (Table 6.6) some differences in morphogenetic and structural values were observed. LER, LSR and IER were higher in this period, while LAR and TBR were similar or slightly lower than in winter 98. Plants and tillers showed higher lengths of live leaf than in winter 98, with tillers of higher stem lengths and plants of higher tiller numbers.

Table 6.9. Effect of grazing intensity on morphogenetic and structural characteristics of signal grass during winter 1999.

	Low	Medium	High	s.e.d	P	Covariate	Coefficient s.e.
LEAF MORPHOGENETIC							
Leaf appearance rate (l d^{-1})	0.06	0.06	0.07	0.006	0.246	LLLp*	$1.23 \times 10^{-5} \pm 0.496 \times 10^{-5}$
Leaf elongation rate (mm d^{-1})	6.9	7.7	6.4	0.69	0.245	LLLp***	$3.37 \times 10^{-2} \pm 0.58 \times 10^{-3}$
Leaf senescence rate (mm d^{-1})	3.6	3.9	3.1	1.011	0.719	LLLt^{NS}	$1.58 \times 10^{-2} \pm 0.451 \times 10^{-2}$
Internode elongation rate(mmd^{-1})	0.8	1.1	0.6	0.21	0.134	LLLp^{NS}	$2.9 \times 10^{-4} \pm 1.57 \times 10^{-4}$
TILLER MORPHOGENETIC							
Tiller appearance rate (t t^{-1} d^{-1})	0.015	0.007	0.011	0.005	0.379	Phosph*	$9.5 \times 10^{-4} \pm 2.7 \times 10^{-4}$
Tiller death rate (t t^{-1} d^{-1})	0.009	0.003	0.006	0.004	0.314	--	--
TILLER STRUCTURAL							
Live leaf length at the end (mm)	450	368	265	155.6	0.582	NS	--
Total no. leaves at the end	7.0	6.2	7.5	1.13	0.577	NS	--
Stem length at the end (mm)	42.8	28.8	22.2	18.54	0.624	*	1.35±0.431
PLANT STRUCTURAL							
No. of live tillers at the end	7.5	7.2	7.6	1.44	0.947	**	1.40±0.170
Live leaf length at the end (mm)	2207	2246	2692	744.3	0.793	*	1.91±0.002

Total d.f.=11. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS: $p>0.05$.

LLLp: Plant live leaf length at the beginning of the period (mm); LLLt: Average tiller live leaf length at the beginning of the period (mm); Phosph.: Soil phosphorus concentration (ppm).

The covariates used in the structural characteristics corresponded to the values of the same variable at the beginning of the period.

Grazing intensity did not affect any of the morphogenetic or structural characteristics, and only structural characteristics of the plant and soil nutrients had a significant effect on the morphogenetic variables. Growth at the plant level was higher in this period than in winter 98, as shown by the higher coefficients of the covariates.

Table 6.10 shows that changes in live leaf length per tiller were more determined by changes in LER. The same occurred with TNTR and changes in TBR, and with LLLpR and LLLtR.

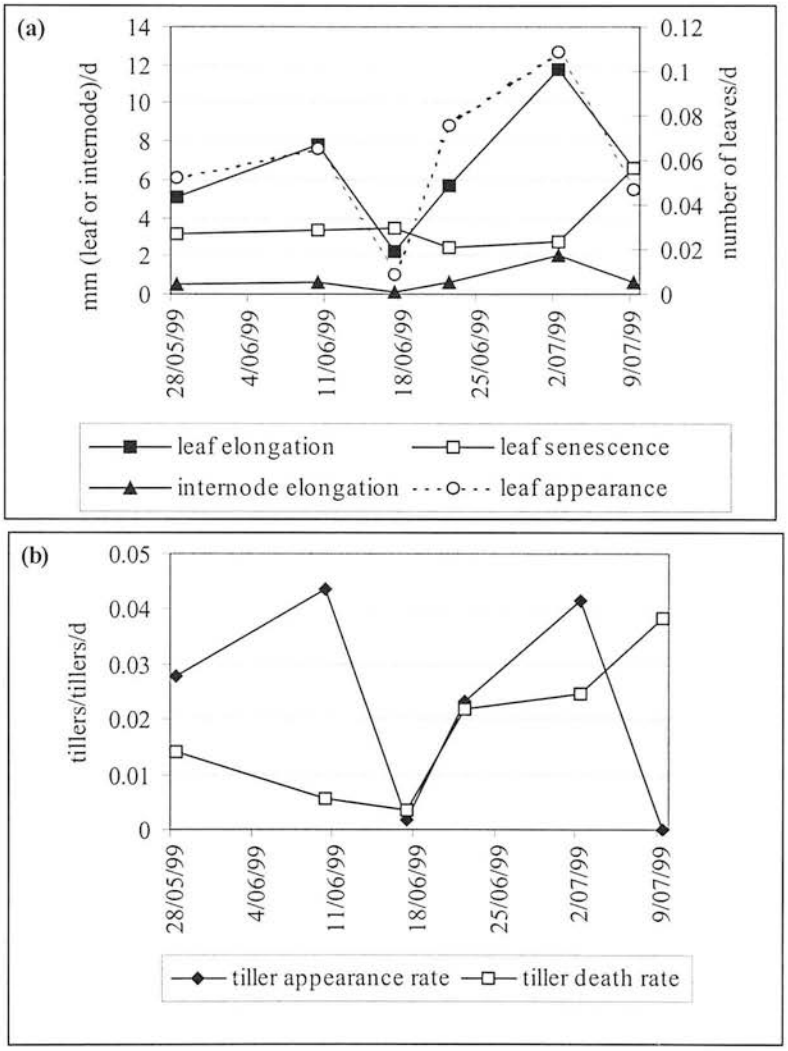

Figure 6.5. Seasonal dynamics of leaf (a) and plant (b) morphogenetic characteristics of signal grass during the winter of 1999. Differences among dates were significantly different ($p<0.01$) for variables in (a) but not for variables.

During the period, LER and LAR showed two significant high values, corresponding to measurement intervals with Eap values higher than the rest (Fig.

6.5a). TBR presented the same seasonal trend to LER and LAR, though differences due to the intervals were not significant. Changes in TDR were also not statistically different (Fig. 6.5b). IER and LSR increased significantly one interval before the last and in the last interval respectively.

Table 6.10. Correlations between morphogenetic variables at the same (second row) and following higher hierarchical level (first row) of the sward for the whole periods, for the different measurement intervals and for the nitrogen and grazing treatments in summer.

Period	LLLtR			TNTR			LLLpR		
/Interval	LER	LSR	LER /LSR	TBR	TDR	TBR /TDR	LLLtR	TNTR	LLLtR /TNTR
WIN98	0.673	-0.693	-0.027	0.872	-0.085	0.413	0.538	0.619	-0.061
2	0.615	-0.610	0.249	0.451	-0.960	-0.183	0.380	0.753	-0.155
3	0.593	-0.837	-0.146	0.902	-0.554	-0.141	0.193	0.222	-0.746
4	0.314	-0.816	0.007	0.720	-0.771	-0.114	0.323	0.167	-0.776
5	0.463	-0.876	0.040	0.507	-0.834	0.053	0.327	0.763	-0.183
6	0.234	-0.956	0.057	0.279	-0.982	-0.091	0.561	0.346	-0.363
7	0.427	-0.881	-0.075	--	--	--	0.616	0.391	-0.460
8	0.732	-0.526	0.140	0.845	-0.500	0.040	0.391	0.204	-0.552
9	0.808	-0.559	-0.018	0.985	-0.296	-0.124	0.040	0.749	-0.367
10	0.565	-0.866	-0.066	0.999	-0.126	-0.074	0.284	0.494	-0.549
11	0.544	-0.907	-0.266	0.626	-0.840	-0.102	0.363	0.328	-0.550
12	0.835	-0.262	0.099	0.996	0.043	0.134	0.214	0.679	-0.358
SUMMER	0.894	-0.437	-0.029	0.697	-0.448	0.157	0.639	0.695	0.178
Oct	0.855	-0.589	-0.154	0.756	0.349	0.516	0.752	0.820	0.332
Nov	0.927	0.086	0.418	0.940	0.378	0.610	0.297	0.654	-0.285
Dec	0.868	-0.088	0.379	0.769	0.415	0.809	0.453	0.804	0.169
Jan	0.917	-0.559	-0.169	0.859	0.295	0.330	0.587	0.381	-0.007
Feb	0.934	-0.442	-0.049	0.880	-0.120	0.135	0.627	0.836	0.291
Apr	0.786	-0.290	0.371	0.911	0.678	0.797	0.850	0.781	0.466
WIN99	0.761	-0.485	0.153	0.853	-0.324	0.218	0.618	0.297	-0.331
1	0.537	-0.736	0.183	0.903	-0.550	-0.164	-0.009	0.652	-0.601
2	0.721	-0.505	0.255	0.953	-0.239	-0.045	0.251	0.436	-0.574
3	0.523	-0.873	0.005	--	--	--	0.898	-0.020	-0.247
4	0.713	-0.575	0.066	0.447	-0.596	0.339	-0.005	0.754	-0.522
5	0.833	-0.272	0.242	0.638	-0.275	0.528	0.723	-0.180	-0.526
6	0.673	-0.547	0.263	--	-0.958	--	0.279	0.450	-0.411
SUMMER									
N0	0.847	-0.383	0.090	0.894	0.556	0.624	0.579	0.595	-0.139
N50	0.859	-0.481	0.000	0.555	-0.058	0.712	0.746	0.481	0.153
N100	0.945	-0.453	-0.137	0.731	0.098	0.468	0.724	0.724	0.238
G1	0.853	-0.537	-0.072	0.781	0.173	0.640	0.624	0.462	-0.293
G2	0.894	-0.477	-0.069	0.729	-0.033	0.368	0.701	0.756	0.311
G3	0.904	-0.337	0.039	0.806	0.384	0.649	0.615	0.505	-0.013

LLLtR: rate of live leaf length change per tiller (mm d^{-1}); LER: leaf elongation rate (mm d^{-1}); LSR: leaf senescence rate (mm d^{-1}); TNTR: rate of change in tiller number per plant (til til^{-1} d^{-1}); TBR: tiller appearance rate (til til^{-1} d^{-1}); TDR: tiller death rate (til til^{-1} d^{-1}); LLLpR: rate of live leaf length change per plant (mm d^{-1})

Tiller survival

The average longevity of tillers born between July and January, interval with known lifespan for all the tillers, was 58 days. Changes in longevity with period and tiller type are shown in figure 6.6. Tiller life was longer for basal tillers born in winter of 1998 (July-September). Aerial tillers were always short-lived, except those born in July (only two tillers). For the periods delimited by grazing events (October-January), the difference in lifespan between basal and aerial tillers was still significant, but lower than in the previous winter.

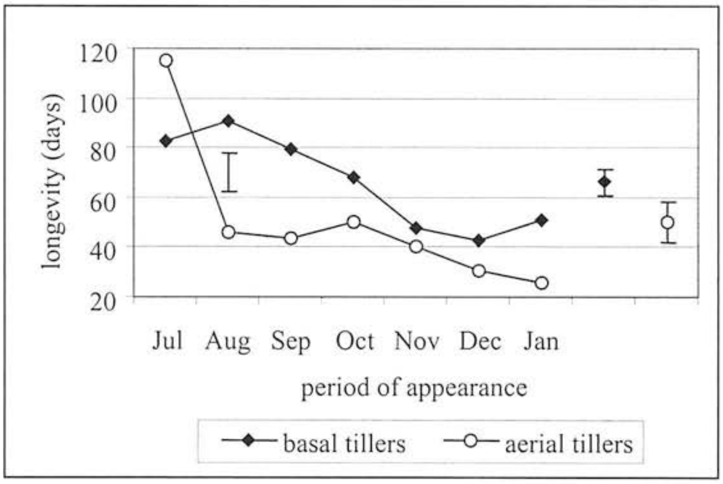

Figure 6.6. Average longevity of tillers depending on the period of appearance and the origin of birth (basal vs. aerial). Vertical bars at the end of the lines indicate s.e.d. of the different periods for each tiller type. The vertical bar between lines indicates s.e.d. between tiller types for each period. Period ($p<0.05$) and tiller type ($p<0.01$) were significant.

Grazing intensity affected tiller longevity: 68.7, 48.8 and 40.4 days of life length for low, medium and high grazing intensities respectively (s.e.d.=6.51; $p<0.001$).

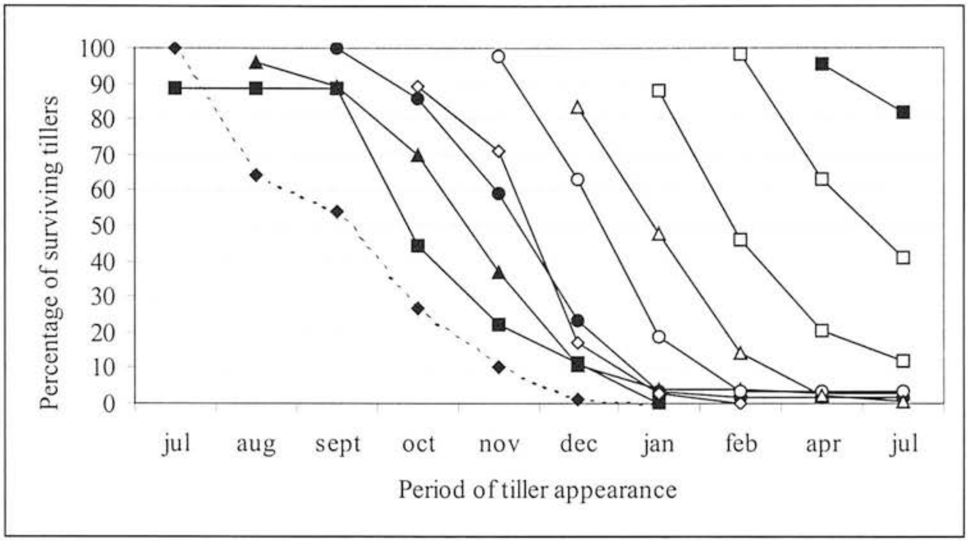

Figure 6.7. Survivorship curves for different cohorts of signal grass tillers according to their period of appearance. The first curve (dashed line) corresponds to tillers marked initially, appeared before July 1998. The number of tillers in each cohort was: initial, 170; July, 9; August, 73; September, 56; October, 65; November, 95; December, 191; January, 117; February, 105 and April, 93.

Figure 6.7 shows the consecutive survivorship curves of tillers appearing in the same period. Fitting the points of these curves to an exponential equation (Pakiding and Hirata, 1999) resulted in an average half-life of tillers of 24 days, with minimum values for tillers born in December (14 days) and maximum values for tillers born in July (42 days). The rates of decline from one period to the next varied mainly depending on the existence of a grazing event between periods (from October onwards). Chi-square tests comparing the proportions of the initial number of tillers in each cohort surviving the following period to that of their appearance, showed that all the cohorts had the same pattern (χ^2=22.14 d.f.=14). When the two following periods to that of their appearance were considered (only for July to February cohorts), not all the tiller cohorts behaved similarly, and three main groups could be defined. Tillers born in July, August and September displayed similar proportional declines of surviving individuals (χ^2=6.34 d.f.=6), which were the lowest of all the cohorts. Tillers born in the February period showed the same proportional decline of surviving individuals to those born in August and September (χ^2=8.30 d.f.=6), but higher to those of the July cohort. Tillers born from October to January had similar declines (χ^2=7.18 d.f.=9), which were the highest of all cohorts. If the proportions of surviving tillers in the next three periods after appearance were considered (only for July to January cohorts), again the July to September cohorts behaved similarly (χ^2=11.34 d.f.=8), with a less accused decline to those of October to January cohorts, these being also similar among them (χ^2=20.79 d.f.=12).

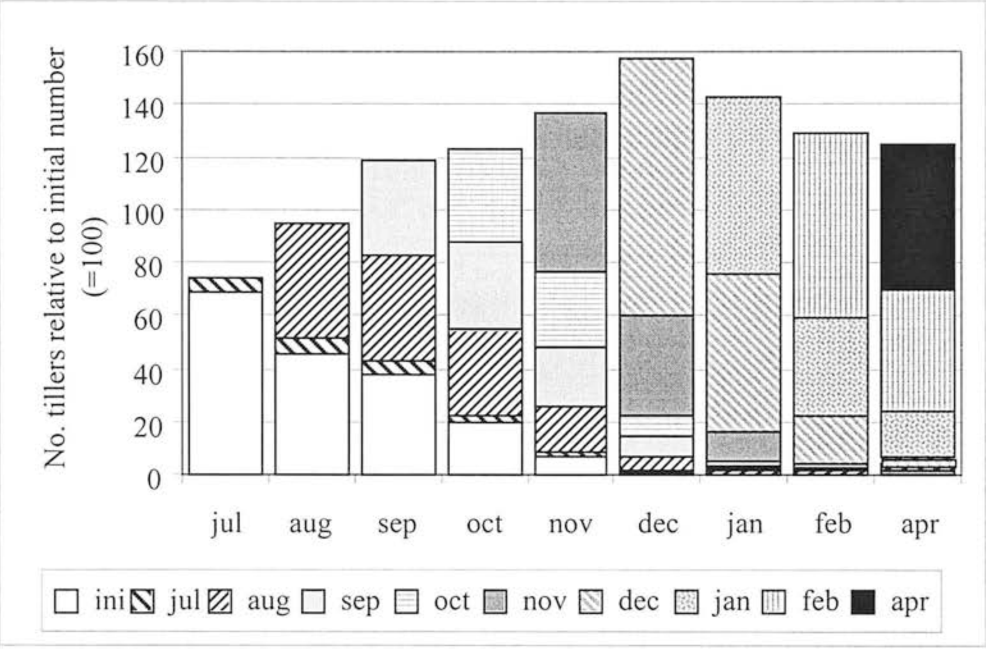

Figure 6.8. Average relative number of signal grass tillers present at each period of the experiment depending on the period in which they appeared (tiller cohorts). The initial number of marked tillers (beginning of July) was set to 100.

Figure 6.8 shows that most tiller cohorts retained a high proportion of their population during the next period to that of their appearance, but this proportion dropped steeply two periods after their appearance, especially for tillers born from October onwards. December was the period with the highest number and proportion (60%) of new tillers, while July showed the lowest proportion of newly appeared tillers with only 7%. From August to November the percentage of tillers having appeared in that or the preceding period was between 50 and 60% of all the existing live tillers, while from December onwards that proportion increased to more than 80% of the total number of tillers. From July until December the number of tillers appearing in each period more than balanced those dying from older cohorts, but from December onwards this trend was reverted (Figure 6.8).

Considering only those tillers appearing during the summer months, differences in tiller survival proportions were studied for plants under different nitrogen fertilisation and grazing intensity regimes (Table 6.11). The only significant difference observed was the higher survivorship observed in the tiller cohorts under low and medium grazing intensities without fertilisation.

Table 6.11. Percentage of tillers surviving the same and following periods to that of their appearance, for plants under different nitrogen fertilisation and grazing intensity regimes during summer (average percentages of tillers appearing from October to February).

N	Grazing	same period	1st next period	2nd next period	3rd next period	4th next period	5th next period
0	low	98	92 a	41 a	0	0	0
0	medium	96	81 ab	28 a	6	5	0
0	high	97	65 ab	16 b	2	0	0
50	medium	95	46 ab	14 b	2	0	0
50	high	93	60 ab	22 b	0	0	0
100	medium	93	54 ab	24 b	8	8	0
100	high	88	40 b	6 b	1	0	0

Different letters within the same column indicate statistical significant differences ($p<0.05$) in the surviving distributions considering the percentages of that column and of all the previous columns (Chi-square tests).

Demography of marked tillers

This section complements the previous analyses of tiller appearance and death rates. In this case more focus is given to the patterns of appearance and death of tillers depending on whether they were basal or aerial.

During the long initial regrowth period (August to September), the majority of tillers appearing were basal, outnumbering the number of basal tillers dying. On the contrary, more aerial tillers died than appeared, resulting in swards with around 90% of basal tillers before the first grazing in October (Figure 6.9).

From October to December the number of basal tillers appearing and dying in each period were very similar, while more aerial tillers appeared than died, increasing their number and proportion successively towards December. The higher number of basal tillers dying than appearing during January and February, and the opposite occurring in the case of aerial tillers, produced a significant higher proportion of aerial tillers. During the last two periods, deaths and births were balanced for basal tillers, while more aerial tillers died than appeared (Figure 6.9).

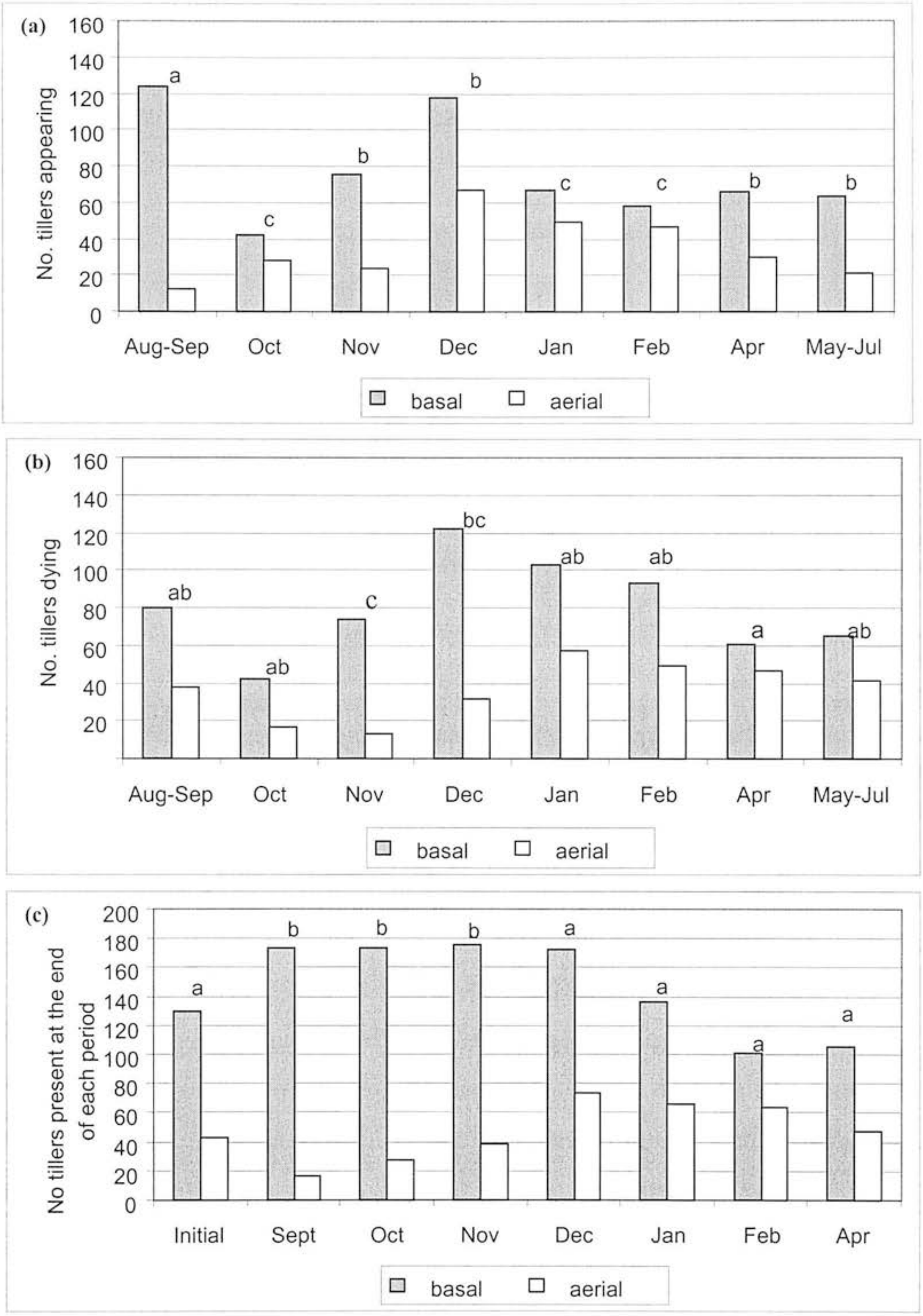

Figure 6.9. **Total number of tillers appearing (a), dying (b) and present (c) during the experiment according to their origin (basal or aerial) and period between grazing events. Different letters above the bars indicate statistically significant different periods with regard to their proportions of basal and aerial tillers (chi-square test).**

The causes of tiller death in a tiller were classified as those directly related to grazing (produced immediately after grazing), those not directly related to grazing and those related to reaching the flowering stage (Figure 6.10).

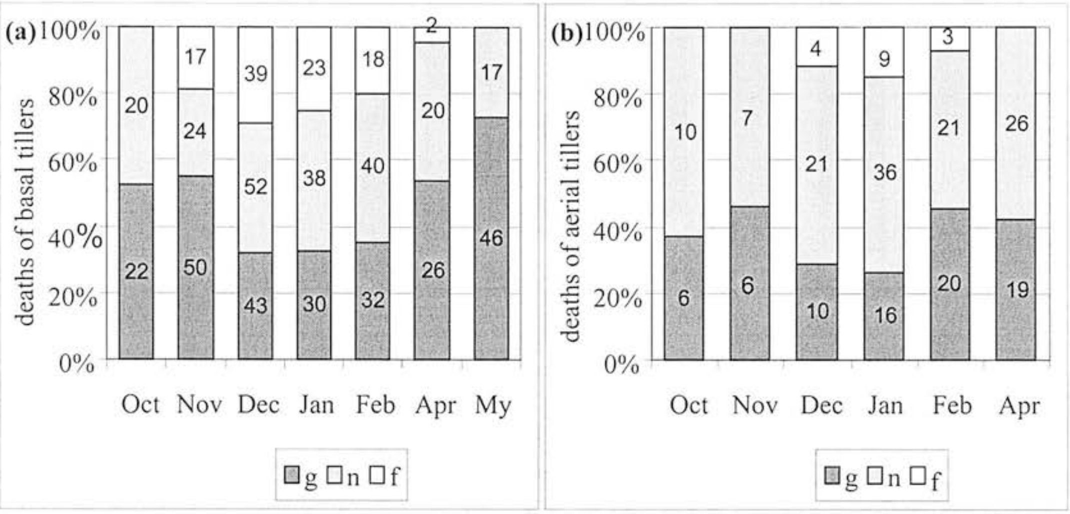

Figure 6.10. Proportion of tillers from basal (a) or aerial (b) origin dying directly from grazing (g), not directly from grazing (n) or flowering (f). Numbers within the column segments are the absolute number of tillers.

The proportions of tiller deaths attributed directly to grazing and not directly to grazing did not change with the period in the case of aerial tillers or basal tillers, except for basal tillers dying in November and in May (χ^2 test; $p>0.05$). These proportions were significantly different in basal and in aerial tillers except for February, with higher proportions of basal tillers dying directly from grazing than aerial tillers. The proportion of tillers dying to flowering was significantly higher in basal than in aerial tillers.

Patterns of appearance of aerial tillers

This analysis was only performed for the periods following grazing, as initially (July-September) the number of aerial tillers born was very small (Figure 6.9). The average number of aerial tillers appearing per parental tiller was 1.37±0.076 (s.e).

Parental tillers at the time of aerial tiller appearance were classified as being alive, dead immediately after grazing, dead not related to grazing or flowering (Figure 6.11).

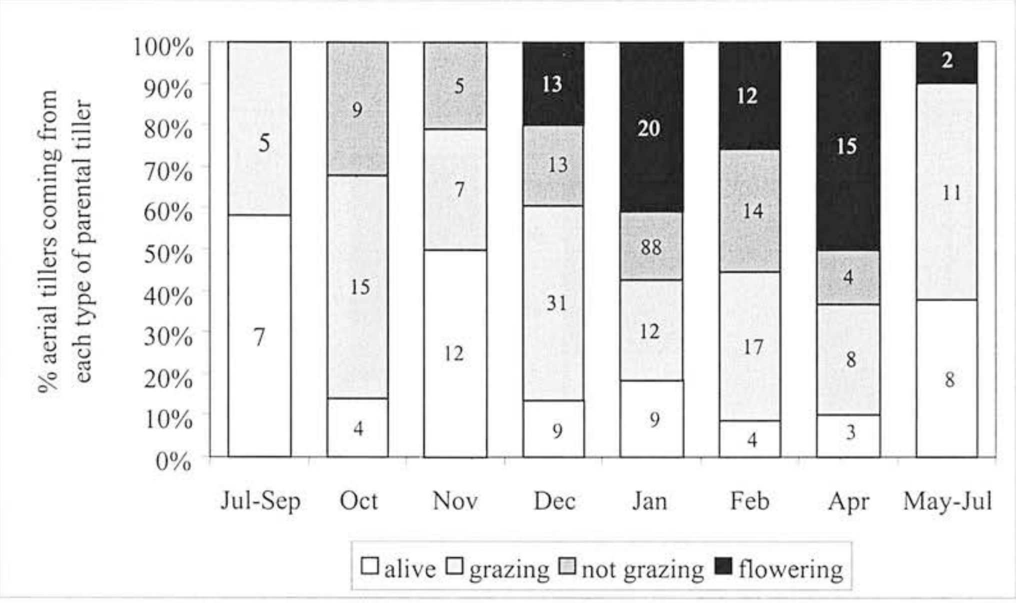

Figure 6.11. Origin of aerial tillers according to the state of their parental tillers and the period of the year. The states of the parental tillers considered are: alive, dead due directly to grazing (grazing) , dead not related to grazing (not grazing) and flowering. Numbers within column segments are absolute tiller numbers

In October, and from December to April, the proportions of aerial tillers coming from living, dead directly due to grazing and dead not directly due to grazing tillers were not significantly different: average values of 18%, 52% and 30% respectively (χ^2=6.84; d.f.=8). In November the number of aerial tillers appearing was the lowest of the short summer regrowth periods, and the number and proportion of aerial tillers originated from living parental tillers was the highest of all periods (Figure 6.11). When considering the four categories of parental tillers, December showed a different distribution in the origin of aerial tillers to the next three periods (January to April). In December the proportion of aerial tillers coming from dead parental tillers due directly to grazing (47%) was much higher than in the following periods (average of 29%). The distribution of proportions of categories of parental tillers did not change with nitrogen fertilisation, but was significantly different with grazing intensity (Figure 6.12). Aerial tillers under low grazing intensity came mainly from flowering parental tillers, while at high grazing intensity tillers dying due to grazing were the mayor source of aerial tillers. At medium grazing intensities the source of aerial tillers was more homogeneously distributed among the different categories of parental tillers.

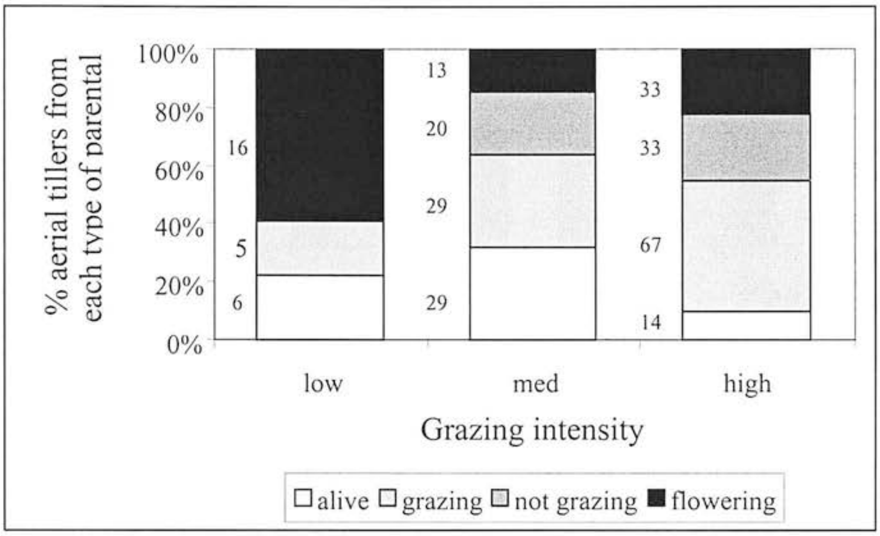

Figure 6.12. Origin of aerial tillers according to the state of their parental tillers and the grazing intensity from October to July. The states of the parental tillers considered are: alive, dead due directly to grazing (grazing) , dead not related to grazing (not grazing) and flowering. Numbers beside column segments are absolute numbers of aerial tillers.

Flowering tillers

A total of 115 marked tillers (10%) reached the flowering or flowering emergence stage. Of these only 16 were aerial, representing 5% of the total number of aerial tillers monitored. Seventy two tillers were seen to complete the flowering stage, while 43 were decapitated, broken or disappeared before flower emergence was detected. The average number of spikes per tiller was 1.74 (±0.63 s.d.), and the average number of flowers per tiller was 47 (±25.2 s.d.). Flowering occurred between mid November and the end of March (in between grazing periods of November, December, January and February). This time was characterised by nearly continuous high soil water availability.

The total number of leaves per flowering tiller decreased significantly ($p<0.001$) with the period of birth of the tillers (Figure 6.13). The minimum number of leaves, around 10, was achieved for tillers born during the flowering season, from November to January. On the contrary, stem length per flowering tiller (up to the flag leaf) did not differ with period of tiller appearance.

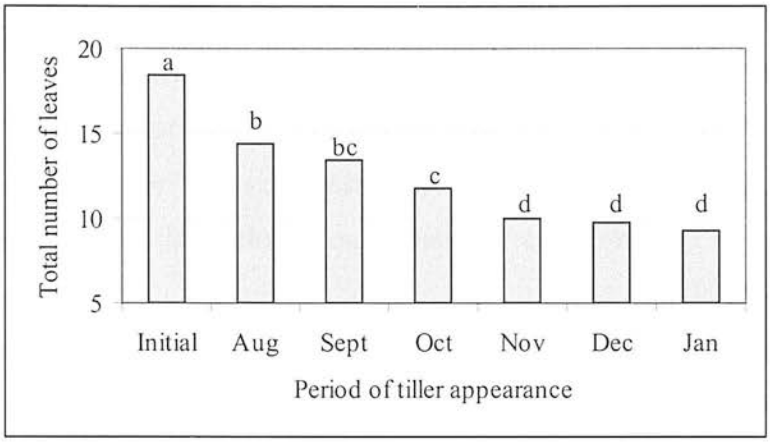

Figure 6.13. Average total number of leaves of flowering tillers according to the period in which they were born. Different letters refer to means significantly different (Tukey's test; interval of confidence of 95%). Initial are tillers born before July of 1998.

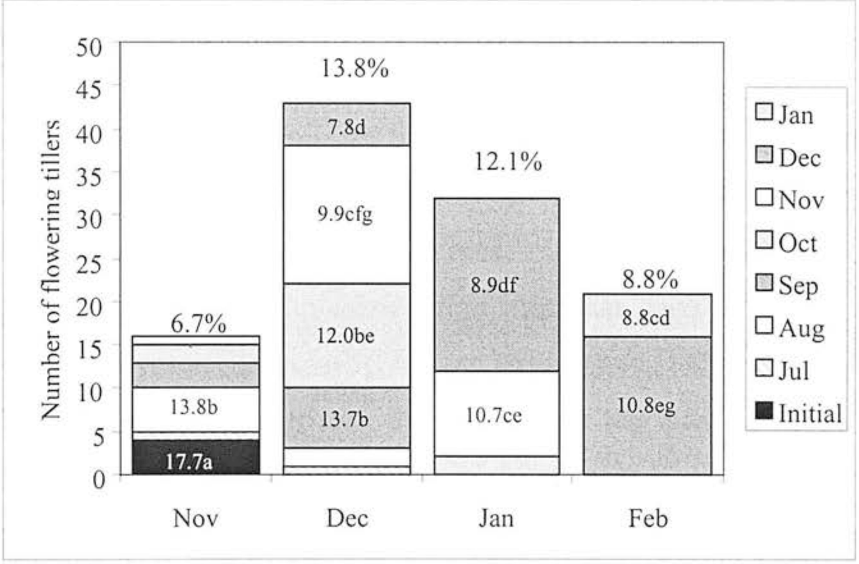

Figure 6.14. Number of flowering tillers per period, and according to the period in which they were born. Numbers in each segment are the mean number of leaves per tiller. Different letters after the numbers indicate significant different means (Tukey's test; 95% interval of confidence). Values above the columns are the percentage of reproductive tillers over the total number of tillers alive monitored. Initial refers to tillers born before July 1998.

Aerial tillers had fewer total number of leaves when flowering than basal tillers, either comparing the total populations of both types of tillers (9.3 vs. 11.4; $p<0.01$), or only those tillers born in December (8.7 vs. 10.0; $p<0.05$).

The number and proportion of flowering tillers over the total number of tillers in each period were highest during December and January (Figure 6.14). During November and December tillers flowering were more heterogeneous regarding to their age, while most of those flowering in January and February were born in the two previous periods.

Grazing intensity affected the proportion of tillers reaching the flowering stage ($p<0.05$). Low grazing intensities allowed a relatively large proportion of tillers (21%) to reach this stage, while medium and high stocking rates reduced markedly this proportion (9 and 8% respectively).

6.4 Discussion

The sward patch

In general, the pasture was dominated by signal grass. Only in soils with the lowest organic matter contents signal grass gave way to other species of the other broad-leaved group, mainly weeds. It was also in these plots where seedling regeneration was greatest, possibly due to the existence of abundant gaps in which light of a certain quality could reach the ground (Stur and Humphreys 1988).

The levels of soil organic matter were in all cases very low (range of 0.4-2.6%), but within that range signal grass density values were very sensible to them. Considering the high proportion of the sand fraction in these soils, the effects that organic matter had on their physical properties, such as keeping its structure, water stable aggregate and water holding capacity were probably more important than its contribution to the level of soil nutrients (Macedo, 1997). The texture of these soils makes them very susceptible to erosion, especially caused by wind, a major environmental problem of the region where the experiment was located.

Grazing management in these degraded soils should be aimed at recovering the herbaceous vegetation cover as soon as possible, in order to reduce wind erosion. It seems especially important to allow signal grass seedling regeneration and establishment, making the most of the negative correlation existing between seedlings density and the soil organic matter content. This would imply reducing or postponing grazing in the most degraded paddocks during the beginning of the wet season, until new plants are well established. Grazing should be resumed when possible, as the most important positive effect of livestock on soils is the supply of organic matter of good quality (Brock and Hay, 1993). Nevertheless, further studies on the competitive interactions between new signal grass plants and weed species and the effects of grazing on this balance would be required to define the best management approach.

Signal grass tiller densities found (750-1100 tillers m^{-2}) were much lower than those found in Nigeria (3400 tillers m^{-2}) in newly established, fertilised and ungrazed swards of an area with similar climate, and with similar standing herbage biomass content (Okeagu and Agishi, 1990). Another experiment in the same location (Okeagu *et al.,*1989), but with older pastures, higher fertilisation rates and regrowth periods, produced much lower tiller densities, average of 270 tillers m^{-2}, but the average standing herbage content was also much higher (11300 kg ha^{-1}). Similar tiller densities and herbage content to our study were found in established swards in Queensland, 20 days after cutting at ground level and removing the litter (Stur and Humphreys 1988), though the contribution of new seedlings was higher. The tiller densities observed in the permanent quadrats differed in some periods significantly from tiller densities in surrounding areas of the plots, especially in July 1999 (Figure 6.1a, and see chapter 4), though in both cases the range of values was similar. Similar differences between permanent and adjacent plots have been also observed in temperate pastures (Matthew *et al.,* 1989), and are usually attributed to the frequent handling of the permanent plots (Hay *et al.,* 2000).

The relationships found in the first winter between changes in the density of growing points of signal grass, legumes and other broad-leaved species, and their interactions with nitrogen, support the positive effect of legumes upon grass growth through nitrogen transfer, and the relative advantage of grasses over legumes when external sources of nitrogen are applied (Parsons and Chapman, 2000). These relationships were not found in subsequent periods. One explanation for it could come from the added effects of grazing, with the multiple possible actions on the permanent quadrats, like active defoliation selection for certain species, urine deposition (CIAT, 1982) or treading. These actions could have changed the balance between the species, but unfortunately they were not monitored in this experiment. The decrease in the number of growing points, other than signal grass, and especially of legumes from the second half of summer onwards, was also remarkable. Again, this pattern was not observed in the surrounding of the permanent quadrats (Figure 4.6b) and its causes are difficult to find without monitoring the morphogenesis of the legume species.

Signal grass tiller populations consisted predominantly of tillers appearing from basal nodes (basal tillers), though the proportion of tillers appearing from aerial nodes (aerial tillers) was important. Aerial tiller proportion increased linearly, from 0 to 40%, with increases in tiller density from 500 to 2000 tillers m^{-2}. Clavero (1997) also observed an increase in the proportion of aerial tillers with increasing number of tillers per plant in dwarf elephant grass (*Pennisetum purpureum* cv. Mott), both values positively related to cutting height. In our study the proportion of live leaf defoliated per plant affected positively the proportion of aerial tillers. This could seem to disagree with the results of Clavero (1997), but the patterns of defoliation were different. Cutting height was probably positively related with the number of remaining aerial nodes per tiller after the cut. In our study the proportion of live leaf defoliated per tiller did not affect the number of remaining aerial nodes per tiller, because the stem was usually cut only at its apical portion (see chapter 5). The appearance of an average of 1.4 aerial tillers per parental tiller explained the increase in tiller density with the appearance of aerial tillers.

Tiller appearance, persistence and death

Appearance and death of tillers followed a similar pattern throughout the year, except at the beginning of the experiment (mid winter), when tiller death rate was much higher than tiller birth rate (Figure 6.3). At this time swards were ungrazed, so these deaths were solely the result of environmental stress, especially of a prolonged dry period. In general, higher tillering and death rates occurred during summer and were lower during winter. The high tillering and death rate values found during summer in this study (around 0.020 tillers $tiller^{-1}d^{-1}$ for both rates), were slightly higher than those found in the temperate grasses *Agrostis stolonifera* and *Lolium perenne* (Bullock *et al.,* 1994a), and much higher than those of the tropical grass *Paspalum notatum* (Pakiding and Hirata, 1999).

During the first winter (August-September 1998) tillering was very high after the few sporadic rain events (Figure 6.4b). Nitrogen application at this time increased tillering rate by approximately 60%. Most of the new vegetative tillers born came from basal nodes, which indicates that axillary buds from basal nodes stayed alive during the dry season, and that readily available nitrogen in the soil enhanced their activation. The recovery of swards from basal axillary buds after dry periods has also been observed in other tropical grasses, even under longer dry seasons (Coates, 1997). The positive effect of nitrogen specifically upon site filling has also been reported for *Lolium perenne* (Van Loo *et al.,* 1992). The positive tillering response to nitrogen application during winter but not in summer could be explained by lower nitrogen mineralisation rates from the soil organic matter during winter, as temperatures were lower. Also the low leaf area index at this time implied that light reaching the ground was not a limiting factor for buds activation. The significant effect of LAR on tillering should be considered the other way around: new born tillers showed higher LAR, as seen from the negative relation between LAR and tiller size (Table 6.5). The high increase in the number of tillers per plant with nitrogen fertilisation at this time (Table 6.6) was mainly a consequence of the increase in tillering rate, as tiller deaths were not affected by nitrogen application.

From October onwards, tillering and tiller death rates were equally affected by the proportion of plant defoliated during grazing (Table 6.7). The significative effect of defoliation on tiller death was mainly caused by the decapitation of the apical meristems (chapter 5), which was mainly a function of the apical meristems' height, as has been also proved in tropical legumes (Clements, 1989). The longer life duration of tillers born in September, compared to those born in later months (Figure 6.7), can be explained by the lower internode elongation rates in September and October compared to those from November onwards (Figure 6.2b). This resulted in September tiller cohorts with shorter stems for the same age, and thus less accessible apical meristems to the grazers. In any case, the use of the apex height of a grass species as an indicator of resistance to grazing (Humphreys, 1991) is biased, as it only predicts tiller death rates (Clements, 1989). The tillering capacity of the plant after being defoliated should also be considered. The appearance of aerial tillers from living parental tillers (Figure 6.11) confirms the recent reassessment that tiller recruitment is not a direct consequence of the release of apical dominance (Murphy and Briske, 1992). Instead, it was observed that appearance of aerial tillers was more related to the excision or disappearance of the sheaths that covered the axilar tiller buds, thus changing their light environment and promoting their activation, as stated by Deregibus *et al.* (1985). Deinum *et al.* (1996) also observed a positive relation between tillering rate and light intensity in living seedlings of *Brachiaria brizantha* and *Panicum maximum* var. *trichoglume*.

Besides defoliation, during the summer months tiller death was also affected by the period, while tillering was not (Table 6.7). Tiller death rate was higher than tiller appearance rate during the second half of summer (Table 6.8), mainly by the higher death rate of basal tillers (Figure 6.9). There was not an apparent change in the causes of tiller death (Figure 6.10), or in the structure of basal tillers during that period. Plant fragmentation, active during this period (see chapter 4), could have enhanced tiller death rates, as tillers belonging to small plants are more susceptible to die (Brock *et.al.,* 1996; Coates, 1997).

During the second winter tiller death rates decreased to the levels observed in the previous winter, while tillering rates were lower than the average values of the previous winter. This was clearly reflected in that live leaf production per plant was mainly determined by live leaf production per existing tillers, and not by changes in tiller numbers, contrary to what occurred in the previous winter and summer (Table 6.10). The reason for the lower tillering rates in winter 99 was probably the light limitation at the basal nodes level, as canopies were more developed.

The rates of change in tiller numbers per plant in the winters was better defined by tiller birth rates, and similarly defined by tiller birth and death rates in the summer (Table 6.10). This suggests that when grazing does not occur (winters), the signal grass population is mainly regulated by tiller appearance, especially sensible to climatic and light conditions. Instead, during summer, defoliation affects similarly tiller birth and death rates, and its effect is more important than that of climate. Similar patterns were observed by Bullock *et al.* (1994a). They found that the effect of weather on tiller density of temperate grasses was more attributable to its effect on tiller birth rate than to its effect on tiller death rate. They also noticed that the intensity of summer grazing increased similarly tiller birth and death rates, with the overall result of not affecting tiller densities.

The high tiller birth and death rates observed in signal grass resulted in the lowest tiller lifespans and half-lives of grass species of similar studies (Korte 1986; McKenzie 1997; Pakiding and Hirata 1999). Tiller persistence was especially low during summer, when grazing frequency was higher. Aerial tillers showed very short lives, with few aerial tillers surviving the next regrowth period to that of their appearance. In spite of their high position in the canopy, death of aerial tillers was less directly related to grazing than death of basal tillers (Figure 6.10), as many of them did not even reach the next grazing event. These facts suggest that new aerial tillers were less supported by their parental tillers than new basal tillers, with the distance from the source of nutrients (i.e. roots, basal nodes) as a possible explanation for this weaker relation.

Flowering tillers

Signal grass tillers needed to develop a minimum number of leaves to reach flowering (Figure 6.13). This supports the use of the phyllochron as a tool to study ontogenetic development in grasses (Moore and Moser, 1995). However, the presence of tillers with much higher number of leaves before flowering suggests the existence of external factors controlling the activation of flower development in the apical meristem. The climatic requirements for floral initiation in signal grass are not yet well understood. It occurs during summer in tropical areas of the southern hemisphere (Stur, 1986) and has been inferred to be a quantitative long-day plant, flowering everywhere in the longer days of the year, and more vigorously at high than at low tropical latitudes (Hopkinson *et al.*, 1996). It is yet to know precisely if floral initiation is activated by the photoperiod or by other conditions like soil water availability or temperatures, with higher values during summer.

Leaf morphogenetic and structural characteristics

Leaf appearance rate was negatively affected by tiller age (Table 6.5), represented here by the tiller total number of leaves (TNL). If only the values for non-fertilised swards were considered, not including the first three periods with low Eap values, a significant ($p<0.001$; $R^2=0.32$) quadratic regression was obtained between LAR and TNL, for TNL values between 2 and 12. From TNL between 2 and 9 the relation between LAR and TNL was linear. Decreases of LAR with TNL have been also observed in other tropical grass species (Wilson, 1976; Gomide and Gomide, 1997). Successive increases in sheath length have explained partially increases in LAR with TNL in other grasses (Bonnett 1998; Duru and Ducrocq 2000a,b). This could be the case in our study, as sheath length did increase successively in new leaves until reaching a plateau at a TNL close to 9 (see Figure 4.12c; but considering the different leaf numbering).

The positive effect of defoliation on LAR (Table 6.7) has also been reported in other tropical grasses (Hirata, 2000). It could be related with sheath excisions, that automatically increases LAR, as the growing leaf had to elongate a shorter distance to appear, and the next sheaths would also be smaller (Casey *et al.*1999; Duru and Ducrocq 2000b). It could also be explained by a reduction in canopy leaf area, as light depletion has also been observed to reduce LAR (Skinner and Nelson 1994b).

In addition to previous sheath length, the other main factor regulating LAR is the leaf elongation rate (Duru and Ducrocq 2000b). This agrees with the high correlations found between LAR and LER for the whole experiment. The importance of leaf elongation and TNL on LAR depended on the magnitude of their values, which varied along the year. Thus, the contradictory LAR values observed in the winter periods with respect to LER values (lower LAR and higher LER values in the second winter compared to the first winter) could be explained by the higher effect that tiller structure (TNL) had on LAR in the second winter. LAR values in summer, period without soil water limitations, were in the order of those found in growth rooms for the same range of average temperatures (see table 3.1).

Leaf elongation rate was not directly affected by any structural characteristic of the tiller. The significative effects showed by some structural variables in some periods (Table 6.9) should rather be interpreted as the natural correlation existing between amount of live leaf per tiller and LER when, as it was the case, leaf senescence rate showed much less variation than LER. The independence of LER to intrinsic tiller characteristics has been observed in some grass species (Bultyink *et al.,* 1999; Duru and Ducrocq 2000a,b), and was previously observed under controlled environments (chapter 3), but it is not general for all grasses (Bultynck *et al.,* 1999).

Summer LER values were similar to those obtained in controlled environments for similar daily average temperatures (see Table 3.2). An important difference between these two experiments was that LER was measured at the leaf level in the controlled environments and at the tiller level in this experiment. If the occurrence of the short time gap without noticeable leaf elongation between the growth of consecutive leaves (chapter 3) applied also in the field, LER at the leaf level would be higher than that measured here at the tiller level. This would indicate that the low radiation levels in the controlled rooms compared to those experienced in the field (less than half the summer radiation levels), could have limited leaf elongation considerably, though other factors related to soil nutrients should also not be discarded.

LER responded slightly to nitrogen fertilisation in winter, but not during the summer period of higher growth. Mazzanti *et al.* (1994) observed also low responses of LER to nitrogen fertilization in *Festuca arundincea* under continuous grazing, suggesting a possible positive interaction between tiller size and nitrogen fertilization. This could have been the case in our study, as most of the tillers used in the calculation of LER in the summer periods corresponded to new ones.

Leaf senescence rate showed always much lower values than LER, especially during summer. The high positive effect of the amount of live leaf in the tiller on LSR (Tables 6.6, 6.7 and 6.9), points to the existence of a large proportion of young or severely defoliated tillers with none or very little mature leaf in the senescing stage. The effect that defoliation had on LSR during summer (Table 6.7) was only caused by the reduction in live leaf length, as could be seen when grazing intensity and tiller live leaf length were considered at the same time (Table 6.9). The rates of leaf senescence observed in other tropical grasses under similar field conditions to those of this study, indicate that a minimum of 40-60 days (wet season) have to pass before leaf senescence starts to increase significantly in new tillers (Wilson and t'Mannetje, 1978; McIvor, 1984). The short tiller lifespan observed in this study explains the low values of leaf senescence rate in living tillers.

From measured tillers, the balance between LER and LSR resulted in average green lamina lengths of 65-80% of the cumulative leaf elongation at the moment of introducing the cattle in the plots (end of regrowth period), with the lower percentages corresponding to winter periods and the higher to the summer periods. Real proportions of green lamina at the end of the regrowth periods were considerably lower because the LER and LSR measurements came from those tillers that kept active growth for the whole of the regrowth periods. In order to obtain the real proportion of green leaf lamina, tillers that died during the regrowth period (10-36% of the living tillers at the beginning of each period) should also be considered. Finally, the efficiency of defoliation (see table 5.2) and the grazing activity of the cattle, producing direct tiller deaths (19-31% of the tillers alive at the beginning of grazing) would provide the efficiency of utilisation (Parsons and Chapman, 2000) and the senescing rate at the sward patch level.

6.5 Conclusions

The productivity of the signal grass pastures analysed was the result of the integration of processes acting at different levels of sward organisation.

Soil degradation, measured through its organic matter content, affected the plant community structure of the pasture, with higher weed invasion the more degraded the soil was. Legumes and nitrogen at the start of the grow season acted positively upon signal grass tiller density, but interacted negatively between them.

Signal grass tiller density showed minimum values at the end of winter and maximum values in mid-summer. The overall year tiller density balance was not affected by the grazing intensity and nitrogen levels applied in this study. Increases in tiller density above a relatively fixed threshold could only come from increases in the participation of aerial tillers.

Signal grass tillers showed very short lifespans, which were mainly defined by the duration of the rotational cycles. Basal tillers in summer died mainly due to apex decapitation, while many aerial tiller deaths were not related to grazing.

High tiller death rates were normally counteracted by high tiller appearance rates, both related in a similar way to grazing, but also differently to climate and canopy structure characteristics.

Two consecutive periods were detected with imbalance between tiller deaths and births, and thus potentially producing changes in the population. The first period of high tiller deaths and no tiller births corresponded to mid winter, when soil water availability was at its minimum. The second period of high tiller births and very low tiller deaths occurred after the first rains, and with open canopy structures.

Leaf appearance rate could be explained by leaf elongation rate and by the total number of leaves in the tiller.

Leaf elongation rate was found independent of tiller structure, while leaf senescence rate was mainly dependent on the amount of live leaf per tiller.

Due to the high tiller turnover rates, characteristic of these swards, their efficiency of utilisation was equally dependent on leaf senescence produced by death tillers and that produced by living tillers.

Chapter 7 General discussion

7.1 Introduction

The previous chapters have analysed and discussed different structural and morphogenetic aspects of signal grass and their responses to different external variables. This chapter aims to integrate the results of the different studies performed in order to attain a global understanding of the population structure and dynamics of this grass species at its different hierarchical levels (section 7.2). The resulting population model will further allow the identification of the environments and management options capable of producing changes in the sward state that could affect its present and future productivity and persistence. In this way the pasture can be viewed as made of different homogeneous cohorts (of plants, tillers or phytomers) defined by their size or age. Each cohort is characterised by unique responses to the environment and pasture management, and this will be reflected in different contributions to the leaf tissue flow components of the sward (gross production, senescence and leaf harvested). Based on the results of the experiments performed, section 7.3 proposes a simple tiller cohort conceptual model and quantifies the participation of each cohort in the leaf tissue flow of the sward community. Finally, the advantages and limitations concerning the methodology and applicability of this research are discussed, and further solutions to overcome some of its problems are suggested (section 7.4).

7.2 Morphogenesis and structure of signal grass at different levels

Using the hierarchical model suggested in chapter 2 (Figure 2.1), the results obtained at each level of organisation from each of the experiments performed are summarised and discussed in this section. When comparing results from the destructive (chapter 4) and non-destructive (chapter 5 and 6) experiments, a consideration is made regarding the naming of the regrowth periods. Regrowth periods in the destructive experiment were named after the month when samples were taken (end of the regrowth period; Table 4.1). In the case of the non-destructive experiment regrowth periods were named usually after the month corresponding to the first date of measurement (start of the regrowth period; Table 6.1). Regrowth period names of Table 6.1 will be used when comparing results from both experiments. For example, values considered as of January according to Table 4.1, will be named as of December (Table 6.1) when using information from both the destructive and the non-destructive experiments.

7.2.1 The Phytomer

Regulation processes at the leaf level

The growth of a grass phytomer is a process regulated both intrinsically by the tiller and by external conditions. Morphogenetic processes at the phytomer level were mainly studied under controlled environments (see chapter 3). Recent research on phytomer growth has found that its intrinsic regulation can be explained by a coordination between the growth at a lower level of organisation, the cell, and the structure at a higher level of organisation, the tiller (Skinner and Nelson, 1994a; Gastal and Durand, 2000; Schnyder *et al.*, 2000). Although growth at the cell level was not considered in this study, the regulation of the phytomer growth was related to known cell morphogenetic processes. It is important to remark that grass cell growth studies refer to a very few number of temperate grass species, and that the universality of these processes to account for what occurs in tropical grasses is just starting to be tested (Nelson, 2000).

Figure 7.1 summarises the cell morphogenetic processes occurring during the first stage of phytomer growth characterised by active cell division. The integration of the three cell rates represented, cell division rate, cell expansion rate and number of cells elongating at the same time, results in the elongation rate at the higher scale level of the phytomer (LER). Once the phytomer reaches a size determined by the length of the previous sheath, a higher scale of regulation at the tiller level, cell division rate will stop and LER will be a function of the remaining two rates. It is at this time when the existing negative relationship between LER and leaf elongation duration (LED; see equation 3.3) is best noticed: for constant cell elongation durations, common under conditions without water limitations, LED will be negatively related to the number of cells elongating at the same time. The increase in LED with the size of the previous sheath (see equation 3.3) is explained by the longer duration of the initial cell division stage, which results in a higher number of cells produced. The different behaviour with the level of environmental factors (temperature, soil nitrogen level) of the three cell rate processes that define the LER can result in different relationships between LER and LED, represented by different slopes or intercepts in their regression equations. These changes were not observed in the experiment under controlled conditions, presumably because of their absorption by the effect of the previous sheath length.

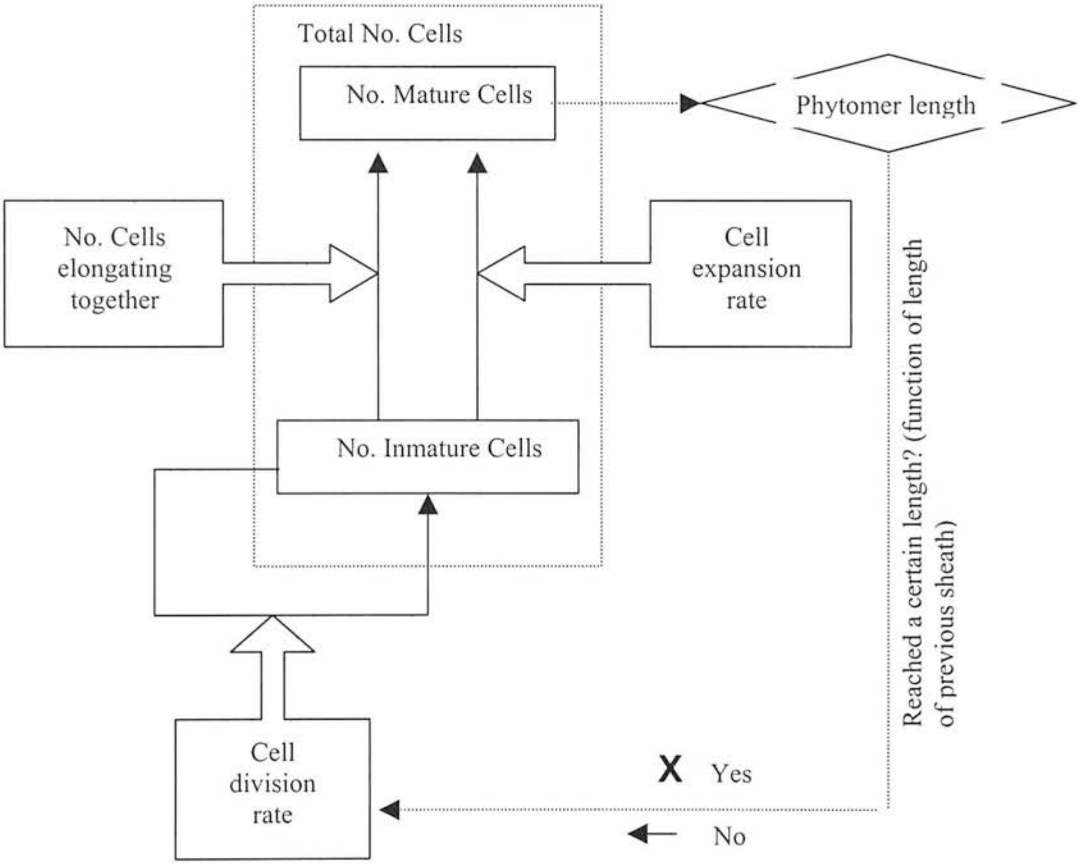

Figure 7.1. Schematic representation of initial phytomer growth according to cell morphogenetic characteristics.

The appearance of a new leaf, measured by the leaf appearance rate (LAR) was also partially regulated by intrinsic tiller mechanisms, following a documented relationship with LED (see equation 3.4 and figure 3.6). In any case, this relationship showed a degree of flexibility, even when the phyllochron (i.e. the inverse of LAR) was corrected to the estimated plastochron (i.e. the inverse of the interval between the initiation of successive leaves; see equation 3.5). The mechanisms explaining the variability in this relationship, which defines the number of leaves growing simultaneously, is not yet well understood (Nelson, 2000).

Blade length was mainly regulated intrinsically by the tiller structure, and only minor modifications, possibly linked with variations in cell expansion rates, were due to external environmental conditions. Internode elongation (IER) followed different patterns and had different effects on the growth of the other components of the phytomer than those observed in other tropical grasses (Cruz and Boval, 2000). Results obtained suggested the role of internode length in compensating for the previously established sheath length (see equation 3.9). A close association was also found between leaf and internode elongation rate values in the same phytomer, suggesting cell production rate as the common initial cause of this relationship. There is yet scarce information regarding the processes regulating internode growth, though its importance in canopy structure and light use efficiency and the repercussions in grazing behaviour and herbage quality are remarkable.

For some of the processes and regulations discussed, it is difficult to draw unquestionable conclusions without studying leaf growth at the cell level. The use of mechanistic simulation models considering the several possible sources of growth regulation in the different stages observed at the cell level (Durand *et al.*,1999) is a promising way to find out the mechanisms involved. In any case, the data obtained in the different experiments of this thesis would serve as a valuable source of validation information for this type of simulation models when considering signal grass growth.

Environmental and grazing effects

All morphogenetic variables of signal grass were dramatically restrained by low water availability in the soil during the first winter of the field experiment. The comparison of these values with those corresponding to the next winter, with lower temperatures but higher water availability, showed that LER and IER were more sensible to water stress than LAR (see figure 6.2b). Nevertheless, these three morphogenetic variables were highly correlated during all the year, with higher values during summer and lower in winter, which agreed with the isolated effects of temperature in the growth rooms (see table 3.1). Nitrogen fertilisation in the field only affected LER, but only after sporadic rain events at the end of the first winter (see table 6.6). It was suggested that at that time, when temperatures were lower, the low doses of nitrogen applied contributed significantly to growth due to the probably low soil mineralisation rates. The lack of response of other phytomer morphogenetic variables to nitrogen in the field was in accord to what occurred in the growth rooms (see table 3.1). During summer nitrogen application did not affect any of the morphogenetic variables, as at this time nitrogen coming from soil mineralisation was probably much larger than the amounts applied.

Leaf senescence rate (LSR) increased during the hot and humid summer period and showed minimum values during winter. The balance between LER and LSR, the rate of change in live leaf per tiller, was more affected by LER in summer and in the second winter, while similarly affected by LER and LSR during the first winter (see table 6.10), when LER values were lowest.

Defoliation affected all phytomer morphogenetic variables except LER (see table 6.7), though that effect was mainly the result of changing the size/age population structure of the sward. The more intense the defoliation was, the younger and smaller were the tillers of the sward, and thus the higher the LAR and the lower the IER and LSR. This was clearly reflected in the effects of grazing intensity on the phytomer structural variables blade, sheath and internode length (see table 4.2), and on tiller heights (see figure 5.7). The fact that grazing intensity did not affect LER during the duration of the field experiment indicates that sward changes in gross productivity due to defoliation intensity were not to be found at the phytomer level. The length of the resting periods was in all cases long enough to compensate for the negative short-term effects of defoliation on pasture growth (Richards, 1993).

7.2.2 The tiller

Signal grass tillers have very short lifespans and the persistence of their population relies on high rates of tiller appearance. This section synthesises the results of the previous chapters on structure of tillers along their morphological and ontogenetic development, and their morphogenetic characteristics tiller appearance and death rates.

Structure and development of signal grass tillers

Signal grass tillers show two distinctive morphological characteristics common to many other tropical grass species: the existence of long internodes at any stage of their development and, partly consequence of it, the existence of aerial tillers.

In signal grass only the first 2-3 phytomers of a tiller do not elongate their internodes, and the following do usually produce internodes of successive increasing length up to leaf numbers 7-8 (see figure 4.6). Depending on the density of basal tillers in the sward, on its standing biomass and on their position in the plant, signal grass tillers adopt a more or less erect growth form. The pastures used in this study produced erect tillers and the species had a caespitose growth form (see figure 4.2). Only in the case of tillers left uncut for long periods or, on the other extreme, when grazing is continuously very intense and with a noticeable proportion of bare ground, signal grass tillers adopt a more stoloniferous growth form. In these cases rooting at the nodes usually occurs when soil conditions are adequate. In this study stoloniferous growth and rooting at the nodes did not occur.

The other conspicuous morphological characteristic of signal grass is the relative abundance of aerial tillers. Aerial tillers are usually produced from living phytomers of basal tillers with excised leaves and nodes exposed to a convenient light radiation. Leaf defoliation was the main cause of their appearance. Natural leaf senescence, though finally producing a detachment of the leaf sheath from the stem, did not activate aerial nodes to produce new tillers. Death of the axilar bud along with the associated leaf senescence was the most probable cause of this tillering inhibition. Though the proportion of parental tillers dying before the appearance of daughter aerial tillers was very high, the existence of a significant proportion of living parental tillers (see figure 6.11) reaffirmed the invalidity of the apical dominance concept as a theory explaining tillering activation (Murphy and Briske, 1992). Nevertheless, decapitated basal tillers from grazing were the main source of new aerial tillers, especially as the pattern of tiller decapitation only involved the apical portion of the stem, leaving all mature aerial nodes intact (see chapter 5). Aerial tillers reached more than 20% of the total tiller population (see figures 4.10; 6.1a), but their lifespan was very short (see figure 6.6), with many of them dying in the same regrowth period in which they appeared (<30 days), mainly of causes not directly related to grazing (see figure 6.10). This fact is reflected in the low structural values shown by aerial tillers in comparison to basal tillers (see figure 4.11). Aerial tillers thus seemed to be scarcely supported by their plants, which partially could be explained by their small size during the periods of aerial tiller abundance (see figure 4.12).

On the other hand, tillers born from basal nodes (basal tillers) showed average stem lengths (see figure 4.11) similar to those characteristic of decapitated tillers (see table 5.3). In this way, tiller height readjusted to grazing intensity (see figure 5.7) as an avoidance mechanism (Briske, 1996) to tiller decapitation, in such a manner that the proportion of tillers decapitated was a constant fraction (31%) of the proportion of tillers defoliated (see chapter 5). The proportion of green leaf harvested per defoliated tiller was similar irrespective of grazing intensity (see table 5.2), and was defined by the height of the apical meristem (see table 5.4).

Flowering tillers started to appear in November, showed its peak in December and lasted until February. The proportion of tillers flowering was negatively affected by grazing intensity (see chapter 6). Tillers needed a minimum of 8 leaves to flower (see figures 6.13 and 6.14), indicating that some were only two months old when they flowered. The trigger activating flower initiation at the apical meristem was not clear, but considering a minimum of two months from tiller emergence to flowering (Stur and

Humphreys, 1987), it coincided with a nearly uninterrupted period with high water availability in the soil.

Tiller appearance and death

The longevity of axilar buds seemed to depend on their location. Axilar buds from basal nodes, corresponding to the oldest phytomers of basal tillers, showed long lifespans. This was inferred from the appearance of a large number of basal tillers at the end of the first winter (see figure 6.9) from parental basal tillers born in the previous wet season. Basal nodes were located at ground level, well protected by the soil from excessive desiccation and near reserve organs of the plant. On the other hand, axilar buds from aerial nodes were able to produce tillers only for a limited period and, if the associated leaves senesced, they usually did so due to the loss of protection from external conditions.

The appearance of tillers was initially controlled by water availability, as observed in the first winter of the field experiment (see figure 6.4). Very low nitrogen applications produced a higher rate of basal bud activation immediately after the occurrence of the first punctual rains (see table 6.6). Once water is not limiting, the next external factor that controls tiller appearance rate is light, as has already been described for the case of aerial tillers. Finally, limitations in the number of active buds per mature tiller was the factor controlling tillering rate when there were no water or light restrictions. Axilar bud longevity may be a function of the level of resources in the plant environment. During the dry period tillers restrained their growth but they probably prolonged the life of their axilar buds. On the contrary, during the season of plant active growth longevity of axilar buds seemed to be limited.

Death of tillers was classified as being caused directly by grazing or cutting, not caused directly by those factors but affecting vegetative tillers, or being a result of reaching the flowering stage. The majority of tillers dying as a direct effect of grazing corresponded to decapitated tillers (see chapter 5). In microswards tiller stem length was the main factor determining the survival of tillers after cutting (data not shown from experiment of chapter 3). While in basal vegetative tillers the proportions of deaths related directly or not to grazing were similar, in aerial tillers deaths not linked to grazing were more numerous (see figure 6.10). In the case of microswards, mortality along the regrowth period was similar for newly appeared tillers and tillers that were not decapitated in the previous cut. Under these controlled conditions, nitrogen had an important effect on reducing the death rate during the regrowth periods. In average, more than half the existing tillers in the microswards at the beginning of a regrowth period died (data not shown from experiment of chapter 3). Very high death proportions could also be observed under field conditions in summer, with tiller average half-lives of 24 days (see figure 6.7). During winter, tiller death rate decreased because of the longer resting periods, and was only high during the driest period at the start of the experiment (see figure 6.3).

Defoliation from grazing produced similar effects on tiller appearance and death rates (see table 6.7), indicating the capacity of signal grass to compensate at this level from the gradient of grazing damage tested. However, a seasonal effect on tiller death produced higher death rates than birth rates at the end of the summer, mainly of basal tillers (see figure 6.9) and probably related to the death of flowering tillers and the fragmentation of large plants (see chapter 4).

The relative importance of tiller turnover and leaf turnover in the efficiency of utilisation of the sward was highlighted in the discussion of chapter 6. Tiller turnover of signal grass under grazing is very high compared to other forage grass species. This implies that leaf senescence from dead tillers is an important component in the calculation of utilisation efficiencies and herbage tissues flows and pools at the sward level.

7.2.3 The plant

The structure of the plant was only explicitly analysed in the destructive experiment (see chapter 4). The results from this experiment will be considered along with the results on tiller appearance and death rates commented in the previous section.

The average size of plants was very sensitive to the season of the year. In this respect three main periods could be distinguished. The first period (September-November; names of periods according to table 6.1) corresponded with high vegetative growth after the dry season. The beginning of this period was characterised by the appearance of a large number of seedlings (see figure 4.12), but with lower importance than the appearance of new vegetative tillers (see chapter 6). Plant weight was usually better explained by the number of tillers per plant than by the average tiller weight, corresponding to higher importance of tillering than growth per tiller in defining growth per plant (see table 6.10). Plants reached high number of tillers and weights and had high proportion of live blade and increasing proportions of live stem in their composition (see figure 4.15).

The second period corresponded with the flowering season (December-March). The highest proportion of reproductive tillers (see figure 4.10), highest structural values for reproductive tillers (see figure 4.11), and highest inflorescence weight per plant (see figure 4.15) occurred in December (January in figures from chapter 4), but flowering was still very active in the following two months. During December the highest tillering rates were recorded (see table 6.8 and figure 6.9), but also the lowest proportions of young tillers per plant, especially in plants with reproductive tillers (see figure 4.14), underlining the importance of the low lifespan of new tillers at that time (see figure 6.7). The large size of plants with reproductive tillers and the steep decrease in size of plants without reproductive tillers during these months (see figure 4.13) suggests that plant fragmentation processes were very active and probably a consequence of the completion of flowering or of the decapitation of large tillers. Tillering was high but death rate was still higher during January and February (see table 6.8), preventing the increase in the number of tillers per plant.

The last period corresponded to the end of summer and of the flowering season. Plants did increase their weight but at the expense of increasing their proportions of dead material (see figure 4.15) and not of recruiting new tillers (see table 6.10).

Plant weight, organ composition and number of tillers per plant were not significantly affected by grazing intensity, though an effect on the amount of live leaf components per plant did probably exist, as it was the case at the tiller level. Grazing intensity affected the proportion of live leaf defoliated per plant, and this was mainly related to the proportion of tillers defoliated rather than to the proportion of live leaf defoliated per tiller (see table 5.2).

The plant level of organisation of the sward is apparently more stable than the tiller level. Plant mortality seemed to occur mainly in summer as a consequence of the widespread plant fragmentation processes linked to tiller ontogenetic changes. Plant appearance through seedling was restricted to the end of winter, when the first important rains occurred after a long dry season. The quantitative importance of seedling regeneration could have accounted for up to 11% of the tiller population at the beginning of summer (see chapter 4). It should be considered an important regeneration path especially in degraded pastures and in the drier areas of the potential distribution of signal grass.

7.2.4 The community and population

Signal grass was always the main species in the swards, with more than 75% of the existing growing points or herbage dry weight (see figure 6.1b and figure 4.15). Soil organic matter was a good indicator of pasture degradation as it was positively correlated with signal grass tiller density and negatively with weed species density (see chapter 6). Litter biomass was the largest component in weight of the sward (50-70% of total aboveground biomass), but it showed a tendency to decrease along the growing season (see figure 4.15).

The population of signal grass plants in the sward showed a skewed size distribution, with few plants of high weight or number of tillers and a large number of small plants. This type of distribution is characteristic of monospecific stands and was not seriously affected by season or grazing intensity.

Tiller density values were highest at the beginning of summer (see figure 4.10), but afterwards the trends observed were different in the permanent quadrats and outside them (see figure 6.1a). The frequent handling of tillers in the permanent quadrats was considered as the main reason for their higher density values. Tiller density in summer was positively related to the proportion of aerial tillers, indicating an upper threshold value for density of basal tillers (see chapter 6).

Tiller density was not affected by grazing intensity in any of the field experiments. Nitrogen did not affect tiller density during summer (see table 6.7), but it did so during the first winter, interacting with the small populations of legumes and weed species existing in the sward.

The vertical structure of the sward canopies showed a positively skewed distribution (see figure 5.5 and figure 5.6), similar to that observed for the sizes of signal grass plants. The sward vertical stratum with maximum green leaf biomass was related with the average height of the apical meristems.

Tiller density and tiller size did not hold any clear relationship of the -3/2 self-thinning type. However, a productivity index calculated according to the distance of tiller density-size co-ordinates of sward patches to an arbitrary -3/2 logarithmic line was very highly correlated with sward herbage biomass. Reversible tiller size - density changes with season were observed with reference to a unique sward productivity level. However, increasing grazing intensity produced a slight decrease in sward potential productivity, signalling a probable initial process of pasture degradation (see chapter 4). The use of tiller-size density values in pasture monitoring is considered to be a robust estimator of its productivity and degradation state, as integrates directly concepts of community and population ecology that separately give only partial and ambiguous sights of the real state of the community (Westoby, 1984).

7.3 Defining leaf tissue turnover and pasture utilisation using a conceptual demographic model

Leaf tissue turnover in grazed swards is a result of the processes of leaf growth, leaf senescence and leaf consumption by the grazers. The values of these processes can be calculated using the morphogenetic variables leaf elongation and senescence rate investigated in this study (chapters 3 and 6). Leaf consumption was also quantified in chapter 5. Leaf tissue turnover flows affect the leaf material pools green herbage, dead herbage and herbage consumed. The efficiency of pasture utilisation is defined as the amount of herbage consumed divided by the sum of green herbage and dead herbage produced in a time unit (Parsons and Chapman, 2000). The efficiency of utilisation is an important variable when analysing the sustainability of a grazing system (Rickert, 1996). This author defines the maximum sustainable stocking rate as the long-term stocking rate that does not cause pasture degradation, based on the critical level of forage utilisation. Thus, to diagnose pasture degradation and relate it to forage utilisation, there is also a need to choose a reliable pasture indicator of degradation from the structure of any of the levels of pasture organisation. This choice must be based on previous knowledge of the dynamics of the structural sward variables in order to define their plasticity and resilience both to grazing and to other environmental variables (Chapman and Lemaire, 1993). The productivity index (Matthew *et al.* 1995; see chapters 2 and 4) seems a promising indicator of pasture degradation, as it links biomass and tiller density values that compensate under normal pasture conditions. However, due to the structural heterogeneity of tropical pastures at the tiller level (chapter 4), this index needs further investigation under these circumstances, probably accounting for the different tiller types existing in the sward. In this section a demographic model considering different tiller cohorts according to their origin, age, size and ontogeny is briefly conceptualised. The objective of this model is to determine the behaviour of each of the tiller cohorts with reference to leaf tissue flow dynamics, and to investigate the tiller population flows among the different cohorts.

A conceptual demographic model

The tiller population of the sward would be organised in tiller cohorts according to their origin, age, occurrence of defoliation (size) and ontogeny. The time step considered would be flexible, depending on the interval between defoliations, as this is assumed to be the main factor regulating the sward structure.

According to their origin, tillers would be classified as basal and aerial. Their different position in the canopy, growth rates and, especially, their different longevity (see figure 6.6) justifies this division.

Tiller age cohorts would be defined by the average total number of leaves (or nodes) per tiller. The number of tiller age cohorts would depend on tiller longevities, defined by the tiller survivorship curves (see figure 6.7). As a result of the high tiller turnover rates of signal grass, three age cohorts would seem appropriate to characterise its tiller population age structure. After each time step surviving tillers of a cohort would move on to the next higher age cohort. Tillering rates, specified for each cohort, and the appearance of seedlings would define the pool of new tillers. In chapter 6 tillering was only associated with specific tiller types in the case of new aerial tillers (see figures 6.11 and 6.12). For new basal tillers considerations regarding the longevity of basal axilar buds could be grossly estimated.

Within tillers of the same age a further division between those defoliated and those not defoliated in the previous grazing event should be considered, as defoliation, along with age, defines the amount of life leaf existing in a tiller (see table 5.2). In this way, tiller size and age, regarded to be important factors in plant population dynamics (Watkinson, 1997), would be explicitly considered. The proportion of tillers defoliated from a cohort would be a function of the age of the tillers (height defined by their total number of leaves) and of the stocking rate (see table 5.2). Defoliation would act in order, starting with the cohorts of higher age and continuing in younger cohorts whenever all the tillers of the previous cohort had been defoliated. A limit on leaf defoliation would be imposed on a determined proportion of the cohort of new basal tillers, as very young tillers would always be out of the reach of the herbivores (see table 5.3). Initially there is no reason to consider more than one category of defoliated tillers, as the proportion of leaf defoliated per tiller is not significantly affected by grazing intensity (see table 5.2).

Finally, ontogeny would be only considered for old basal tillers, as flowering of aerial tillers is rare (see figure 6.10). The relationship between tiller leaf number and the development of inflorescence (see figure 6.13), coupled with the determination of the external conditions to trigger flower initiation at the tiller apex, would allow for the calculation of the probability of flowering in a vegetative cohort. Figure 7.2 shows a very simplified version of the model, with only new and old tillers, and not defining explicitly the origin of new tillers. Data from the experiments was used to assign tiller densities in each of the tiller cohorts for the different grazing intensities.

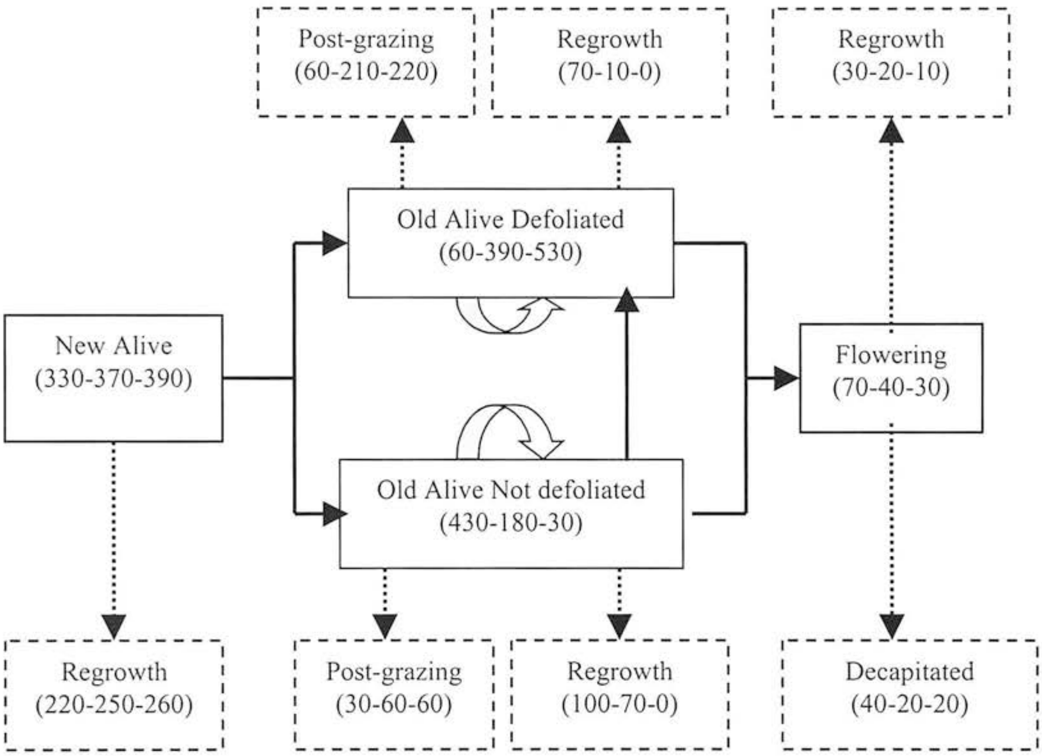

Figure 7.2. Tiller demography of signal grass swards distributed in different tiller cohorts according to age (new and old), ontogeny and size (defoliated and not defoliated) during one regrowth cycle in summer. Boxes with solid lines indicate cohorts of living tillers, while boxes with dashed lines correspond to dead tillers. Values between brackets are tiller densities (tillers/m^2) for increasing stocking rates (1,2,3 animal units/ha/yr).

Each of the arrows of figure 7.2 represents a tiller demographic flow that would be differently regulated by external and intrinsic plant factors. Dashed arrows indicate tiller death rates for each cohort. They consider two distinct periods within the time step of the model: the time just after grazing and the regrowth period until the next grazing would take place (i.e. beginning of next time step). These periods are linked with the main causes of tiller death.

Considering the number of tillers in each tiller cohort (figure 7.2), it is then possible to calculate the gross leaf production, the production of senescent leaf and, knowing the amount of green leaf consumed by the livestock, the efficiency of utilisation of the pasture (table 7.1).

Table 7.1. Efficiency of leaf utilisation of signal grass pastures for one rotational cycle (30 days) in summer, and three stocking rates. Leaf gross production, senescence and tiller densities (between brackets) of different tiller cohorts.

	1 AU/ha/yr	2 AU/ha/yr	3 AU/ha/yr
	Green leaf biomass at and after grazing (kg/ha)		
Herbage allowance	1380 (890)	1390 (980)	1290 (980)
Basal tillers	1110 (670)	1090 (700)	1000 (700)
Aerial tillers	190 (180)	250 (240)	260 (250)
Reproductive tillers	80 (70)	50 (40)	30 (30)
Green leaf consumed	270	690	770
Green leaf consumed/A.U.	11kg/AU/d	14 kg/AU/d	10 kg AU/d
Residual green leaf herbage	1110	700	520
	Gross leaf production in the period (kg/ha)		
Living new tillers	520	590	620
Living old tillers	890	970	940
Dead new tilllers	170	200	210
Dead old tillers	160	60	0
Total gross leaf production	1740	1820	1770
	Leaf senescence in the period (kg/ha)		
Old dead post-grazing	100	200	200
New dead regrowth	170	200	210
Old dead regrowth	330	120	0
New alive	160	180	190
Old alive	710	430	400
Total leaf senescence	1470	1130	1000
Efficiency of utilization	**16%**	**38%**	**43%**

Values obtained from results of experiments from chapters 3 to 6. For all grazing intensities tiller densities and standing green herbage biomass before grazing did not change from one rotational cycle to the next.

The results from figure 7.2 and table 7.1 should be considered as gross estimations. They were calculated from results obtained in the previous chapters on different aspects of the tiller population dynamics and the leaf tissue flows during one summer regrowth cycle. They are mostly intended for showing the structure of the model proposed but their results do not differ greatly from what was observed in the field.

The efficiency of pasture utilisation values obtained were lower than those considered to produce pasture degradation in tropical and subtropical areas (Rickert, 1996). Green leaf biomass consumed per animal unit per day showed values that were somewhat larger than those obtained from intake studies based on alkanes in the same area (M. Herrero, personal communication). Either an active defoliation selection by the cows for the tillers marked within the permanent quadrats, or a biased underestimation of green leaf biomass from leaf length values remaining in the tillers after grazing, could explain these high consumption values. In any case, it is seen that during summer efficiency of pasture utilisation was low, and this was reflected in the stability of tiller density and green herbage biomass values from one rotational cycle to the next.

Although the stocking rates tested did not produce any short-term sign of pasture degradation (e.g. sharp decreases in tiller density or herbage biomass), it is interesting to observe the changes with grazing intensity in the relative contribution of the different cohorts to the final leaf gross production (table 7.1). Cohorts of old tillers were most important when considering leaf tissue flows. The higher production of dead leaf at the low stocking rate was mainly a consequence of the higher death rates of old tillers during the regrowth period and the higher number of non defoliated living old tillers.

From the asymptotic trend in the amount of green leaf consumed with stocking rate, animals under the high stocking rates were probably starting to find difficulties in satisfying their nutritional requirements. At the high stocking rate the density of old non-defoliated living tillers was very low, indicating that higher stocking rates than those tested would result in cows starting to defoliate tillers from the new living tillers cohort. This would probably increase the inefficiency of the defoliation process from the point of view of animal productivity (i.e. decrease in bite sizes), but also could affect tiller regeneration. Defoliation of new tillers can increase their mortality significantly. The death of these tillers may not be balanced by the appearance of new ones, as axillary buds from young tillers may not be mature enough to produce new tillers. Higher stocking rates than those tested would have been needed to produce a clear evidence of pasture degradation, at least for the time scale used in the experiments. The results show that during summer tissue turnover was fast and more or less balanced at all the levels of the pasture organisation. It is in the dry periods of winter when grazing, even at low stocking rates, may produce a higher damage to the pasture. At this time tiller and leaf regeneration are stopped and grazing, apart from defoliating, may remove the protective environment that litter and attached dead leaves exert on axilar buds, thus endangering the tiller recruitment characteristic of the end of winter. The use of models considering different tiller classes is a promising tool to identify the different responses of the pasture to external disturbances. It is thought that many of the apparent contradictory responses of tropical pastures to similar grazing managements (see example in Fisher and Kerridge, (1996)) might be explained if the pasture is studied at this level of detail.

7.4 Advantages, limitations of the study and further proposed research

Field research in biological complex systems has been traditionally divided between those studies aimed at analysing the responses of organisms to their natural environment (descriptive), and those, hypothesis-driven, in which the environment is modified and the responses of the organisms to that modification are quantified. Experimental limitations of practical nature are still a major constraint defining the type and extent of information gathered on the organisms of concern in each of these research approaches. Descriptive studies, usually involving more environmental effects, but with less variance in the short term and many times with high correlations among them, tend also to consider a greater number of response variables on the organism behaviour, usually in a reductionist way (Sackvillle Hamilton and Harper, 1989). This approach has the advantage of integrating partial aspects of the dynamics of the organism that could explain its overall behaviour, and of identifying those aspects more sensible to the environmental conditions. Descriptive studies do usually suffer the drawback of being limited in the size of the sample analysed, as many of the measurements on the response variables are time consuming. Smaller samples produce larger standard errors, and the possibility of missing real effects or relationships is higher (Thomas and Laidlaw, 1993). On the other hand, hypothesis driven studies are focused on detecting the effects or relationships if they exist. Therefore they adjust the sample size according to the variance of the response variable, if that is known from previous experiments. The major drawback of this approach refers to the usual consideration of the environmental variable of interest isolated from other sources of variation, which are normally minimised by the design of the experiment. Such an approach clearly demonstrates the existence of an effect and estimates its magnitude under the conditions of the experiment. However, it does not estimate the relative importance of an effect, in the sense of what proportion of the total observed spatial and temporal variation in the field population is attributable to it (Sackville Hamilton and Harper, 1989).

The idea of this thesis was to perform both descriptive and hypothesis-driven experiments in order to study different complementary aspects of signal grass population dynamics. Some of the experiments were more focused on hypothesis testing (chapter 3), while others were more oriented towards the understanding of the behaviour of signal grass under realistic field and management conditions (chapters 4 to 6).

The effects of nitrogen and temperature were precisely studied keeping other external variables homogeneous (chapter 3), as these two factors were known to define dramatically the morphogenesis and structure of grasses (see chapter 2). Comparison of results from growth rooms with those from the field (chapters 5 and 6) suggested that in the growth rooms other environmental variables were limiting some of the responses of signal grass, but did not seem to affect the trend of those responses with changes in the level of the factors. Reversibly, these relative responses of the plant to the amount of resources available in controlled environments allowed a qualitative interpretation of the nature of the environmental or management effects acting on the plant under field conditions. For example, it was observed that leaf elongation rate in the field was higher in the second winter than in the first winter (see figure 6.2). However, according to the response of LER to temperatures under controlled conditions (see table 3.1), if other external factors were not limiting growth, LER should have been higher in the first winter, as temperatures were higher (see table 6.1). This comparison helped in identifying soil water availability as a limiting factor for leaf elongation during the first winter. Nevertheless, precautions have to be addressed in considering the results from controlled environments isolated from complementary field analyses, as the environment of the plants (e.g., light, soil) was very different to that found in normal field conditions.

The field experiments implied the use of destructive (chapter 4) and non-destructive (chapters 5 and 6) sampling techniques. This complementary approach permitted the analysis of nearly all the morphological levels of organisation of the pasture (Grant and Marriott, 1994). The destructive experiment was essential to understand the dynamics of the grass at the plant level, impossible to study with non-destructive techniques. It also served to compare some variables measured in the non-destructive experiment (chapter 6) and obtain information on the effects of frequent pasture handling in permanent plots. However, this experiment was very labour intensive, and this fact limited the amount of sampling to the minimum considered acceptable (Brock and Fletcher, 1993; Brock *et al.*, 1996). It is thought that this limited sampling was unable to detect many of the real differences in pasture structure caused by the gradient of grazing intensity applied, especially those of higher order like community, population and plant structures. The clear trend observed in the mean values of some of these variables for each of the grazing treatment levels ratifies this impression. An important conclusion drawn is that the greater heterogeneity in the structure of tropical pastures with respect to temperate ones makes sampling recommendations derived from studies on temperate swards need to be considered carefully, if significative effects of management are to be found in tropical pastures.

The non-destructive experiment (chapters 5 and 6) can be considered most similar to what was previously defined as a descriptive study, though effects such as grazing and nitrogen were also considered. The study of the appearance, growth, senescence and ontogeny of individual tillers using the plant as a sampling unit (i.e. group of physiological integrated tillers), could be considered as flawed because of the generalised process of seasonal plant fragmentation observed (see chapter 4). However, this approach is still considered valid as plant structural variables were not generally used to explain any of the response variables considered at the tiller level. In the cases they were used, the interpretation did not relate to direct effects of the plant on the tiller, but to the structure of the nearby canopy. Measuring tillers coming from the same "apparent" plant, as opposed to individual tillers separated among them (as in chapter 3), presented some advantages. Firstly, there was an assurance that whole plants were considered, regarding whether there were one or more than one. Further research evaluating in more detail the causes of plant fragmentation and a mapping of the relative position of tillers within plants could allow to predict visually and precisely the number of plants and the tillers belonging to each one in the field. Secondly, the appearance of new tillers could be measured accurately, and the quantification of the tillering rate could be done easily. Thirdly, the time employed in measuring was reduced, as less time per tiller was needed to locate them within the permanent plot. Finally, the effects of soil variables could be analysed precisely taking soil samples from under each of the selected plants at the end of the experiment. This would have been impractical if tillers were separated, as the number of soil samples would have been excessive. The disadvantage of this sampling approach relates mainly to the reduction in the number of spatial locations sampled for a given number of tillers. This, as in the case of the destructive experiment, could be important in the direct evaluation of grazing intensity on leaf defoliation (chapter 5), and could also produce more biased estimations of pasture structure, especially at the higher levels of organisation. Another disadvantage observed was a consequence of using a fixed sampling unit, the plant, to be followed in detail throughout the year. Labour limitations, and especially the initial determination of the sample size during the season of low growth, resulted in marked differences in the frequency of measuring intervals depending on the season of the year, as the sample sizes also changed markedly. This fact did not affect the validity of the measurements as, in the worst scenario, they were performed at least at the beginning and end of undisturbed growth periods (i.e. resting periods between grazing events). Nevertheless, it prevented the measurement of leaf morphogenetic characteristics at the leaf level, which could have only been performed using more frequent measurements.

A proposed improved methodology to the continuous plant and tiller monitoring performed would still consider the measurement of tillers grouped in plant units. However, more plants per plot would be measured, and measurements would be performed at different levels of intensity. Thus, fast measurements such as tiller appearance, death, number of total and green leaves per tiller and flowering occurrence could be taken at regular intervals of medium frequency (every 10-15 days). High frequency measurements on leaf characteristics (every 2-3 days), necessary for calculation of leaf morphogenetic variables, could be taken on selected tillers in each plant according to their stage of development, and for limited periods (e.g. during 30 days) at different seasons of the year. Low frequency measurements (every 1 to 6 months) would involve high order structural variables such as tiller density or vertical canopy structure.

As Hay *et al.* (2000) point out, once there is a good, not necessarily quantitative, understanding of the demography of a species, it is possible to make a better interpretation of some simple measurements in other experiments with the same species. This study has provided a qualitative comprehensive model of the population dynamics of signal grass. It has identified processes and states that enable the species to persist in the sward, but also situations in which the pasture persistence could be threatened. The relative short duration of the experiments and the limited scope of management and environmental conditions tested do not allow for accurate long-term predictions under different environmental and managerial circumstances. However, the research approach considered in this thesis will hopefully contribute to a better understanding of the demography and productivity of one of the most used forage grass species in the tropics. Thus, it could help in defining future research objectives and management strategies with the objective of producing more sustainable livestock farming systems.

References

Agnusdei, M.G., Mazzanti, A., Cordero, J., and Wade, M.H. 1997. Leaf appearance rate of native and introduced grasses under different intensities of continuous grazing (Argentina). *Proceedings of the XVIIIth International Grassland Congress.* Winnipeg-Saskatoon, Canada. pp 29.13-14.

Allard, G., Nelson, C.J. and Pallardy, S.G. 1991. Shade effects on growth of tall fescue: I. Leaf anatomy and dry matter partitioning. *Crop Science,* **31:** 163-167.

Alvim, M.J., Botrel, M. de A., Verneque, R.S. and Savati, J.A. 1990. Aplicacao de nitrogenio em accesos de *Brachiaria.* 1. Efeito sobre a producao de materia seca. *Pasturas Tropicales,* **12(2):** 2-6.

Andrew, M.H. and Mott, J.J. 1983. Annuals with transient seed banks: the population biology of indigenous *Sorghum* species of tropical north-west Australia. *Australian Journal of Ecology,* **8:** 265-276.

Anslow, R.C. 1966. The rate of appearance of leaves on tillers of the graminae. *Herbage Abstracts,* **3:** 150-155.

Arias, J. E., Dougherty, C.T., Bradley, N. W. Cornelius, P.L. and Lauriault, L.M. 1990. Structure of tall fescue swards and intake of grazing cattle. *Agronomy Journal,* **82:** 545-548.

Bahmani, I., Hazard, L., Varlet-Grancher, C., Betin, M., Lemaire, G., Matthew, C. and Thom, E.R. 2000. Differences in tillering of long- and short-leaved perennial ryegrass genetic lines under full light and shade treatments. *Crop Science,* **40:** 1095-1102.

Barthram, G.T. and Grant, S.A. 1984. Defoliation of ryegrass-dominated swards by sheep. *Grass and Forage Science,* **39:** 211-219.

Begg, J.E. and Wright, M.J. 1962. Growth and development of leaves from intercalary meristems of *Phalaris arundinacea. Nature* (London), **194:** 1097-1098.

Belanger, G. 1998. Morphogenetic characteristics of timothy grown with varying N nutrition. *Canadian Journal of Plant Science,* **78:** 103-108.

Bircham, J.S. and Hodgson, J. 1983. The influence of sward conditions on rates of herbage growth and senescence in mixed swards under continuous grazing management. *Grass and Forage Science,* **38:** 323-331.

Black, J.L. and Kenney, P.A. 1984. Factors affecting diet selection by sheep. II Height and density of pasture. *Australian Journal of Agricultural Research*, **35:** 565-578.

Boddey, R.M., Rao, I.M. and Thomas, R.J. 1996. Nutrient cycling and environmental impact of *Brachiaria* pastures. In: *Brachiaria: Biology, Agronomy and Improvement.* J.W. Miles, B.L. Maass and C.B. do Valle (eds.). Chapter 5. CIAT – EMBRAPA. Cali, Colombia. pp. 72-86.

Bonnett, G.D. 1998. Rate of leaf appearance in sugarcane, including a comparison of a range of varieties. *Australian Journal of Plant Physiology,* **25:** 829-834.

Boval, M., Cruz, P., Peyraud, J.L. and Penning, P.D. 1999. The effect of herbage allowance on daily intake by Creole heifers tethered on natural *Dichanthium* spp. pasture. *Grass and Forage Science,* **55:** 201-208.

Briske, D.D. 1989. Vegetation dynamics in grazed systems: a hierarchical perspective. *Proceedings of the XVIth International Grassland Congress.* Nice, France. pp. 1829-1833.

Briske, D.D. 1996. Strategies of plant survival in grazed systems: a functional interpretation. In: *The ecology and management of grazing systems.* J. Hodgson and A.W. Illius (eds.). Chapter 2. CAB International. Wallingford, Oxon, UK. pp. 37-67.

Briske, D.D. and Silvertown, J.W. 1993. Plant demography and grassland community balance: the contribution of population regulation mechanisms. In: *Grasslands for our world.* M.J. Baker (ed.). SIR Publishing, Wellington. New Zealand. pp. 105-111.

Brock, J.L., Hay, M.J.M., Thomas, V.J. and Sedcole, R.J. 1988. Morphology of white clover (*Trifolium repens* L.) plants in pastures under intensive sheep grazing. *Journal of Agricultural Science, Cambridge,* **111:** 273-283.

Brock, J.L. and Fletcher, R.H. 1993. Morphology of perennial ryegrass (*Lolium perenne*) plants in pastures under intensive sheep grazing. *Journal of Agricultural Science, Cambridge,* **120:** 301-310.

Brock, J.L. and Hay, R.J.M. 1993. An ecological approach to forage management. In: *Grasslands for our world.* M.J. Baker (ed.). SIR Publishing. Wellington, New Zealand, pp. 55-64.

Brock, J.L., Hume, D.E. and Fletcher, R.H. 1996. Seasonal variation in the morphology of perennial ryegrass (*Lolium perenne*) and cocksfoot (*Dactylis glomerata*) plants and populations in pastures under intensive sheep grazing. *Journal of Agricultural Science, Cambridge,* **126:** 37-51.

Bullock, J.M., Clear Hill, B. and Silvertown, J. 1994a. Tiller dynamics of two grasses - responses to grazing, density and weather. *Journal of Ecology,* **82:** 331-340.

Bullock, J.M., Mortimer, A.M. and Begon, M. 1994b. Physiological integration among tillers of *Holcus lanatus*: age dependence and responses to clipping and competition. *New Phytologist,* **128:** 737-747.

Bultynck, L., Fiorani, F. and Lambers, H. 1999. Control of leaf growth and its role in determining variation in plant growth rate from an ecological perspective. *Plant Biology,* **1:** 13-18.

Carriere, M. 1989. Les communautes vegetales saheliennes en Mauritanie (region de Kaedi). Analyse de la reconstitution annuelle du couvert herbace. These de Doctorat. Université de Paris-Sud. 238 p.

Casal, J.J., Sánchez, R.A. and Deregibus, V.A. 1987. The effect of light quality on shoot extension growth in three species of grasses. *Annals of Botany,* **59:** 1-7.

Casey, I.A., Brereton, A.J., Laidlaw, A.S. and McGilloway, D.A. 1999. Effects of sheath tube length on leaf development in perennial ryegrass (*Lolium perenne* L.). *Annals of Applied Biology,* **134:** 251-257.

Casler, M.D. 1998. Breeding cool-season grasses. In: *Grass for dairy cattle.* J.H Cherney and D.J.R. Cherney (eds). CABI Publishing, Wallingford, UK. pp. 23-48.

Chacón, E. and Stobbs, T.H. 1976. Influence of progressive defoliation of a grass sward on the eating behaviour of cattle. *Australian Journal of Agricultural Research,* **27:** 709-727.

Chapman, D.F and Lemaire, G. 1993. Morphogenetic and structural determinants of plant regrowth after defoliation. In: *Grasslands for our world.* M.J. Baker (ed.). SIR Publishing, Wellington. New Zealand. pp 93-104.

CIAT 1982. Animal excreta as nutrient pools. In: *Tropical pastures program annual report 1981.* Centro Internacional de Agricultura Tropical, Cali, Colombia. pp. 186-191.

CIAT 1999. Project IP-5. Tropical grasses and legumes: optimising genetic diversity for multipurpose use. *Annual report 1999.* Centro de Investigacion Agricola Tropical. Cali, Colombia.

Clavero, T. 1997. Tiller dynamic of dwarf elephant grass (*Pennisetum purpureum* cv. *Mott*) uder defoliation. *Proceedings of the XVIII International Grassland Congress.* Winnipeg-Saskatoon. Canada. pp22: 31-32.

Clements, R.J. 1989. Rates of destruction of growing points of pasture legumes by grazing cattle. *Proceedings. of the XVIth. International Grassland Congress.* Nice, France. pp. 1027-1028.

Coates, D.B. 1997. The recovery of Nixon Sabi grass pastures following severe drought. *Tropical Grasslands,* **31:** 67-72.

Colvill, K.E. and Marshall, C. 1984. Tiller dynamics and assimilate partitioning in *Lolium perenne* with particular reference to flowering. *Annals of Applied Biology,* **104:** 543-557.

Cowan, R.T., Davison, T.M. and Shephard, R.K. 1986. Observations on the diet selected by Friesian cows grazing tropical grass and grass-legume pastures. *Tropical Grasslands*, **20:** 183-191.

Cowan, R.T. and Lowe, K.F. 1998. Tropical and subtropical grass management and quality. In: *Grass for dairy cattle.* Cherney J.H. and Cherney D.J.R. (eds.). CABI Publishing, Wallingford, UK. pp. 101-136.

Crawley, M.J. 1997. The structure of plant communities. In: *Plant Ecology.* M.J. Crawley (ed.). Chapter 14. Blackwell Science. Oxford, UK. pp. 475-531.

Cruz, P. and Boval, M. 2000. Effect of nitrogen on some morphogenetic traits of temperate and tropical perennial forage grasses. In: *Grassland Ecophysiology and Grazing Ecology.* G. Lemaire, J. Hodgson, A. de Moraes, P.C. de F. Carvalho and C. Nabinger (eds.). Chapter 8. CABI Publishing, Wallingford, Oxon, UK. pp. 151-168.

Curll, M.L. and Wilkins, R.J. 1982. Frequency and severity of defoliation of grass and clover by sheep at different stocking rates. *Grass and Forage Science,* **37:** 291-297.

Davies, A. 1974. Leaf tissue remaining after cutting and regrowth in perennial ryegrass. *Journal of Agricultural Science (Cambridge),* **82:** 165-172.

Davies, A. 1988. The regrowth of grass swards. In: *The Grass Crop. The Physiological Basis of Production..* M.B. Jones & A. Lazenby (eds.) Chapman & Hall. London. pp. 85-127.

Davies, A. 1993. Tissue turnover in the sward. In: *Sward measurement handbook.* A. Davies, R.D. Baker, S.A. Grant and A.S. Laidlaw (eds.). British Grassland Society. pp. 183-216.

Davies, A., Evans, M.E., and Exley, J.K. 1983. Regrowth of perennial ryegrass as affected by simulated leaf sheaths. *Journal of Agricultural Science (Cambridge),* **101:** 131-137.

Davis, M.H. and Simmons, S.R. 1994. Far-red light reflected from neighboring vegetation promotes shoot elongation and accelerates flowering in spring barley plants. *Plant, Cell and Environment,* **17:** 829-836.

Deinum, B., Sulastri, R.D., Zeinab, M.H.J. and Maasen, A., 1996. Effects of light intensity on growth, anatomy and forage quality of two tropical grasses (*Brachiaria brizantha* and *Panicum maximum* var. *trichoglume*). *Netherlands Journal of Agricultural Science,* **44:** 111-124.

Deregibus, V.A., Sánchez, R.A. and Casal, J.J. 1983. Effects of light quality on tiller production in *Lolium* sp. *Plant Physiology,* **72:** 900-912.

Deregibus, V.A., Sánchez, R.A., Casal, J.J. and Trlica, M.J. 1985. Tillering responses to enrichment of red light beneath the canopy in a humid natural grassland. *Journal of Applied Ecology,* **22:** 199-206.

Derner, J.D. and Briske, D.D. 1998. An isotopic (^{15}N) assessment of intraclonal regulation in C_4 perennial grasses: ramet interdependence, independence or both? *Journal of Ecology,* **86:** 305-314.

Derner, J.D. and Briske, D.D. 1999. Intraclonal regulation in a perennial caespitose grass: a field evaluation of above- and below-ground resource availability. *Journal of Ecology,* **87:** 737-747.

Dougherty, C.T., Bradley, M.W., Cornelius, P.L and Lauriault, L.M. 1992. Allowance-intake relations of cattle grazing vegetative tall fescue. *Grass and Forage Science*, **47:** 211-219.

Durand, J.L., Schauffele, R. and Gastal, F. 1999. Grass leaf elongation rate as a function of developmental stage and temperature: a morphological analysis and modelling. *Annals of Botany,* **83:** 577-588.

Duru, M. and Ducrocq, H. 2000a. Growth and senescence of the successive grass leaves on a tiller. Ontogenetic development and effect of temperature. *Annals of Botany,* **85:** 635-643.

Duru, M. and Ducrocq, H. 2000b. Growth and senescence of the successive grass leaves on a cocksfoot tiller. Effect of nitrogen and cutting regime. *Annals of Botany,* **85:** 645-653.

Ferragine, M.D.C., Monteiro, F.A. and Da Silva, S.C. 2001. Leaf appearance rate in *Brachiaria decumbens* grown in nitrogen and potasium rates. In: *Proceedings of the XIX International Grassland Congress*. Sao Paulo. Brazil. pp. 69-70.

Fisher, M.J. and Kerridge P.C. 1996. The agronomy and physiology of *Brachiaria* species. In: *Brachiaria: Biology, Agronomy and Improvement.* J.W. Miles, B.L. Maass and C.B. do Valle (eds.). Chapter 3. CIAT – EMBRAPA. Cali, Colombia. pp. 43-52.

Fisher, M.J., Rao, I.M., Thomas, R.J. and Lascano, C.E. 1996. Grasslands in the well-watered tropical lowlands. In: *The ecology and management of grazing systems.* J. Hodgson and A.W. Illius (eds.). Chapter 14. CAB International. Wallingford, Oxon, UK. pp. 393-425.

Flores, E.R., Laca, E.A., Griggs, T.C. and Demment, M.W. 1993. Sward height and vertical morphological differentiation determine cattle bite dimensions. *Agronomy Journal*, **85:** 527-532.

Fulkerson, W. J. and Slack, K. 1994. Leaf number as a criterion for determining defoliation time for *Lolium perenne.* 1. Effect of water-soluble carbohydrates and senescence. *Grass and Forage Science*, **49:** 373-77.

Gastal, F., Belanger, G. and Lemaire, G. 1992. A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. *Annals of Botany,* **70:** 437-442.

Gastal, F. and Nelson, C.J. 1994. Nitrogen use within the growing leaf blade of tall fescue. *Plant Physiology,* **105:** 191-197.

Gastal, F. and Durand, J.L. 2000. Effects of nitrogen and water supply on N and C fluxes and partitioning in defoliated swards. In: *Grassland Ecophysiology and Grazing Ecology.* G. Lemaire, J. Hodgson, A. de Moraes, P.C. de F. Carvalho and C. Nabinger (eds.). Chapter 2. CABI Publishing, Wallingford, Oxon, UK. Pp. 15-39.

Gautier, H., Varlet Grancher, C., and Baudry, N. 1998. Comparison of horizontal spread of white clover (*Trifolium repens* L.) grown under two artificial light sources differing in their content of blue light. *Annals of Botany (London),* **82:** 41-48.

Genstat 5 Committee 1989. Genstat 5 Reference Manual. Clarendon Press, Oxford, UK.

Giraldo, L.M., Lizcano, L.J., Gijsman, A.J., Rivera, B. and Franco, L.H. 1998. Adaptación del modelo DSSAT para simular la producción de *Brachiaria decumbens. Pasturas Tropicales,* **20(2):** 2-12.

Gomide, J.A. 1989. Morphological and physiological growth aspects of three tropical grasses. *Proceedings of the XVIth International Grassland Congress*, Nice, France. pp. 1055-1056.

Gomide, A. and Gomide, C.A.M. 1997. Morphogenesis and growth analysis of *Panicum maximum* cultivars. *Proceedings of the XVIIIth International Grassland Congress.* Winnipeg-Saskatoon, Canada. pp. 7, 65-66.

Grant, S.A., Barthram, G.T. and Torvell, L. 1981. Components of regrowth in grazed and cut *Lolium perenne* swards. *Grass and Forage Science*, **36:** 155-168.

Grant, S.A., Barthram, G.T., Torvell, L., King, J. and Smith, H.K. (1983). Sward management, lamina turnover and tiller population density in continuously stocked *Lolium perene* dominated swards. *Grass and Forage Science,* **38:** 333-344.

Grant, S.A. and Marriott, C.A. 1994. Detailed studies of grazed swards - techniques and conclusions. *Journal of Agricultural Science, Cambridge,* **122:** 1-6.

Gutteridge, R.C. 1985. Survival and regeneration of four legumes oversown into native grasslands in northeast Thailand. *Journal of Applied Ecology,* **22:** 885-894.

Hacker, J.B. and Jank, L. 1998. Breeding tropical and subtropical grasses. In: *Grass for dairy cattle.* J.H. Cherney and D.J.R. Cherney (eds.). CABI Publishing, Wallingford, UK. pp. 49-72.

Harper, J.L. 1977. *Population Biology of Plants*. Academic Press. London, UK.

Harper, J.L. 1978. Plant relations in pastures. In: *Plant Relations in Pastures.* J.R. Wilson (ed.). Chapter 1. CSIRO. East Melbourne, Australia. pp. 3-15.

Hay, M.J.M., Jones, R.M and Orr, D.M. 2000. Plant population dynamics in grasslands. In: *Field and Laboratory Methods for Grassland and Animal Production Research.* L. t'Mannetje and R.M. Jones (eds.). Wallingford, Oxon, UK: CAB International. pp. 123-149.

Hendon, B.C. and Briske, D.D. 1997. Demographic evaluation of a herbivory-sensitive perennial bunchgrass: does it possess an Achilles heel?. *Oikos,* **80:** 8-17.

Hendricksen, R. and Minson, D.J. 1980. The feed intake and grazing behaviour of cattle grazing a crop of *Lablab purpureus*. *Journal of Agricultural Science, Cambridge,* **95:** 547-554.

Hendricksen, R. and Minson, D.J. 1985. Growth, canopy structure and chemical composition of *Lablab purpureus* cv. Rongai at Samford, S.E. Queensland. *Tropical Grasslands,* **19:** 81-87.

Hendrickson, J.R. and Briske, D.D. 1997. Axillary bud banks of two semiarid perennial grasses: occurrence, longevity, and contribution to population persistence. *Oecologia,* **110:** 584-591.

Hernández Garay, A., Matthew, C. and Hodgson, J. 1999. Tiller size/density compensation in perennial ryegrass miniature swards subject to differing defoliation heigths and a proposed productivity index. *Grass and Forage Science,* **54:** 347-356.

Herrero, M., Dent, J.B. and Fawcett, R.H. 1998. The plant/animal interface in models of grazing systems. In: *Agricultural Systems: Modelling and Simulation.* R.M. Peart and R.B. Curry (eds.). Marcel Dekker Inc. New York. pp. 495-542.

Herrero, M., Fawcett, R.H., Silveira, V., Busqué, J., Bernués, A. and Dent, J.B. 2000. Modelling the growth and utilisation of kikuyu grass (*Pennisetum clandestinum*) under grazing 1. Model definition and parameterisation. *Agricultural Systems*, **65:** 73-97.

Hirata, M. 2000. Effects of nitrogen fertiliser and cutting height on leaf appearance and extension in bahia grass (*Paspalum notatum*) swards. *Tropical Grasslands,* **34:** 7-13.

Hodgson, J. 1985a. The significance of sward characteristics in the management of temperate sown pastures. In: *Proceedings of the XVth International Grassland Congress.* Japan. pp. 31-34.

Hodgson, J. 1985b. The control of herbage intake in the grazing ruminant. *Proceedings of the Nutrition Society*, **44:** 339-346.

Hodgson, J. 1990. *Grazing management. Science into practice.* Longman. Harlow, UK.

Hodgson, J., Cosgrove, G.P. and Woodward, S.J.R. 1997. Research on foraging behaviour: progress and priorities. *Proceedings of the XVIIIth International Grassland Congress,* Winnipeg-Saskatoon, Canada. pp 109-118.

Hopkinson, J.M., de Souza, F.H.D., Diulgheroff, S., Ortiz, A. and Sánchez, M. 1996. Reproductive physiology, seed production and seed quality of *Brachiaria.* In: *Brachiaria: Biology, Agronomy and Improvement.* J.W. Miles, B.L. Maass and C.B. do Valle (eds.). Chapter 8. CIAT – EMBRAPA. Cali, Colombia. pp. 124-140.

Humphreys L. R. 1991. *Tropical pasture utilisation.* Cambridge University Press. Cambridge, UK.

Hutchings, M.J. 1997. The structure of plant populations. In: *Plant ecology.* M.J. Crawley (ed.). Chapter 11. Blackwell Science. Oxford. UK. pp 325-358.

Illius, A.W., Gordon, I.J., Milne, J.D. and Wright, W. 1995. Costs and benefits of foraging on grasses varying in canopy structure and resistance to defoliation. *Functional Ecology*, **8:** 894-903.

Ivory, D.A. and Whiteman, P.C. 1978. Effect of temperature on growth of five subtropical grasses. I. Effect of day and night temperature on growth and morphological development. *Australian Journal of Plant Physiology,* **5:** 131-148.

Jones, M.B. 1988. Water relations. In: *The Grass Crop. The Physiological Basis of Production.* M.B. Jones and A. Lazenby (eds.). Chapman & Hall. London. pp. 205-242.

Jones, R.J. and Jones, R.M. 1997. Grazing management in the tropics. In: *Proceedings of the XVIIIth International Grassland Congress.* Winnipeg-Saskatoon, Canada. pp. 535-542.

Keller-Grein, G., Maass, B.L. and Hanson, J. 1996. Natural variation in *Brachiaria* and existing germplasm collections. In: *Brachiaria: Biology, Agronomy and Improvement.* J.W. Miles, B.L. Maass and C.B. do Valle (eds.). Chapter 2. CIAT – EMBRAPA. Cali, Colombia. pp. 16-35.

Korte, C.J. 1986. Tillering in 'Grasslands Nui' perennial ryegrass swards. 2. Seasonal pattern of tillering and age of flowering tillers with two mowing frequencies. *New Zealand Journal of Agricultural Research,* **29:** 629-638.

Laca, E.A. and Lemaire, G. 2000. Measuring sward structure. In: *Field and laboratory methods for grassland and animal production research.* L. t'Mannetje and R.M. Jones (eds.). Chapter 5. CAB International. Wallingford, Oxon, UK. pp. 103-121.

Larcher, W. 1995. *Physiological Plant Ecology*. Springer. Berlin, Germany.

Lascano, C.E. 1991. Managing the grazing resource for animal production in savannas of tropical America. *Tropical Grasslands,* **25:** 66-72.

Lemaire, G. and Chapman, D. 1996. Tissue flows in grazed plant communities. In: *The ecology and management of grazing systems.* J. Hodgson and A.W. Illius (eds.). Chapter 1. CAB International. Wallingford, Oxon, UK. pp. 3-36.

Lemaire, G. and Gastal, F. 1997. N uptake and distribution in plant canopies. In: *Diagnosis of N Status in Crops.* G. Lemaire (ed.). Advanced Series in Agricultural Sciences. Springer-Verlag, Berlin, pp. 3-43.

Lemaire, G. and Millard, P. 1999. An ecophysiological approach to modelling resource fluxes in competing plants. *Journal of Experimental Botany,* **50:** 15-28.

Lemaire, G. and Agnusdei, M. 2000. Leaf tissue turnover and efficiency of herbage utilization. In: *Grassland Ecophysiology and Grazing Ecology.* G. Lemaire, J. Hodgson, A. de Moraes, P.C. de F. Carvalho and C. Nabinger (eds.). Chapter 14. CABI Publishing, Wallingford, Oxon, UK. pp. 265-287.

Lemaire, G. 2001. Ecophysiology of grasslands: dynamic aspects of forage plant populations in grazed swards. *Proceedings of the XIX International Grassland Congress.* Sao Pedro, Brazil. pp 1-19.

L'Huillier, P.J., Poppi, D. P. and Fraser, T.J. 1984. Influence of leaf green distribution on diet selection by sheep and the implications for animal performance. *Proceedings of the New Zealand Society of Animal Production,* **44:** 105-107.

Lonsdale, W.M. and Watkinson, A.R. 1983. Plant geometry and self thinning. *Journal of Ecology* **71:** 285-297.

Ludlow, M.M. and Charles-Edwards, D.A. 1980. Analysis of the regrowth of a tropical grass/legume sward subjected to different frequencies and intensities of defoliation. *Australian Journal of Agricultural Research,* **31:** 673-692.

Ludlow, M.M., Stobbs, T.H., Davis, R. and Charles-Edwards, D.A. 1982. Effect of sward structure of two tropical grasses with contrasting canopies on light distribution, net photosynthesis and size of bite harvested by grazing cattle. *Australian Journal of Agricultural Research,* **33:** 187-201.

Macedo, M.C.M. 1997. Sustainability of pasture production in the savannas of tropical America. *Proceedings of the XVIIIth International Grassland Congress.* Winnipeg-Saskatoon, Canada. pp. 391-400.

Mannetje, L.'t and Ebersohn, J.P. 1980. Relations between sward characteristics and animal production. *Tropical Grasslands,* **14:** 273-287.

Matthew, C., Xia, J.X., Hodgson, J. and Chu, A.C.P. 1989. Effect of late spring grazing management on tiller age profiles and summer-autumn pasture growth rates in a perennial ryegrass (*Lolium perenne* L.) sward. *Proceedings of the XVI International Grassland Congress.* Nice. France. pp 521-522.

Matthew, C., Chu, A.C.P., Hodgson, J. and Mackay, A.D. 1991. Early summer pasture control: what suits the plant? *Proceedings of the New Zealand Grassland Association,* **53:** 73-77.

Matthew, C., Lemaire, G., Sackville-Hamilton, N.R. and Hernández Garay, A. 1995. A modified self-thinning equation to describe size/density relationships for defoliated swards. *Annals of Botany,* **76:** 576-587.

Matthew, C., van Loo, E.N., Thom, E.R., Dawson, L.A. and Care, D.A. 2001. Understanding shoot and root development. *Proceedings of the XIX International Grassland Congress.* Sao Pedro, Brazil. pp 19-27.

Mazzanti, A, Lemaire, G. and Gastal, F. 1994. The effect of nitrogen fertilization upon the herbage production of tall fescue swards continuously grazed with sheep. 1. Herbage growth dynamics. *Grass and Forage Science,* **49:** 111-120.

McIvor, J.G. 1978. The effect of cutting interval and associate grass species on the growth of *Stylosanthes* species near Ingham, north Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry,* **18:** 546-553.

McIvor, J.G. 1984. Leaf growth and senescence in *Urochloa mosambicensis and U. oligotricha* in a seasonally dry tropical environment. *Australian Journal of Agricultural Research,* **35:** 177-187.

McIvor, J.G., Jones, R.M and Taylor, J.A. 1993. Tropical pasture establishment. 4. Population dynamics of sown species in developing pastures. *Tropical Grasslands,* **27:** 302-313.

McKenzie, F.R. 1997. Influence of grazing frequency and intensity on the density and persistence of *Lolium perenne* tillers under subtropical conditions. *Tropical Grasslands,* **31:** 219-226.

Mears, P.T. and Humphreys, L.R. 1974. Nitrogen response and stocking rate of *Pennisetum clandestinum* pastures. I. Pasture nitrogen requirement and concentration, distribution of dry matter and botanical composition. *Journal of Agricultural Science,* **83:** 451-68.

Miles, J.W., Maass, B.L., and do Valle C.B. (eds.). 1996. *Brachiaria: Biology, Agronomy and Improvement.* CIAT – EMBRAPA. Cali, Colombia.

Milne, J.A., Hodgson, J., Thompson, R., Souter, W.G. and Barthram, G.T. 1982. The diet ingested by sheep grazing swards differing in white clover and perennial ryegrass content. *Grass and Forage Science,* **37:** 209-218.

Moore, K.J. and Moser, L.E. 1995. Quantifying developmental morphology of perennial grasses. *Crop Science,* **35:** 37-43.

Mott, J.J., Ludlow, M.M., Richards, J.H. and Parsons, A.J. 1992. Effects of moisture supply in the dry season and subsequent defoliation on persistence of the savanna grasses *Themeda triandra*, *Heteropogon contortus* and *Panicum maximum*. *Australian Journal of Agricultural Research*, **43:** 241-260.

Mueller, R.J. and Richards, J.H. 1986. Morphological analysis of tillering in *Agropyrum spicatum* and *Agropyrum desertorum*. *Annals of Botany,* **58:** 911-921.

Murphy, J.S. and Briske, D.D. 1992. Regulation of tillering by apical dominance: chronology, interpretive value and current perspectives. *Journal of Range Management,* **45:** 419-429.

Murtagh, G.J., Kaiser, A.G. and Huett, D.O. 1980. Summer growing components of a pasture system in a subtropical environment. 2. Relationships between pasture and animal production. *Journal of Agricultural Science, Cambridge,* **94:** 665-674.

Murtagh, G.J., Halligan, E.A., and Greer, D.H. 1987. Components of growth and dark respiration of Kikuyu (*Pennisetum clandestinum* Chiov.) at various temperatures. *Annals of Botany (London),* **59:** 149-157.

Nelson, C.J. 2000. Shoot morphological plasticity of grasses: leaf growth vs. tillering. In: *Grassland Ecophysiology and Grazing Ecology*. G. Lemaire, J. Hodgson, A. de Moraes, P.C. de F. Carvalho and C. Nabinger (eds.). Chapter 6. CABI Publishing, Wallingford, Oxon, UK. Pp. 101-126.

Nelson, C.J. and Zarrough, K.M. 1981. Tiller density and tiller weight as yield determinants of vegetative swards. In: *Plant Physiology and Herbage Production. Occasional Symposium No. 13, The British Grassland Society*. C.E. Wright (ed.). The British Grassland Society, Hurley, UK. pp. 25-29.

Noble, J.C., Bell, A.D. and Harper, J.L. 1979. The population biology of plants with clonal growth: I. The morphology and structural demography of *Carex arenaria*. *Journal of Ecology,* **67:** 983-1008.

Noy-Meir, I. and Briske, D.D. 1996. Fitness components of grazing-induced population reduction in a dominant annual, *Triticum dicoccoides* (wild wheat). *Journal of Ecology,* **84:** 439-448.

O'Connor, T.J. 1993. The influence of rainfall and grazing on the demography of some African savanna grasses: a matrix modelling approach. *Journal of Applied Ecology,* **30:** 119-132.

Okeagu, M.U., Asiribo, O.E., and Akinola, J.O. 1989. Effect of nitrogen application on growth characteristics of signal grass (*Brachiaria decumbens*). *Journal of Agricultural Science (Cambridge),* **113:** 373-376.

Okeagu, M.U. and Agishi, E.C. 1990. Effect of row spacing and rate of applied nitrogen on the productivity of signal grass (*Brachiaria decumbens* Stapf.). *Tropical Agriculture (Trinidad)*, **67:** 178-182.

Ong, C.K. 1978. The physiology of tiller death in grasses. 1. The influence of tiller age, size and position. *Journal of the British Grassland Society,* **33:** 197-203.

Ong, C.K, Marshall, C., and Sagar, G.R. 1978. The physiology of tiller death in grasses. 2. Causes of tiller death in a grass sward. *Journal of the British Grassland Society,* **33:** 205-211.

Ong, C.K and Baker, C.K. 1985. Temperature and leaf growth. In: *Control of leaf growth.* N.R. Baker, W.J. Davies and C.K. Ong (eds.). Cambridge University Press, Cambridge, UK. pp. 175-200.

Pakiding, W. and Hirata, M. 1999. Tillering in a bahia grass (*Paspalum notatum*) pasture under cattle grazing: results from the first two years. *Tropical Grasslands,* **33:** 170-176.

Parsons, A.J. and Robson, M.J. 1980. Seasonal changes in the physiology of S24 perennial ryegrass (*Lolium perenne* L.). 1. Response of leaf extension to temperature during the transition from vegetative to reproductive growth. *Annals of Botany,* **46:** 435-444.

Parsons, A.J. and Chapman, D.F. 2000. The principles of pasture growth and utilization. In: *Grass. Its production and utilization.* 3rd edition. A. Hopkins (ed.) British Grassland Society. Blackwell Science, Oxford, UK. pp. 31-89.

Rao, I.M., Kerridge, P.C. and Macedo, M.C.M. 1996. Nutritional requirements of *Brachiaria* and adaptation to acid soils. In: *Brachiaria: Biology, Agronomy and Improvement.* J.W. Miles, B.L. Maass and C.B. do Valle (eds.). Chapter 4. CIAT – EMBRAPA. Cali, Colombia. pp. 53-71.

Rezende, C.D., Cantarutti, R.B., Braga, J.M., Gomide, J.A., Pereira, J.M., Ferreira, J.M., Ferreira, E., Tarre, R., Macedo, R., Alves, B.J.R., Urquiaga, S., Cadish, G., Giller, K.E. and Boddey, R.M. 1999. Litter deposition and disappearance in *Brachiaria* pastures in the Atlantic forest region of the South of Bahia, Brazil. *Nutrient Cycling in Agroecosystems,* **54:** 99-112.

Richards, J.H. 1993. Physiology of plants recovering from defoliation. In: *Grasslands for our world.* M.J. Baker (ed.). SIR Publishing, Wellington, New Zealand. pp. 46-54.

Rickert, K.G. 1996. Stocking rate and sustainable grazing systems. In: *Grassland Science in Perspective.* A. Elgersma, P.C. Struik and L.J.G. van der Maesen. Wagenigen University Papers 96-4. Wagenigen Agricultural University. The Netherlands. pp. 29-66.

Rowell, D.L. 1994. *Soil Science. Methods and Applications.* Longman. Harlow, UK.

Sackville Hamilton, N.R. and Harper, J.L. 1989. The dynamics of *Trifolium repens* in a permanent pasture. I. The population dynamics of leaves and nodes per shoot axis. *Proceedings of the Royal Society of London, B,* **237:** 133-173.

Sackville Hamilton, N.R., Matthew, C. and Lemaire, G. 1995. In defence of the -3/2 boundary rule: a re-evaluation of self-thinning concepts and status. *Annals of Botany,* **76:** 569-577.

Schnyder, H. and Nelson, C.J. 1988. Diurnal growth of tall fescue leaf blades. I. Spatial distribution of growth, deposition of water, and assimilate import in the elongation zone. *Plant Physiology,* **86:** 1070-1076.

Schnyder, H., Schaufele, R., de Visser, R. and Nelson, C.J. 2000. An integrated view of C and N uses in leaf growth zones of defoliated grasses. In: *Grassland Ecophysiology and Grazing Ecology.* G. Lemaire, J. Hodgson, A. de Moraes, P.C. de F. Carvalho and C. Nabinger (eds.). Chapter 3. CABI Publishing, Wallingford, Oxon, UK. Pp. 41-60.

Schwinning, S. and Parsons, A.J. 1996a. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *Journal of Ecology,* **84:** 799-814.

Schwinning, S. and Parsons, A.J. 1996b. A spatially explicit population model of stoloniferous N-fixing legumes in mixed pasture with grass. *Journal of Ecology,* **84:** 815-826.

Sheath, G.W. and Clark, D.A. 1996. Management of grazing systems: temperate pastures. In: *The ecology and management of grazing systems.* J. Hodgson and A.W. Illius (eds.). Chapter 11. CAB International. Wallingford, Oxon, UK. pp. 301-323.

Sheehy, J.E., Gastal, F., Mitchell, P.L., Durand, J.L., Lemaire, G. and Woodward F.I. 1996. A nitrogen-led model of grass growth. *Annals of Botany,* **77:** 165-177.

Silsbury, J.H. 1970. Leaf growth in pasture grasses. *Tropical Grasslands,* **4:** 17-36.

Simoes, M. and Baruch, Z. 1991. Responses to simulated herbivory and water stress in two tropical C4 grasses. *Oecologia,* **88:** 173-180.

Simon, J.C. and Lemaire, G. 1987. Tillering and leaf area index in grasses in the vegetative phase. *Grass and Forage Science,* **42:** 373-380.

Skinner, R.H. and Nelson, C.J. 1992. Estimation of potential tiller production and site usage during tall fescue canopy development. *Annals of Botany,* **70:** 493-499.

Skinner, R.H. and Nelson, C.J. 1994a. Epidermal cell division and the coordination of leaf and tiller development. *Annals of Botany (London),* **74:** 9-15.

Skinner, R.H. and Nelson, C.J. 1994b. Effect of tiller trimming on phyllochron and tillering regulation during tall fescue development. *Crop Science,* **34:** 1267-1273.

Skinner, R.H. and Nelson, C.J. 1995. Elongation of the grass leaf and its relationship to the phyllochron. *Crop Science,* **35:** 4-10.

Stobbs, T.H. 1973. The effect of plant structure on the intake of tropical pastures. 1. Variation in the bite size of grazing cattle. *Australian Journal of Agricultural Research,* **24:** 809-819.

Stobbs, T.H. 1975. Factors limiting the nutritional value of grazed tropical pastures for beef and milk production. *Tropical Grasslands,* **9:** 141-150.

Stur, W.W. 1986. Reproductive development of the apex of *Brachiaria decumbens* Stapf. *Annals of Botany,* **58:** 569-575.

Stur, W.W. and Humphreys, L.R. 1987. Tiller development and flowering in swards of *Brachiaria decumbens. Annals of Applied Biology,* **110:** 639-644.

Stur, W.W. and Humphreys, L.R. 1988. Defoliation and burning effects on the tillering of *Brachiaria decumbens. Journal of Applied Ecology,* **25:** 273-277.

Takahashi, K. 1992. Role of the leaf sheath in the elongation of the internodes of rice plants. *Japan Journal of Crop Science,* **61:** 235-243.

Thomas, H. and Laidlaw, A.S. 1993. Planning, design, analysis and establishment of experiments. In: *Sward measurement handbook.* A. Davies, R.D. Baker, S.A. Grant and A.S. Laidlaw (eds.). British Grassland Society. pp. 13-37.

Thomas, R.J. and Asakawa, N.M. 1993. Decomposition of leaf litter from tropical forage grasses and legumes. *Soil Biology and Biochemistry,* **25:** 1351-1361.

Toft, N.L., McNaughton, S.J. and Georgiadis, N.J. 1987. Effects of water stress and simulated grazing on leaf elongation and water relations of an East African grass, *Eustachys paspaloides. Australian Journal of Plant Physiology,* **14:** 211-226.

Torsell B.W.R. and Nicholls A.O. 1978. Population dynamics in species mixtures. In: *Plant Relations in Pastures.* J.R. Wilson (ed.). Chapter 15. CSIRO. East Melbourne, Australia. pp. 217-2323-15.

Valerio, J.R., Lapointe, S.L., Kelemu, S., Fernandes, C.D. and Morales, F.J. 1996. Pests and diseases of *Brachiaria* species. In: *Brachiaria: Biology, Agronomy and Improvement.* J.W. Miles, B.L. Maass and C.B. do Valle (eds.). Chapter 6. CIAT – EMBRAPA. Cali, Colombia. pp. 87-105.

do Valle, C.B., Calixto, S. and Amezquita, M.C. 1993. Agronomic evaluation of *Brachiaria* germplasm in Brazil. *Proceedings of the XVIIth International Grassland Congress.* Hamilton, New Zealand. pp. 511-512.

Van Loo, E.N., Schapendonk, A.H.C.M. and de Vos, A.L.F. 1992. Effects of nitrogen supply on tillering dynamics and regrowth of perennial ryegrass populations. *Netherlands Journal of Agricultural Science,* **40:** 381-400.

Vine, D.A. 1983. Sward structure changes within a perennial ryegrass sward: leaf appearance and death. *Grass and Forage Science,* **38:** 231-242.

Volenec, J.J. and Nelson, C.J. 1983. Responses of tall fescue leaf meristems to N fertilization and harvest frequency. *Crop Science,* **23:** 720-724.

Wade, M.H., Peyraud, J.L., Lemaire, G. and Comeron, E.A. 1989. The dynamics of daily area and depth of grazing and herbage intake of cows in a five day paddock system. *Proceedings of the XVIth International Grassland Congress*. Nice, France. pp. 1111-1112.

Walker, B. (1980). Effects of stocking rate on perennial tropical legume-grass pastures. *PhD Thesis*. University of Queensland.

Warrington, I.J. and Kanemasu, E.T. 1983. Corn growth response to temperature and photoperiod. II. Leaf initiation and leaf appearance rates. *Agronomy Journal,* **75:** 755-761.

Watkinson, A.R. Plant population dynamics. In: *Plant Ecology*. M.J. Crawley (ed.). Chapter 12. Blackwell Science. Oxford. UK. pp 359-400.

Westoby, M. 1984. The self-thinning rule. *Advances in Ecological Research,* **14:** 167-225.

Whiteman, P.C. 1980. *Tropical Pasture Science*. Oxford University Press. Oxford, UK.

Wilhelm, W.W. and McMaster, G.S. 1995. Importance of the phyllochron in studying development and growth in grasses. *Crop Science,* **18:** 769-772.

Wilson, J.R. 1976. Variation of leaf characteristics with level of insertion on a grass tiller. I Developmental rate, chemical composition and dry matter digestibility. *Australian Journal of Agricultural Research,* **27:** 343-354.

Wilson, J.R. and Mannetje, L.'t. 1978. Senescence, digestibility and carbohydrate content of Buffel grass and Green Panic leaves in swards. *Australian Journal of Agricultural Research,* **29:** 503-516.

Wilson, R.E. and Laidlaw, A.S. 1985. The role of sheath tube in the development of expanding leaves in perennial ryegrass. *Annals of Applied Biology,* **106:** 385-391.

Woodward, S.J.R. 1998. Quantifying different causes of leaf and tiller death in grazed perennial ryegrass swards. *New Zealand Journal of Agricultural Research,* **41:** 149-159.

Yin, X. and Kropff, M.J. 1996. The effect of temperature on leaf appearance in rice. *Annals of Botany (London),* **77:** 215-221.

Zarrough, K.M., Nelson, C.J., and Sleper, D.A. 1984. Interrelationships between rates of leaf appearance and tillering in selected tall fescue populations. *Crop Science,* **24:** 565-569.