

On the relation between tillering, leaf area dynamics and growth
of perennial ryegrass (*Lolium perenne* L.)



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Promotoren: dr. ir. L. 't Mannetje
hoogleraar in de graslandkunde

dr. ir. R. Rabbinge
hoogleraar in de theoretische produktie-ecologie
met bijzondere aandacht voor de plantaardige
produktie

E.N. van Loo

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area dynamics and growth of
perennial ryegrass (*Lolium perenne* L.)**

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STELLINGEN

1. Verschillen tussen populaties van Engels raaigras in hergroeisnelheid na ontbladeren berusten in belangrijke mate op verschillen in zijspuitvorming en bladstrekkingsnelheid.

Dit proefschrift.

2. Veredeling in Engels raaigras op een hogere bladverschijningssnelheid zal leiden tot genotypen met een hogere spruitverschijningssnelheid en een hogere spruitdichtheid.
3. Selectie van genotypen van Engels raaigras voor hergroeisnelheid moet worden uitgevoerd op basis van de prestatie van genotypen in mengsels.
4. Het specifieke bladoppervlak geeft een indicatie voor de mate waarin bladgroei wordt beperkt door het aanbod van assimilaten of door morfologische beperkingen aan de grootte van het bladapparaat.

Dit proefschrift.

5. De snelheid van hergroei wordt alleen gedurende de eerste drie dagen na volledige ontbladering voor een groot deel bepaald door de hoeveelheid reserves in de stoppel van een graszode.

Dit proefschrift.

6. Op grasland met open plekken moet de stikstofbemesting naar beneden worden aangepast aan de lagere produktiviteit om verdere achteruitgang van de zode en onnodige stikstofverliezen naar het milieu te voorkomen.
7. Verhoging van de stikstofbenutting uit gras door weidend vee is mogelijk via het adagium: "eiwit omlaag en energie omhoog".
8. Het is onverteerbaar dat de eigenschap verteerbaarheid (nog) niet gebruikt wordt voor beoordeling van rassen van Engels raaigras voor de Nederlandse rassenlijst.
9. Een verhoging van de belastingheffing op energie- en grondstoffenverbruik en een verlaging van de belastingheffing op arbeid zal een grote bijdrage leveren aan de werkgelegenheid en aan een vermindering van de uitstoot van voor het milieu schadelijke stoffen.
10. Premiedifferentiatie bij arbeidsongeschiktheidsverzekeringen op basis van iemands individuele gezondheid of gesteldheid ondermijnt de onderlinge solidariteit tussen verzekerden en moet daarom niet worden toegepast. Het is bijvoorbeeld onwenselijk dat sommige particuliere verzekeringsmaatschappijen bij arbeidsongeschiktheidsverzekeringen voor één-ogigen het verlies van hun goede oog uitsluiten of dit risico slechts tegen hogere premies accepteren.
11. Het enige voordeel van het lage aanvangssalaris van AIO's is dat zij daardoor in aanmerking kunnen komen voor een goedkopere huurwoning.

Proefschrift van E.N. van Loo. On the relation between tillering, leaf area expansion and growth of perennial ryegrass (*Lolium perenne* L.).

ABSTRACT

E.N. van Loo. On the relation between tillering, leaf area dynamics and growth of perennial ryegrass (*Lolium perenne* L.). Doctoral thesis, Department of Agronomy, Agricultural University, Wageningen, The Netherlands, viii + 169 pp., English and Dutch summaries.

Modern intensively managed grasslands are subject to sward deterioration as a result of urine scorching, treading, winter mortality and late mowing or grazing. The major species in Dutch grasslands is perennial ryegrass. Deterioration consists of a decreasing presence of this species through death of tillers and plant. This gives rise to open swards which are subject to weed invasion and leads to a reduced productivity and nitrogen recovery. The objectives of this study were to increase the understanding of the recovery potential of open perennial ryegrass swards and of the relation between tiller density and herbage accumulation. Tiller production, tiller mortality, leaf area dynamics and growth as affected by cutting treatment, water availability, nitrogen supply and plant density were studied in field, glasshouse and phytotron experiments. The relative tiller appearance rate was studied as the product of leaf appearance rate and site filling. Leaf appearance rate was highly positively correlated with temperature and negatively with cutting height. Cutting frequency had no effect. Leaf appearance rate was reduced by low water potential and low nitrogen supply. Site filling decreased with decreasing nitrogen supply and increasing leaf area index. Just after defoliation, site filling was lower than later after defoliation, because of low substrate availability to developing tiller buds.

In studying the effect of low and high nitrogen supply, genetic variation was found in nitrogen use efficiency. Populations with a high nitrogen use efficiency and a high herbage accumulation rate, also had a high leaf weight ratio and high tillering capacity.

Models were developed for the simulation of changes in tiller number, leaf area expansion and dry matter accumulation of spaced plants and swards. These models were used for sensitivity analyses of the effect of leaf area expansion rate and leaf weight ratio on regrowth after defoliation and for the analysis of the importance of substrate availability for tillering and regrowth. The field experiments and the sward model showed that even with very low seeding rates, the amount of herbage harvested in the second harvest year is not lower than at very high seeding rates. Therefore, it was concluded that only when a low tiller density coincides with a clumped distribution of tillers and with a high frequency of patches without perennial ryegrass, reseeding of perennial ryegrass swards should be considered.

NOTE

Chapter 4 has been published as a paper in *Annals of Botany*, 70, 511-518.

Chapter 5 has been published as a paper in *Netherlands Journal of Agricultural Science*, 40, 401-419.

Chapter 2, 3, 6 and 7 have been submitted for publication in international journals.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Agricultural background

The current intensive management of perennial ryegrass (*Lolium perenne* L.) dominated grasslands - with high nitrogen inputs and high stocking rates - may easily lead to sward deterioration, for example through urine scorching, treading, poaching and tiller pulling (Edmond, 1966; Keuning, 1981; Wilkins and Garwood, 1986; Tallwin *et al.*, 1986). Also, the risks of a substantial tiller mortality during winter increases with increasing nitrogen fertilizer application rate (Snijders, 1986; Deenen, unpublished). Further, with the increased productivity of grassland, the risk of late mowing has become larger, since the timing of harvesting becomes more critical. Late mowing of swards with a high percentage of reproductive stems may lead to massive tiller death because of decapitation of growing points of tillers.

Sward deterioration may be characterised by a decreasing tiller density and is often accompanied by a more clumped distribution of tillers with an increased frequency of open patches (Prins, 1983). This leads to lower productivity and nitrogen recovery (Deenen, 1990). Further, the occurrence of unwanted plant species (less productive, less digestible or less palatable species) will increase in more open swards. When the presence of perennial ryegrass declines too much, dry matter yields or herbage quality will be so low that reseeding is considered economically advantageous. In the mid-eighties, annually about 10 % of the Dutch grasslands are reseeded (Keuning and Vellinga, 1987). In 70 % of the area resown, the reason for reseeding is the openness of the sward or the frequent occurrence of unwanted species (e.g. couch (*Elymus repens* L.) or annual meadow grass (*Poa annua* L.)). However, reseeding of grassland is expensive. It has been argued that in many cases the costs of reseeding exceed the economic benefits (Wilkins, 1987). Costs of reseeding do not only include the costs for seed and labour, but also the yield reduction during establishment of the sward. The yield reduction of open swards or swards with some unwanted species may be overestimated as it occurs only temporary, since open perennial ryegrass swards may recover. However, little is known about the recovery potential of perennial ryegrass swards.

1.2 Recovery of grassland depends on tillering

Utilisation of grassland in the Netherlands may be of several types: continuous grazing, rotational grazing, cutting for silage or forage feeding. With all these types of grassland utilisation and with a good timing of grazing or cutting, perennial ryegrass will hardly produce any seed heads. Recovery of swards with a reduced tiller density or with open spaces can therefore only occur through tillering of perennial ryegrass. Therefore, studying the tiller density dynamics and the frequency of open patches may provide a better understanding of the recovery potential of swards. Possibly, information on tiller density or frequencies of open patches may help to formulate criteria for decisions on reseeding perennial ryegrass swards.

Both productivity and tiller density of perennial ryegrass depend on the cultivar used, the type of grassland utilisation, fertilizer application rates and environmental conditions (radiation, temperature and water supply). Since tiller size is greatly influenced by these factors, it is as yet not possible to directly relate dry matter accumulation to the tiller density of a sward. Therefore, use of information on tiller density for grassland management decisions is impossible.

1.3 Scope of the thesis

The basic theme of this thesis will be the interaction between tiller dynamics, leaf area dynamics and dry matter accumulation of grass swards or of spaced grass plants. Much is already known about dry matter accumulation of grass swards with a dense and homogeneous distribution of tillers (Lantinga, 1985, 1988). However, the current state of the art in modelling of grassland production does not allow adequate simulation of the effect of tiller density on the productivity of perennial ryegrass.

Tiller density mainly influences dry matter yield of swards through its effect on regrowth after defoliation, especially when the amount of leaf area after defoliation is so small that the net growth rate of the sward is hardly positive or even negative. Simulation models may be used to integrate knowledge on all the interacting processes (tillering, leaf area increase and dry matter accumulation). However, existing models are not adequate in simulating the effect of tiller density on dry matter accumulation. This is partly due to a lack of understanding of the initial leaf area expansion after complete or almost complete defoliation. Improvement or extension of existing models to account for an effect of low tiller density on regrowth after defoliation may help to increase our understanding of grassland productivity. In the following subparagraphs, some tillering and growth parameters and basic relationships that need to be quantified, will be defined. Further, two possible ways in which tiller density or tiller number may affect regrowth after defoliation are proposed.

1.3.1 Tillering parameters

Tillering has been studied in terms of the two factors determining relative tiller appearance rate: i) leaf appearance rate per tiller and ii) site filling. Leaf appearance rate is related to the tiller bud production per tiller, since in the axil of each tiller a tiller bud is produced. Site filling was defined originally by Davies and Thomas (1983) as: 'the number of tillers appearing per tiller during a single leaf appearance rate' and is mathematically defined as the relative tiller appearance rate divided by the leaf appearance rate. With these two parameters, the tiller appearance rate is determined. Understanding tiller density dynamics further requires information on tiller mortality.

1.3.2 Tiller morphology and leaf area expansion rates per tiller

Young tillers are small, not only because they have formed fewer leaves, but also because the first leaves on each tiller usually are smaller than leaves formed later on the same tiller (e.g. Mitchel, 1953). Possibly, the relationship between final leaf size and leaf number on a

tiller is constant under constant environmental conditions. This hypothesis should be put to the test. When the hypothesis can be shown to be acceptable, the relationship may be of use in explaining the plant or crop leaf area expansion rate from tiller number or density.

1.3.3 Relationship between tiller density and herbage production

In classical growth analysis, leaf area dynamics and dry matter accumulation of root and shoot are related to one another. In this classical view, the product of specific leaf area (SLA), leaf weight ratio (LWR) and net assimilation rate (NAR) determines the relative growth rate (RGR) of the plant or crop (Hunt, 1980). Growth models have largely focused on explaining the NAR of a crop from i) light interception, ii) CO₂-assimilation rates and iii) maintenance and growth respiration rates (for example SUCROS87, Spitters *et al.*, 1989). SLA and LWR are introduced in most models as forcing functions of environmental factors and of development stage of the crop. In these models, there was no need for the introduction of effects of tillers. However, as SLA is introduced as a fixed value, leaf area increase is completely determined by leaf growth. Therefore, no allowance is possible for any effect of tiller density or tiller number on leaf area dynamics and growth. One of the aims of this thesis is to study how numbers of tillers, tiller buds and leaf primordia affect leaf area expansion and finally herbage growth.

There are - at least - two possible mechanisms by which tiller number could affect (re)growth: i) the undefoliated part of a tiller (the stubble) may function as a substrate pool from which material may be used during regrowth and the amount of substrate available may then depend on tiller number or density; ii) the crop leaf area expansion may depend on tiller density and on the maximum leaf area expansion rate per tiller. In the first case, the amount of substrate determines the regrowth rate (source limitation of regrowth). In the second case, tiller density determines the crop leaf area expansion rate and thereby the rate of use of substrate in the stubble (sink-limited regrowth). To what extent both situations occur in defoliated grass swards or plants is unclear.

1.4 Aim of the thesis

The aim of this thesis was to integrate existing knowledge on tillering, leaf expansion rates and dry matter accumulation of perennial ryegrass for a better understanding of the relationships between tiller density and dry matter accumulation and of the recovery potential of perennial ryegrass swards. To understand the role of tiller density in leaf area expansion and dry matter accumulation, a model for the seasonal variation of tiller density of perennial ryegrass was developed and an existing model on grassland production was extended to incorporate effects of tiller density and tiller morphology. The development of these models also included identifying and filling gaps in knowledge on tillering parameters, leaf morphological parameters and other growth parameters needed in the models.

1.5 Outline of the thesis

The studies in this thesis are presented in four parts. In Part I (Chapters 2 and 3), two studies are presented in which light, temperature, water and nutrients were non-limiting for growth. In Chapter 2, the effects of cutting frequency and height on tillering, leaf area increase per tiller and growth of spaced plants are described of three cultivars differing in tillering and leaf morphological characteristics. In Chapter 3, a mechanistic simulation model is presented to provide a theoretical basis for explaining the results of Chapter 2. The model comprises modules for tillering, leaf area expansion and dry matter accumulation. The model has been used to study to what extent tillering may be limited by tiller bud production or by an effect of substrate supply on tiller bud development. Further, the model was used to study to what extent regrowth of leaves after defoliation is supported by substrates from i) the substrate pool in the stubble, ii) expansion of parts of leaves that resided in the sheath bundle of a tiller at the time of defoliation (the unemerged part of leaves) or iii) from current assimilation of leaf area remaining in the stubble after defoliation.

Part II (Chapter 4) presents some aspects of tillering, leaf area expansion and growth under various levels of water availability, studied in a hydroponics system using polyethyleneglycol to lower the water potential.

Part III (Chapters 5 and 6) describes effects of nitrogen supply on tillering, leaf area expansion and growth. Two different systems of nitrogen supply were used. In the first study (Chapter 5), the effect was studied of "steady state" plant nitrogen concentrations (Ingestad, 1980) and of fast changes in nitrogen supply on tillering and regrowth of several populations of perennial ryegrass. In the second study (Chapter 6), nitrogen was applied at the start of each growth period (after each harvest). Chapter 6 describes the interaction between effects of nitrogen supply and plant density on tillering, leaf area expansion, light interception and (re)growth. In both chapters, a simple model - based on light interception and a constant radiation use efficiency - is used for the prediction of growth after defoliation and results obtained with the model are compared to experimental results.

In Part IV (Chapter 7), results of two field experiments are presented in which effects of plant density and cutting regime on tillering, tiller mortality, leaf area expansion and dry matter accumulation were investigated. Tiller dynamics and herbage accumulation of a diploid and a tetraploid cultivar are compared. A relationship is presented between tiller density and annual herbage yield. A simulation model has been developed with modules for i) tiller dynamics, ii) leaf area dynamics and ii) herbage accumulation. The model was used i) to explain the seasonal variation in tiller density, iii) to investigate the effect of leaf appearance rate and leaf size on tillering and on the maximum tiller density that may be reached and iii) to explain the relationship between tiller density and herbage production. In the final chapter (Chapter 8), results of the studies are discussed in relation to the aims and questions formulated in this Introduction.

PART I

Potential tillering and leaf area expansion

CHAPTER 2

The response to cutting regimes in tillering, leaf extension and regrowth of perennial ryegrass cultivars with contrasting leaf characteristics

with J.H. Neuteboom

ABSTRACT

Tillering, leaf expansion and regrowth were studied of three cultivars of perennial ryegrass, at two cutting frequencies and three cutting heights. Spaced plants were used to evaluate effects of cutting on tillering and leaf morphology without changing the light intensity and quality experienced by the buds. Tillering rates were lower with weekly than with three-weekly cuts and with cuts at lower height. These effects were generally the same for the three cultivars. Relative tiller appearances rates were analysed as the product of leaf appearance rate and site filling (a measure related to the proportion of buds that grow into tillers). The relationship between leaf size and leaf number was different for the three cultivars and differentially influenced by cutting. A single relationship for the three cultivars was found for the relation between leaf area, width and length. Cultivar differences in growth and harvestable biomass were related to differences in sheath and leaf lengths and in growth parameters. With all cultivars, the specific leaf area (SLA) and fraction of biomass allocated to above a stubble height of 2.5 cm increased with increasing defoliation severity. The capacity to increase SLA and the fraction of biomass allocated to leaves with increasing defoliation severity, both help to compensate for the smaller leaf area remaining after cutting. The lower SLA and fraction of biomass allocated to above 2.5 cm may be explained by an 'overflow' of assimilates towards the stubble when assimilation exceeds the demand of leaves.

2.1 INTRODUCTION

Regrowth of grass depends on many factors, some affecting assimilate supply like the amount of leaf area and reserves remaining in the stubble after defoliation, others affecting assimilate demand for growth, like tiller number and maximum expansion rates per leaf. Experimental data and theoretical models are available for the analysis of effects of severity of defoliation and duration of regrowth on yield (Johnson and Parson, 1983; Parsons, Johnson and Harvey, 1988). However, effects of frequency and height of cutting on regrowth potential through effects on leaf morphology and tiller density are still poorly understood, because "the mechanisms which determine the structure of the sward (e.g., tiller number and size) in response to management are unclear" (Parsons, Johnson and Harvey 1988). Therefore, it is still impossible to simulate more than one regrowth period when no data are available on the initial conditions of a second regrowth period, because the amount

of leaf area remaining in the stubble after defoliation cannot easily be predicted.

How much leaf area remains in the stubble after defoliation depends on cutting height, but also on tiller morphology (leaf width and length, sheath length). Leaf dimensions of successive leaves along a stem often show a progressive increase with position (Dale and Milthorpe, 1983). This has also been shown for grasses (for example for *Festuca arundinacea*: Easton, 1978). However, not much is known about the effect of cutting on the relationship between the position of a leaf on a tiller and leaf and sheath length. It is also still a question whether the relationship found for the main tiller is also valid for younger tillers. If the relationship between leaf size and sheath length and leaf number would be the same for all tillers, the relationship could be used to predict the fraction of leaves and sheaths harvested and the amount of leaf area remaining in the stubble after cutting. This would enable simulation of regrowth of grass without having to measure initial values for stubble weight and leaf area after each defoliation.

As a consequence of repeated cutting of plants, changes can occur in many plant factors, e.g., in leaf expansion rate and leaf morphology, in tillering rates, and in dry matter distribution between root and shoot (Grant, Barthram and Torvell, 1981; Del Pozo, 1963; Hume, 1990). Since leaf expansion and assimilate flow to leaves are partially independent (Johnson and Thornley, 1983), also changes in specific leaf area may be expected. These changes could influence the regrowth potential. The direction and extent of such changes may be different for cultivars differing in leaf and tillering characteristics. Therefore, an experiment was carried out with spaced plants of three perennial ryegrass cultivars with different leaf size and tillering characteristics, at different cutting regimes.

The aim of the study was to increase the understanding of the effect of cutting on regrowth potential as affected by tiller number and leaf morphology. For this purpose, the effect of cutting treatment on the relation between leaf size and leaf number on a tiller was determined and it was determined whether cultivars differed in the response of leaf morphology to cutting. The effect of cutting on relative tiller appearance rates was analysed in terms of its components: leaf appearance rate and site filling. Site filling is defined as the relative tiller appearance rate divided by the leaf appearance rate (Davies, 1974; Neuteboom and Lantinga, 1989) and is related to the proportion of tiller buds that grow into visible tillers (Van Loo, 1992). Finally, also the effect of cutting treatments on specific leaf area and on dry matter distribution to harvestable shoot, stubble and root was assessed.

2.2 MATERIAL AND METHODS

The experiment was carried out in a glasshouse with spaced plants of *Lolium perenne* L. cultivars Barry (diploid lawn grass), Wendy (diploid production grass), Condesa (tetraploid production grass). Wendy is known to have a higher leaf appearance rate than Condesa (Neuteboom, Lantinga and Wind, 1988). On 16 January 1990 (day 1), seeds were sown in

sand. On day 9, 72 plants per cultivar with one tiller and one appeared leaf were selected and placed on eighteen containers (length: 2 m, volume: 40 L) with hydroponics (Steiner nutrient solution, Steiner, 1984). Six blocks were formed, each consisting of three containers. In each block, cultivars were assigned to one of the containers (12 plants per container). On day 28, 36 plants per cultivar were harvested for dissection into root, stubble (< 2.5 cm) and herbage above 2.5 cm stubble height. The other 36 plants per cultivar (six per container) remained on the hydroponics and those plants were subjected to six cutting treatments. A split-plot design was used with cultivars as main factor and cutting treatments as the split factor. The hydroponics were changed weekly and the pH of the hydroponics was kept between 5.0 and 6.0.

Cutting treatments: All combinations of two cutting frequencies (weekly and three weekly cuts) and three cutting heights (2.5, 5 and 7.5 cm) gave six cutting treatments. To avoid complications due to variation in tiller and leaf angle, leaves were held upright and then cut at the desired cutting height. Thus, after cutting, for all tillers the length of leaf sheath plus blade of cut leaves was equal to the cutting height. Seven cuts were harvested with weekly cuts and three with three-weekly cuts. The first cut was on day 28 and the last cut on day 70. At the final cut, the cutting height was 2.5 cm in all cutting height treatments. This was done to enable comparison of the fraction of biomass production allocated to herbage above the same stubble height of the three cutting height treatments.

Environmental conditions: Day/night temperatures were 20/15 °C. Natural daylight was admitted and additional light (400 W Philips SON-T lamps) was used from 6.00 h - 21.00 h. Total daily radiation (PAR) within the glasshouse increased during the experiment from 3.4 MJ.m⁻².d⁻¹ to 4.4 MJ.m⁻².d⁻¹.

Measurements: At the first and final cut, plants were harvested completely, and weights of root, stubble and herbage above cutting height were determined. At the other cutting dates, only herbage yield was determined. Dry weight of plant material was determined after drying at 70 °C for 24 h. Specific leaf area (SLA) was determined from subsamples of the harvested leaves on day 28 and day 70. Leaf area of the subsamples was calculated from leaf blade lengths and leaf blade widths. The area of individual leaves (A, cm²) was calculated as:

$$A = 0.25L \cdot (1.5W_1 + W_2 + W_3 + 0.5W_4) \quad (1)$$

in which L is leaf length (cm) and W_1 , W_2 and W_3 are the leaf widths (cm) at positions 25, 50 and 75 % of the leaf length measured from the base and W_4 is the width at the end of the leaf. Eqn. (1) is based on the assumption that a leaf forms a rectangle from the leaf base to position 1 (where W_1 was measured) and forms a trapezium from position 1 to 2 and from position 2 to 3, and from position 3 to 4 (for uncut leaves W_4 is zero).

At weekly intervals, the number of tillers per plant and the number of newly appeared leaves

on the main and third tiller were counted and the leaf blade and sheath length of all leaves on the main and third tiller were measured. From the time course of the number of tillers per plant and number of appeared leaves on each tiller, the relative tiller appearance rate and leaf appearance rate were calculated. Site filling is defined as the relative tiller appearance rate divided by the leaf appearance rate:

$$F_s = \frac{R_{tar}}{L_A} = \frac{\ln T_2 - \ln T_1}{I_2 - I_1} = \frac{\ln T_2 - \ln T_1}{\Delta t} \frac{\Delta t}{I_2 - I_1} \quad (2)$$

in which, F_s is site filling, L_A is the leaf appearance rate (new leaves per tiller per time interval, d^{-1}), R_{tar} is the relative tiller appearance rate (d^{-1}) and T_1 and T_2 , are plant tiller number and I_1 and I_2 are number of appeared leaves on the main tiller at time t_1 and t_2 , respectively.

From the length measurements, the relation between sheath and blade length and leaf number was determined; the leaf number is counted from tiller base upwards.

2.3 RESULTS

Tillering, site filling and leaf appearance

Final number of tillers per plant were highest with three-weekly cuts at 7.5 cm and lower with weekly cuts or lower cutting heights (Table 2.1). Relatively, the effect of cutting height was strongest with weekly cuts. Cultivars Barry and Wendy had a higher final number of tillers per plant than Condesa. Tiller number of Barry, a lawn grass type, was not so much affected by cutting frequency as of Wendy and Condesa. Only with cuts at 2.5 cm tiller number of Barry was lower with weekly cuts than with three-weekly cuts. Therefore, final tiller number of Barry was higher than of Wendy with weekly cuts at 2.5 and 5 cm.

Differences in final tiller number per plant were caused by differences in relative tiller appearance rate, since all plants had one tiller at the start of the experiment. We analysed the relative tiller appearance rate as the product of site filling and the leaf appearance rate (Table 2.2). The leaf appearance rate was significantly lower at 2.5 than at 7.5 cm cutting height. With three-weekly cuts, the leaf appearance rate of cuts at 5 cm was most close to that of cuts at 2.5 cm, while with weekly cuts leaf appearance rate of cuts at 5 and 7.5 cm were similar. The leaf appearance rate of Condesa was about 15 % lower than of Wendy and Barry until the first cut (Table 2.3). This difference in leaf appearance rate gradually became less as, after the first cut, leaf appearance rate of Wendy and Barry declined while that of Condesa remained stable. As a consequence of the lower mean leaf appearance rate of Condesa, also its mean relative tiller appearance rate was lower (Table 2.2).

Table 2.1. Number of tillers per plant at the last cut (day 70).

Cutting height	Weekly cuts			Three-weekly cuts		
	2.5	5	7.5	2.5	5	7.5 cm
Cultivar						
Barry	99	173	142	120	164	159
Wendy	64	119	179	133	202	227
Condesa	42	85	90	99	106	135
Means	68	126	137	117	157	174

Significance of differences (based on log-transformed data):

Main effects:

Cultivar:

$P < 0.001$

Cutting frequency:

$P < 0.001$

Cutting height:

$P < 0.001$

Interactions:

Cultivar*Frequency: $P < 0.001$

Cultivar*Height: $P = 0.037$

Frequency*Height: $P = 0.011$

Cultivar*Frequency*Height: $P > 0.05$

Site filling can be graphically shown as the slope of the relationship between the logarithm of the number of tillers per plant and the number of leaves appeared per tiller (Fig. 2.1), since no tiller mortality occurred. At first, site filling was maximal at 0.693 (Neuteboom and Lantinga, 1989), but later declined to lower values (see also Fig. 2.2). Site filling was lower with weekly cuts than with three-weekly cuts. The effect of cutting height on site filling depended on the cutting frequency: with weekly cuts site filling was highest with cuts at 7.5 cm, but with three-weekly cuts with cuts at 5 cm (Fig. 2.2). With weekly cuts, site filling steadily declined with time. With three-weekly cuts, site filling was temporarily lower after each of the two cuts, but site filling almost recovered in the second week after cutting (Fig. 2.2B).

Cultivar differences in relative tillering rate, leaf appearance rate and site filling were not significantly influenced by cutting treatments. Therefore, overall means for the cultivars and means for each of the six cutting treatments have been presented (Table 2.2). Averaged over all cutting treatments, Wendy had the highest site filling and Barry had the lowest site filling. The lower site filling of Barry was mainly due to a 9 % lower site filling of Barry than of Wendy and Condesa with cuts at 7.5 cm. With cuts at 2.5 and 5 cm, site filling of Barry was only 5 % lower than of Wendy and Condesa (results not shown).

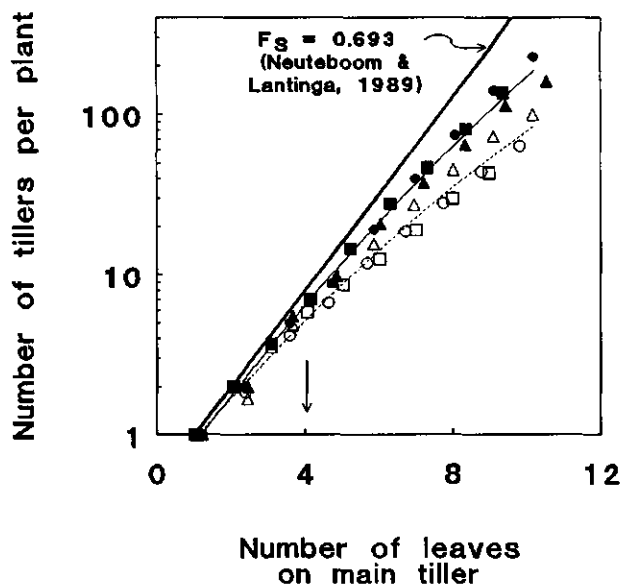


Fig. 2.1. Relationship between number of tillers per plant and number of leaves appeared on the main tiller. Three-weekly cuts at 7.5 cm: Δ Barry, \bullet Wendy, \blacksquare Condesa. Regression line: $\ln T = -0.0136L^2 + 0.737L - 0.877$, $R^2_{adj} = 0.9949$, $SD = 0.128$, T = number of tillers, L = number of leaves on the main tiller. Weekly cuts at 2.5 cm: Δ Barry, \circ Wendy, \square Condesa. Regression line: $\ln T = -0.0138L^2 + 0.0647L - 0.725$, $R^2_{adj} = 0.9887$, $SD = 0.155$. Each point is a mean for six plants. The arrow indicates approximately the leaf number at the moment of the first cut.

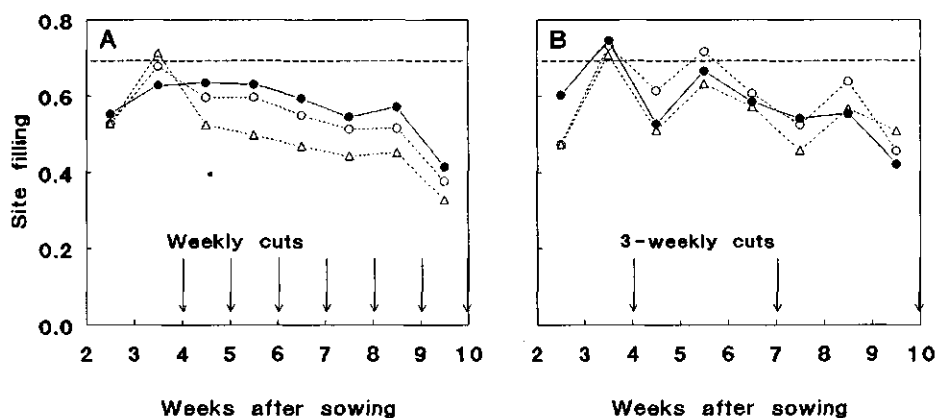


Fig. 2.2. Site filling. A) weekly cuts B) 3-weekly cuts. Cutting heights: Δ 2.5 \circ 5 \bullet 7.5 cm. Points are means of 18 plants (means over all cultivars). $LSD = 0.06$ ($P = 0.05$). The dashed line indicates the theoretical maximum site filling (0.693).

Table 2.2. Average relative tiller appearance rate (R_{tar} , d^{-1}), leaf appearance rate (L_A , d^{-1}) and site filling (F_s), from day 28 (cut 1) until the end of the experiment (day 70). The leaf appearance rate is the average for the main and third tiller.

	Cultivars			Weekly cuts			Three-weekly cuts		
	Barry	Wendy	Condesa	LSD	Cutting height		Cutting height		LSD
				($P=0.05$)	2.5	5	2.5	5	($P=0.05$)
R_{tar}	0.084	0.085	0.079	0.004	0.066	0.083	0.085	0.084	0.006
L_A	0.159	0.152	0.144	0.005	0.144	0.153	0.156	0.151	0.075
F_s	0.527	0.557	0.545	0.020	0.458	0.542	0.545	0.601	0.030

N.B. Interactions between cultivars and cutting treatments were not significant ($P>0.05$).

Table 2.3. Leaf appearance rate (leaves per tiller per day, d^{-1}) in pre-cutting period, first three weeks after first cut and second three weeks after first cut. Means of the main and third tiller (average of the cutting treatments).

Cultivar	Before 1 st cut	Week 1-3 after 1 st cut	Week 4-6 after 1 st cut	Mean
Barry	0.175	0.161	0.155	0.164
Wendy	0.171	0.154	0.149	0.158
Condesa	0.147	0.145	0.143	0.145
mean	0.164	0.153	0.149	0.155
LSD ($P=0.05$)	0.010	0.008	0.010	0.004

Total dry matter accumulation and accumulation above the cutting height

Since tiller number increased, herbage yields per plant of the successive cuts increased almost exponentially as shown for Wendy in Fig. 2.3A. With weekly cuts at 2.5 cm, there was no significant difference in total dry matter accumulation and herbage harvested per plant between the three cultivars, whereas large differences with three-weekly cuts or with higher cutting heights were substantial (Fig. 2.3B and C). Cumulative dry matter accumulation above the cutting height until day 49 was maximal with cuts at 7.5 cm for Condesa, with cuts at 5 cm for Wendy and with cuts at 2.5 cm for Barry, both with weekly and three-weekly cuts (Fig. 2.3B). Total dry matter accumulation until the last cut was maximal with cuts at 7.5 cm for Wendy and Condesa and with cuts at 5 cm for Barry (Fig. 2.3C). Effects of cutting treatments on dry matter accumulation were very similar to effects on tiller number. However, differences in tiller number per plant were not the only cause of differences in dry matter accumulation, since also accumulation per tiller was strongly influenced by the different cutting treatments (Fig. 2.3D).

Dry matter distribution

The fraction of total biomass above the cutting height ('fraction harvestable') at day 28 was, of course, highest with cuts at 2.5 cm and lowest with cuts at 7.5 cm (Fig. 2.4A). Wendy and Condesa did not differ in this fraction. When cut at 5 and 7.5, Barry showed a lower fraction harvestable compared to Wendy and Condesa. At the final cut, all plants were cut at 2.5 cm. This enabled comparison between the cutting treatments of the fraction of dry matter accumulation occurring in the stubble below 2.5 cm and to shoot above 2.5 cm. With all cultivars, a shift in this distribution occurred towards a higher fraction allocated to shoots above 2.5 cm with the more frequent cuts at the shorter heights and to a lower fraction to shoots above 2.5 cm with the more lenient cutting treatments (Fig. 2.4B). Again, the effect of cutting height was strongest with weekly cuts. With Condesa a higher fraction of dry matter accumulation occurred in shoot above 2.5 cm than with Barry and Wendy (except with weekly cuts at 5 and 7.5 cm where this fraction was similar for Wendy and Condesa).

The fraction of total dry matter accumulation occurring in roots (= incremental root weight ratio) was approximately 20 % for all treatments and cultivars. Only with weekly cuts at 2.5 cm (for all three cultivars) and with three-weekly cuts at 7.5 cm for Barry and Wendy, the incremental root weight ratio was lower (Fig. 2.4D). With weekly cuts at 2.5 cm, the reduction of the incremental root weight ratio compared to three-weekly cuts at 7.5 cm was greatest for Condesa, intermediate for Wendy and only very small for Barry. Since the incremental root weight ratio was not very much influenced by the cutting treatments, the effect of cutting on the fraction of dry matter accumulation occurring in the stubble below 2.5 cm showed a pattern that was the reverse of the effect on the fraction going to shoot above 2.5 cm (Fig. 2.4C).

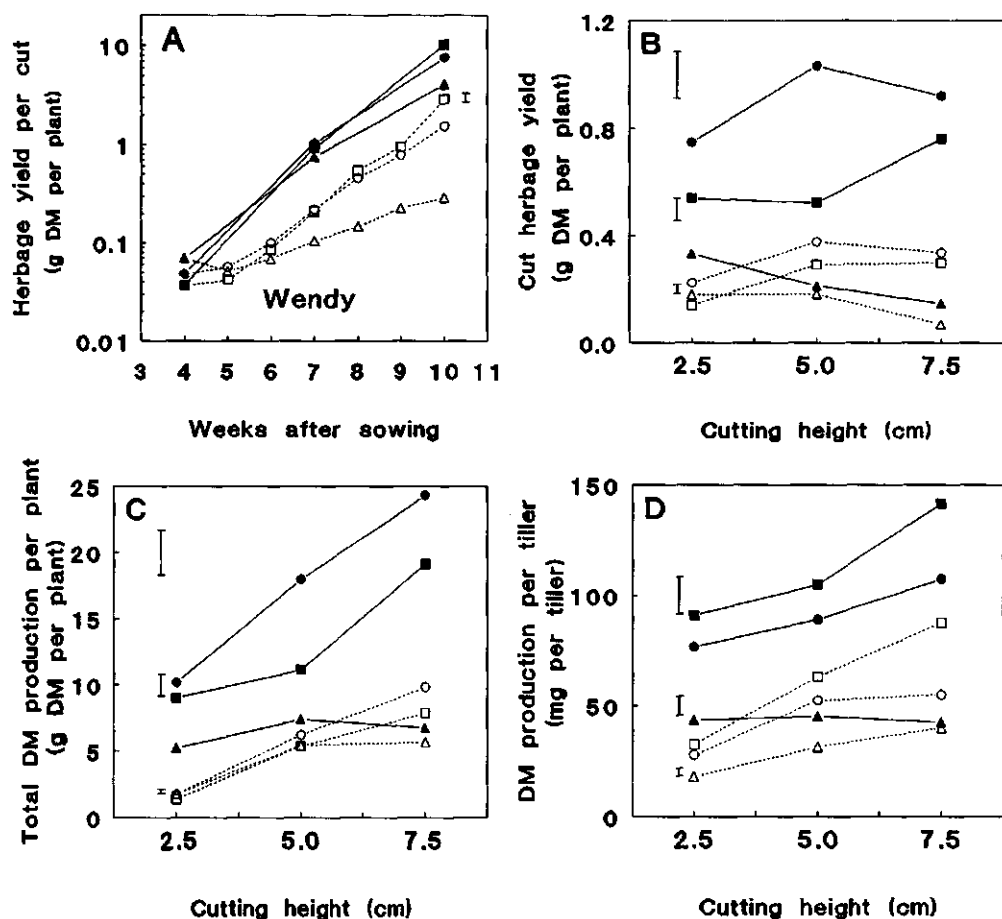


Fig. 2.3. Effect of cutting treatments on total dry matter accumulation and accumulation above the cutting height per plant and per tiller. A. Harvested herbage per cut of Wendy. B. Cultivar differences in total harvested herbage of cut 2, 3 and 4 for weekly cuts and of cut 2 of three-weekly cuts. C. Total dry matter accumulation. D. Total dry matter accumulation per tiller present at day 70. In A: Three-weekly cuts: ▲ 2.5, ● 5, ■ 7.5 cm cutting height. Weekly cuts: ▲ 2.5, ○ 5, □ 7.5 cm cutting height. In B, C and D: three-weekly cuts: ▲ Barry, ● Wendy, ■ Condesa. Weekly cuts: ▲ Barry, ○ Wendy, □ Condesa. Bars indicate LSD ($P=0.05$). In B, C and D the LSD is proportional to the mean, because the quantities were log-normally distributed.

Specific leaf area

At the first cut on day 28, the specific leaf area of Barry was 294, of Wendy was 303 and of Condesa was 266 $\text{cm}^2 \text{g}^{-1}$. With three-weekly cuts, SLA at the final cut was lower than at the first cut, and with weekly cuts, SLA was higher at the final cut than at the first cut, for all cultivars (Fig. 2.5). With weekly cuts, SLA of all cultivars increased with decreasing cutting heights. Wendy even had a 1.6 times higher SLA with weekly cuts at 2.5 cm compared to cuts at 7.5 cm. With three-weekly cuts, only SLA of Wendy was higher with cuts at 2.5 cm than with cuts at 5 and 7.5 cm. SLA of Barry and Condesa was not greatly influenced by cutting height with three-weekly cuts.

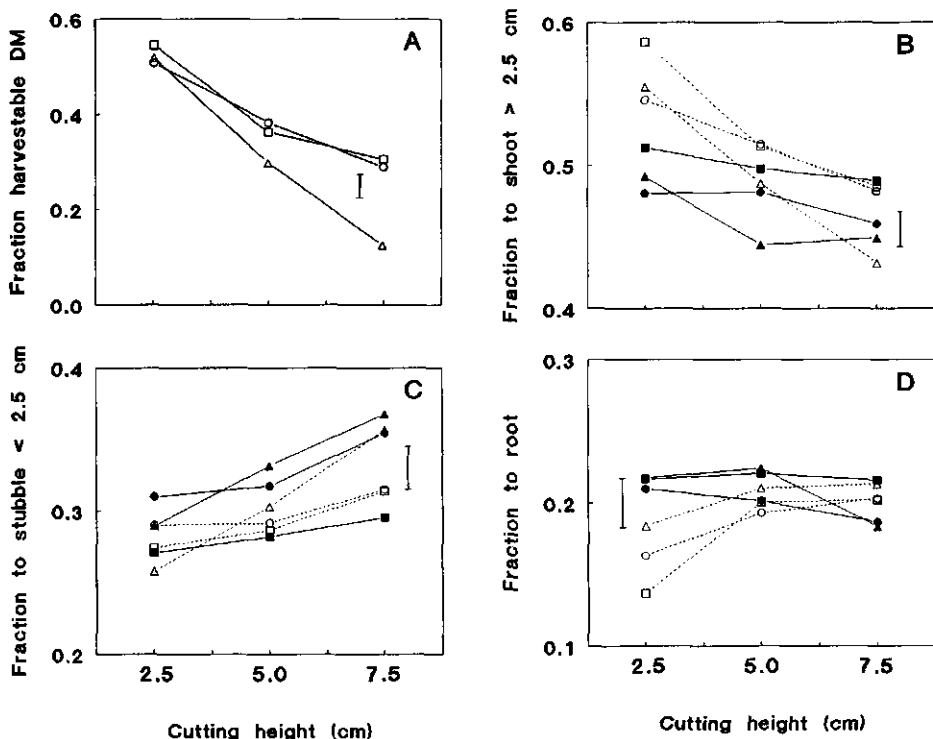


Fig. 2.4. Dry matter distribution. A) Fraction of total plant dry weight above cutting height ('fraction harvestable DM') at the first cut (day 28). Δ Barry, \circ Wendy, \square Condesa. B), C), D) Fraction of dry matter accumulation occurring above 2.5 cm, occurring in the stubble (<2.5 cm) and in roots. Three-weekly cuts: Δ Barry, \bullet Wendy, \blacksquare Condesa. Weekly cuts: Δ Barry, \circ Wendy, \square Condesa. Each point is based on means of six plants. Bar indicates LSD ($P=0.05$). F5

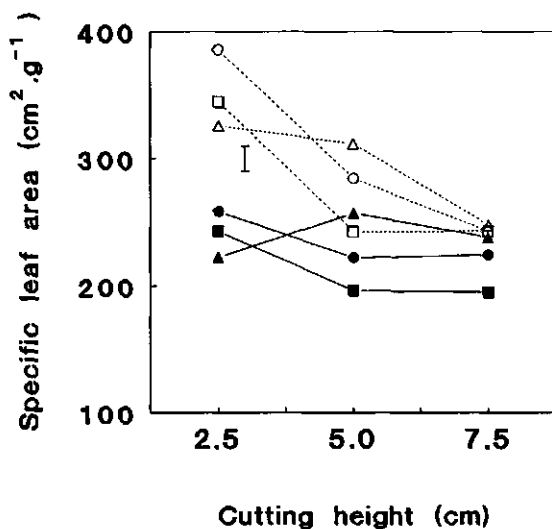


Fig. 2.5. Specific leaf area at the final cut. Weekly cuts: Δ Barry, \circ Wendy, \square Condesa. Three-weekly cuts: Δ Barry, \bullet Wendy, \blacksquare Condesa. Points are means of 6 plants. Bar indicates LSD ($P=0.05$).

Effects on leaf morphology

The increase of SLA and fraction allocated to above 2.5 cm stubble height with more frequent cuts at lower height enabled plants to maintain the leaf area expansion rate when the growth rate declined as a consequence of the removal of leaf area. However, the increase of both could not prevent that the leaf expansion rate and leaf size declined with increasing defoliation severity (Fig. 2.6). Leaves attained their maximal length with three-weekly cuts at 7.5 cm. With this treatment, all cultivars showed an increase in leaf length of successive leaves on the main tiller until the end of the experiment (shown for Wendy in Fig. 2.7A and 2.7.B). The final leaf blade length of the first leaf of Barry was 5.4 cm, of Wendy was 7.4 cm and of Condesa was 9.2 cm (average for the first and third tiller). With three-weekly cuts, the increase of the leaf lengths of successive leaves on the main tiller was greatest for Condesa and smallest for Barry as can be inferred from the length of the ninth leaf (Fig. 2.6).

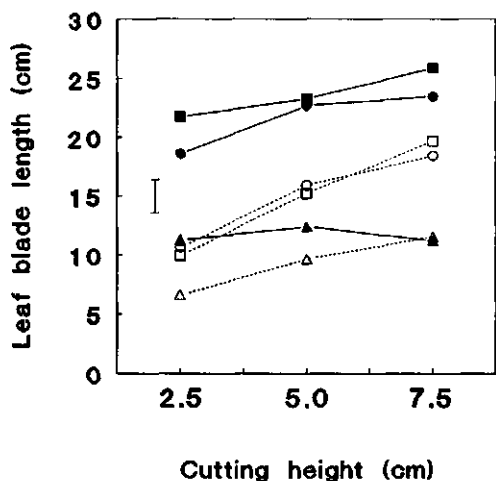


Fig. 2.6. Effect of cutting treatments on final leaf blade length of leaf 9. Three-weekly cuts: ▲ Barry, ● Wendy, ■ Condesa. Weekly cuts: △ Barry, ○ Wendy, □ Condesa. Points are means of 6 replicates. Bar indicates LSD ($P=0.05$).

Cutting at 5 cm did hardly change the total leaf blade length compared to cuts at 7.5 cm (shown for Wendy in Fig. 2.7A and B). Weekly cuts and cuts at 2.5 cm reduced the leaf lengths of all leaves formed after the first cut. With weekly cuts at 2.5 cm, leaf length of Barry was less influenced than leaf length of Wendy and Condesa, which showed a decrease with time and leaf number, while leaf length of Barry remained constant with that treatment. With three-weekly cuts, leaves which were expanding at the time of the cut, did not reach their potential leaf size, since they often reached a smaller total length than their preceding and succeeding leaves (Fig. 2.7B). Generally, a steady increase of the sheath lengths of successive leaves occurred, which was disturbed when sheath lengths became larger than the cutting height (results shown for Wendy, Fig. 2.7C and D). With lower cutting heights, sheath length was reduced, but still showed a tendency to increase with leaf number. Sheath lengths of Barry were so small that the increase of sheath length with leaf number was only disturbed by weekly cuts at 2.5 and 5 cm (Table 2.4). Sheath lengths of Wendy and Condesa on the main tiller were similar and similarly affected by weekly cuts. At weekly cuts at 2.5 cm, however, sheath lengths of Condesa were relatively more reduced than of Wendy.

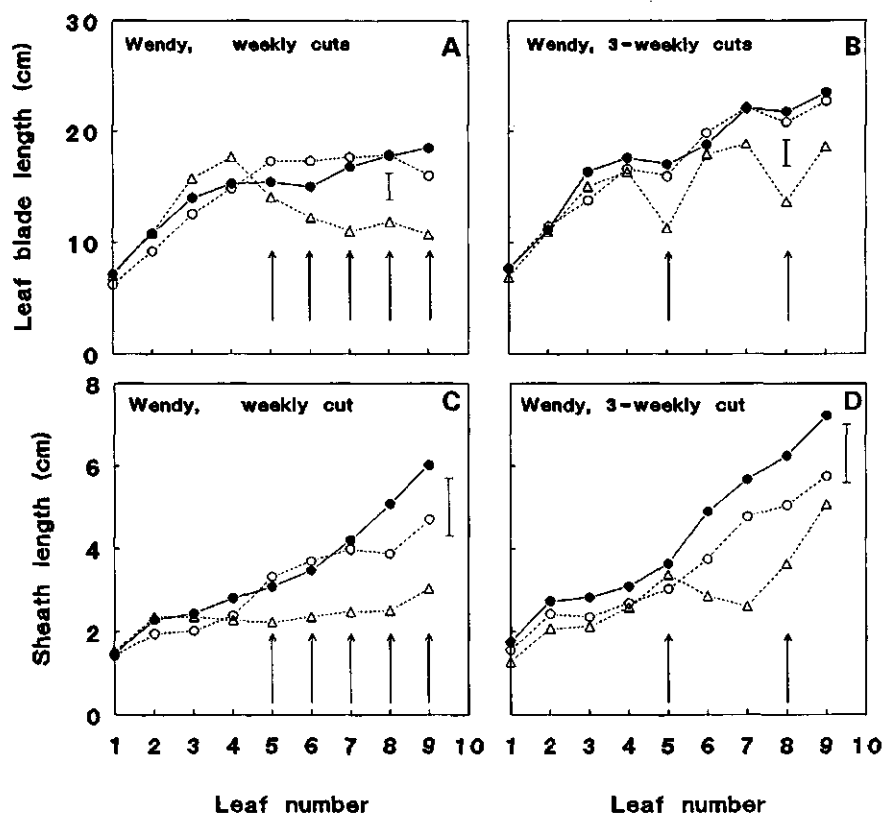


Fig. 2.7. The relationship between leaf size and leaf number on the main tiller of Wendy. A. Final leaf blade length, weekly cuts. B. Final leaf length, three-weekly cuts. C. Sheath length, weekly cuts. D. Sheath lengths, three-weekly cuts. Cutting heights: Δ 2.5 \circ 5 \bullet 7.5 cm. Arrows indicate leaf numbers of leaves that were cut while still expanding. For cut leaves final leaf blade length is the length before the cut plus the increase in length after the cut. Bar indicates LSD ($P=0.05$).

Table 2.4. Sheath length of the ninth leaf (cm) on the main tiller.

	Weekly cuts			3-weekly cuts			
	Cutting height 2.5	5	7.5	2.5	5	7.5 cm mean	
Cultivar							
Barry	2.0	2.5	3.2	3.2	3.6	3.3	3.0
Wendy	3.0	4.7	6.0	5.1	5.8	7.2	5.3
Condesa	1.8	4.4	5.0	6.1	5.2	6.7	4.8
mean	2.3	3.9	4.7	4.8	4.8	5.7	

LSD_{cultivar} = LSD_{height} = 0.7; LSD_{frequency} = 0.5

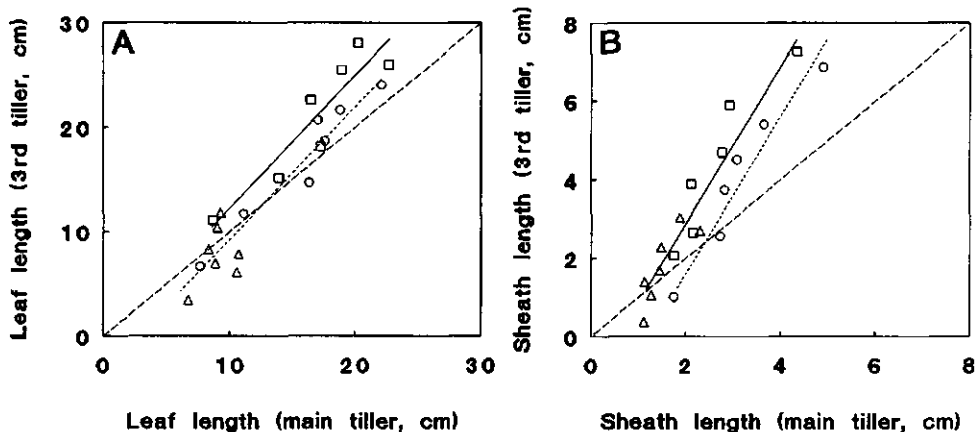


Fig. 2.8. Relationship between leaf morphology of leaves with the same leaf number on tiller number 3 and on the main tiller, for three-weekly cuts at 7.5 cm. A. Final leaf blade length. B. Sheath length. Δ Barry; \circ Wendy; \square Condesa. Leaf number 1 to 7 have been used. Dashed lines represent the 1:1 line. Regression lines (solid and dotted): $L_3 = 1.3L_1 - 3.5 + 3.0C$; $R^2_{adj} = 0.907$, $SD = 2.3$; $C = 0$ for Barry and Wendy, $C = 1$ for Condesa; $SL_3 = 2.0SL_1 - 1.2 - 1.2W$, $R^2_{adj} = 0.921$, $SD = 0.6$; $W = 0$ for Condesa and Barry, $W = 1$ for Wendy. L_1 and SL_1 are leaf blade and sheath lengths with the same leaf number on tiller 1; L_3 and SL_3 are leaf blade and sheath lengths on the third tiller.

Fig. 2.8A shows that the relation between leaf length with three-weekly cuts at 7.5 cm and leaf number was approximately the same for the main tiller as for the third tiller. There is a tendency that leaves on the third tiller were somewhat larger than the leaves on the main tiller for the higher leaf numbers. Sheath lengths of leaves of the same leaf number were generally longer on the third tiller than on the main tiller, in particular in the case of Condesa (Fig. 2.8B). Leaf width increased with leaf length (Fig. 2.9A). When averaged over all cutting treatments, mean leaf width of Barry was lowest, of Wendy was intermediate and of Condesa was highest. The relation between leaf width and leaf blade length could be described by one linear relation for the three cultivars (Fig. 2.9A). This relation was used to derive a quadratic relation between area per leaf and leaf blade length (Fig. 2.9B).

2.4 DISCUSSION

Genotype comparisons of regrowth capacity

It was expected that genetic differences in regrowth capacity would be expressed most strongly with frequent cuts at low cutting height. However, with weekly cuts at 2.5 cm cultivar differences in growth and harvestable yield were generally small, while cultivar differences with three-weekly cuts at 7.5 cm were largest. Davies (1974) used the ratio of growth with cutting to undisturbed growth as a measure of the extent to which a genotype was set back by cutting, in order to arrive at a measure that is independent of the inherent growth rate of the genotype. Here a similar measure may be calculated: the ratio of biomass produced with weekly cuts at 2.5 to biomass produced with three-weekly cuts at 7.5 cm.

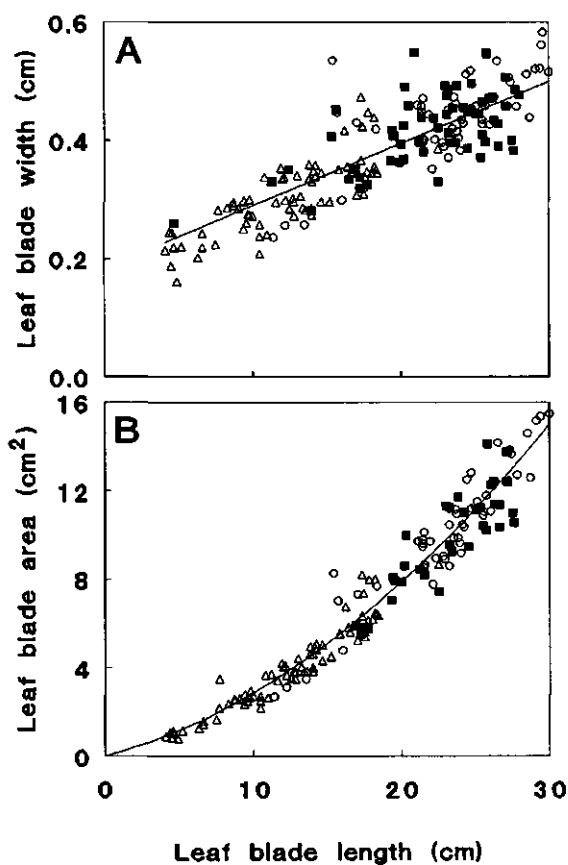


Fig. 2.9. Relationship between area per leaf, leaf width and leaf blade length of intact leaves at the final cut. A) Mean leaf width versus leaf length. Regression line: $W_{est} = 0.185 + 0.0105 * L$, $R^2_{adj} = 0.67$; W_{est} = estimated mean leaf width (cm) and L = leaf blade length (cm). B) Leaf area (A) versus leaf blade length. Line indicates $A = W_{est} * L$. Δ Barry, \circ Wendy, \blacksquare Condesa.

This ratio is 0.264 for Barry, 0.074 for Wendy and 0.073 for Condesa. For weekly cuts at 5 cm compared to three-weekly cuts at 7.5 cm, these ratios are 0.79 for Barry, 0.255 for Wendy and 0.283 for Condesa. So, Barry was set back least by frequent cuts. This is caused by the relatively lower harvestable fraction through the short sheaths and leaf blades of Barry.

Effect of cutting on tillering and leaf appearance

Tillering rates in grasses can be influenced by, among other factors, assimilate availability (Dayan, Van Keulen and Dovrat, 1981), hormones (Jewiss, 1972; Jinks and Marshall, 1982) and light quality (Simon and Lemaire, 1987; Casal, Sanchez and Gibson, 1990). In a sward, all of these will be influenced by cutting. In the present study, effects of cutting on light quality were eliminated as far as possible by the use of spaced plants.

More frequent cuts at lower heights increasingly reduced tillering rates, partly through effects of cutting height on leaf appearance rate (i.e., tiller bud formation per tiller), partly through effects of cutting height and frequency on site filling. This was also found by Hume (1990) for both prairie grass (*Bromus willdenowii* Kunth) and perennial ryegrass, with single plants. Also, Davies (1974) found that with increasing defoliation severity site filling and leaf appearance rate of perennial ryegrass became smaller. However, reduced tillering rates have

not been generally found in the field when different cutting treatments were compared. Jones (1981), for example, found a higher tiller density with continuous grazing compared to infrequent cutting. In the field, cutting reduces the leaf area index and therefore increases the red/far-red ratio perceived by tiller buds. Since an increased red/far-red ratio stimulates site filling (Casal, Sanchez and Gibson, 1990), more frequent cuts at lower cutting heights result in *higher* tillering rates in swards.

Also, leaf appearance rates are not always negatively affected by cutting in swards. In the field, Grant *et al.* (1981) found higher leaf appearance rates with higher grazing pressure, although the effect was transient and disappeared after some weeks. Grant *et al.* (1981) supposed that the temporarily increased leaf appearance rate could be due to increased light levels reaching soil level with more severe defoliation, since at higher light levels the leaf appearance rate is higher (Mitchell, 1953). Also, the absence of a negative effect on leaf appearance rate of a low cutting height with the dense swards in the glasshouse experiment of Grant *et al.* (1981), may have been caused by the positive side effect of an increased irradiance of the tiller bases with the low cutting height. In our experiment, no such side effects of cutting treatments on irradiance level near the tiller bases occurred and effects of cutting on leaf appearance rate and site filling may be explained by direct effects of the cutting treatments on for example assimilate availability or hormonal effects of cutting.

Effects of cutting on assimilate availability occur as a consequence of differences in residual leaf area after cutting and/or the amount of reserves in the stubble with the different treatments. Whether a possible effect of assimilate availability to growing tiller buds alone is sufficient for explaining the effect cutting on site filling, will be examined in a next paper (Chapter 3) in which a model will be presented for tillering, leaf area expansion and regrowth after defoliation.

Potential tiller and leaf size and effects of cutting on leaf size

The size of individual tillers increased with the age of the tiller as the number of leaves increased and - except in the most severe cutting treatment (high frequency and lowest height) - as the size of leaves increased with leaf number on the tiller. The size of tillers was maximal with three-weekly cuts at 7.5 cm. With that cutting treatment, leaf blade lengths were not much higher than with three-weekly cuts at 5 cm, so the leaf size attained with three-weekly cuts at 7.5 cm will probably be close to the potential leaf size. The size that a leaf will attain depends on the number of cells produced by the leaf meristem and the final size these cells will reach. Probably, an increase in the leaf primordium size is responsible for the increase of leaf length with leaf number on a tiller. Results of this study indicate that the relationship between potential leaf blade length and leaf number on a tiller is approximately the same for older and younger tillers. This opens the possibility of using that relation to simulate the increase of leaf area per plant on the basis of this relationship and the age distribution and the total number of tillers on a plant. Sheath lengths on the third tiller were larger than on the main tiller at the same leaf number (Fig. 2.8B).

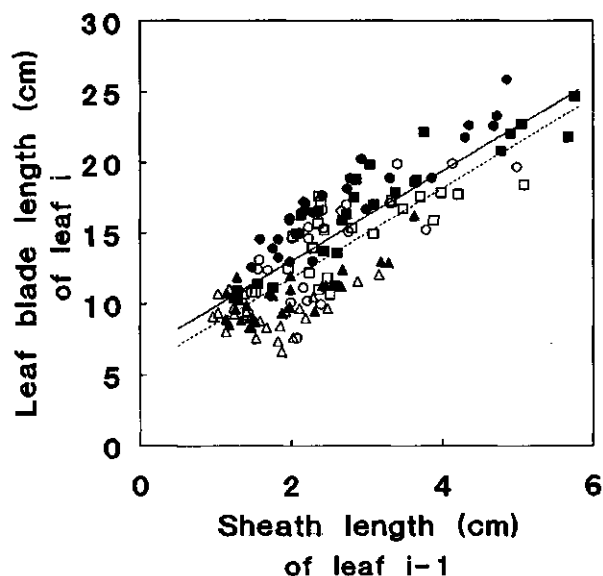


Fig. 2.10. The relationship between final leaf blade length and the length of the sheath tube (sheath length of the previous leaf number). Three-weekly cuts: \blacktriangle Barry, \bullet Wendy, \blacksquare Condesa. Weekly cuts: \triangle Barry, \circ Wendy, \square Condesa. Each point is based on means (of six replicates) for a leaf number on the main tiller of one of the six treatments. Regression lines: solid line, three-weekly cuts; dashed line, weekly cuts; $Y = 6.73 - 1.28F + 3.18X$, Y = leaf blade length of leaf number i on the main tiller (cm), X = sheath length of leaf number $i-1$ (cm), $F = 0$ for three-weekly cuts and $F = 1$ for weekly cuts. $R^2_{adj} = 0.697$, $SD = 2.38$.

The higher the frequency of defoliation and the lower the cutting height, the more leaf and sheath length were reduced compared to three-weekly cuts at 7.5 cm. It is not easy to explain this effect of cutting on final leaf size by an effect of cutting on leaf primordium size, since cutting also affected the final length of leaves that were already expanding. It is also hardly conceivable that the leaf extension zone itself is directly changed by cutting, since elongation of leaves takes place in the basal part of leaves. For leaves growing in sheaths of 7.5 cm the elongation zone is confined to the basal 20 to 30 mm (Schnyder, Keo and Kuhbauch, 1989). With cuts at 5 and 7.5 the elongation zone is therefore not removed. With cuts at 2.5 cm, part of the elongation zone may have been removed. The effect of cutting on the final leaf blade length may also be mediated by an effect of the length of the sheath tube in which that leaf developed, since we found a positive correlation between leaf blade length and the length of the sheath tube (Fig. 2.10A). Such a positive correlation between leaf and sheath length has also been reported by Grant *et al.* (1981). An alternative hypothesis for the negative effect of low cutting heights on leaf length and elongation could be that assimilate availability reduces leaf elongation. However, the increased leaf elongation duration with

shade treatments of tall fescue (*Festuca arundinacea* L.) found by Allard, Nelson and Pallardy (1991) does not support the hypothesis of a negative effect of a reduced assimilate availability on leaf extension.

Tiller morphology (sheath and leaf blade length, blade width and thickness) has an important impact on the fraction of leaf remaining in the stubble and the fraction of shoot that can be harvested above the cutting height. Cultivars differed in the increase of sheath length of successive leaves and also showed a different response of the sheath length to cutting treatments. In the field, a fast adaptation of the sheath length to a change in defoliation height is important to maintain a stable harvestable fraction and residual leaf area after defoliation.

Barry (a cultivar for lawns), did not show a capacity to increase its sheath length with less severe defoliations. Also, Barry could hardly increase its leaf length with less frequent cuts or cuts at higher height. The fraction of dry matter accumulation occurring above the cutting height was therefore less for Barry than for Wendy and Condesa, particularly at higher cutting heights. Although Wendy and Condesa showed almost the same leaf and sheath lengths on the main tiller, the fraction of harvestable shoot of Wendy was often somewhat lower than of Condesa. This was caused by the longer sheaths of younger tillers of Condesa (Fig. 2.8B). Furthermore, Wendy had a larger proportion of younger tillers than Condesa, because the relative tiller appearance rate of Condesa was lower than of Wendy. Leaves on younger tillers have a lower leaf number and therefore had shorter sheaths than leaves on older tillers. Therefore, relatively less shoot material will have been harvested above the cutting height of younger than of older tillers.

Changes in SLA: a plastic response to cutting and duration of growth

When leaf expansion rate and assimilate flow to leaves are not strictly related, a rise in specific leaf area (SLA) may be expected when the total assimilation rate is suddenly reduced by defoliation. This rise in SLA will be larger when the cut is more often or more severe. Indeed, we found higher values of SLA with more frequent cuts at lower cutting heights. Therefore, we conclude that leaf expansion was at least partially independent of assimilation supply to the leaf. Cutting reduced total leaf lengths most when high values of SLA were reached, suggesting that at high values of SLA leaf expansion depended on assimilate supply. The capacity to increase SLA after defoliation can be regarded as a good characteristic of a cultivar, because it enables a faster leaf expansion after defoliation. This gives a faster regrowth rate and better competitive capacity. Wendy showed the largest capacity to increase SLA with frequent and severe defoliation.

With prolonged growth duration after cutting (when assimilates become abundantly available), a reduction of SLA would be an advantage. The capacity to decrease SLA in that situation would reflect the capacity to increase or maintain the sink size of leaves or, in other words, the capacity to use leaves as a storage organ. Storing more carbohydrates in the

leaves instead of in the stubble would increase the fraction harvestable shoot. Condesa showed the lowest SLA at infrequent cuts at high cutting height and also allocated a larger proportion of dry matter to above 2.5 cm.

Our results on SLA are in agreement with results of Sheehy, Cobby and Ryle (1980) who showed that SLA was high just after cutting and declined with time after cutting. Such a pattern with time would result in a higher SLA with more frequent cuts. However, Grant, *et al.* (1981) showed the reverse trend of SLA: an increase of SLA with time. Grant *et al.* (1981) reported a lower SLA with higher cutting heights, which is in agreement with the present results. When leaf area expansion is independent of assimilation rate, perhaps an increase of SLA with time after defoliation is also possible in swards. In swards, the mean assimilation rate per unit leaf area declines and the crop respiration rate increases with time after defoliation. Also the fraction of assimilates allocated to roots may increase with time after defoliation as crop photosynthesis increases (Sheehy *et al.*, 1980). Therefore, the availability of assimilates to new leaf area could decrease with time in a sward, which would lead to higher values of SLA.

Since SLA may depend on time after cutting and on assimilate supply to leaves, use of SLA as a constant parameter to derive the rate of increase of leaf area from leaf growth in regrowth models for grass does not seem valid. Perhaps, SLA based on *structural* (mainly cell wall) material will prove to be more constant, since a large part of the variation in SLA based on total leaf dry matter is caused by variation in the ratio of structural to non-structural material (see also Chapter 4).

Dry matter distribution between harvestable shoot, stubble and root

According to the theory of the functional equilibrium between roots and shoots, a higher allocation of assimilates to shoots may be expected after defoliation (Brouwer, 1963; Thornley, 1972; review by Wilson, 1988). The response of dry matter distribution to cutting regime found in this study was not completely in agreement with such functional balance theories, since only with the weekly cuts at 2.5 cm a decrease was found in the fraction of dry matter accumulation occurring in roots. The distribution of dry matter *within* the shoot responded much more to the different cutting treatments: with three-weekly cuts at 7.5 cm a higher fraction of dry matter accumulation occurred in the stubble than with weekly cuts at 2.5 cm, with intermediate cutting treatments showing intermediate results. This was also found by Simons, Davies and Troughton (1972) when comparing the effect of cutting at 2 or 5 cm. As was found in the present study, also Simons *et al.* (1972) found that the effect of cutting on above ground biomass distribution was biggest for the cultivar with the highest leaf appearance rate and smallest leaves. The results of Simons *et al.* (1972) and our results can only be directly compared for the first cut, where we measured the fraction of biomass above the cutting height (Fig. 2.4A). For the period after the first cut, we determined the *incremental* fraction of biomass production allocated to the shoot above and below 2.5 cm. For this purpose, we determined the cumulative dry matter accumulation above 2.5 cm

stubble height. Dividing this accumulation above 2.5 cm by the total dry matter accumulation since the first cut gave the incremental fraction of dry matter accumulation occurring above 2.5 cm. Using this method it was possible to decide that a real shift in dry matter distribution (between root, stubble below 2.5 cm and herbage above 2.5 cm cutting height) had occurred. By only comparing the fraction of biomass allocated to above the cutting height at different cutting heights this is not possible, since the fraction above the cutting height will be lower with higher cutting height due to the limited size of leaves.

The higher fraction of dry matter accumulation occurring in the stubble with higher cutting heights can be explained by an "overflow" of substrate from leaves to the stubble when substrate availability is high. This will occur when the sink size of growing leaves is larger than the source size. The process of storage of assimilates in the stubble when assimilates are abundant and of usage of stored assimilates during regrowth is very important for the grass crop, since the initial regrowth after defoliation depends on availability of substrate in the stubble when little leaf area remains in the stubble after defoliation.

Chapter 3

A regrowth model for spaced plants of perennial ryegrass incorporating effects of tillering and leaf morphology

with E.A. Lantinga

ABSTRACT

A model for the regrowth after defoliation of perennial ryegrass is presented in which sink-source relations are explicitly simulated. The model was used to evaluate differences between cutting treatments (weekly *versus* three-weekly cuts at 2.5, 5 and 7.5 cm) in tillering and regrowth found in a glasshouse experiment with spaced plants of perennial ryegrass. In the model, the tillering rate is a function of bud formation and development, and of substrate availability to buds. The observed relationship between leaf size and leaf number of the low frequency/high cutting height treatment in the experiment was used to calculate the potential leaf expansion rate. In the model, this potential rate determines the substrate demand of growing leaves (sink size). The actual expansion rates and allocation of dry matter to leaves are less than the potential rates when substrate availability (source size) is not sufficient.

With the simulation model, we could explain the increase in fraction of dry matter allocated to shoot above 2.5 cm, the increase in specific leaf area (SLA) and the reduced tillering that occurred especially with the high frequency/low cutting height treatment in the experiment. The model predicts that the specific leaf area (SLA) will increase just after defoliation until a maximum is reached and will decline thereafter. This temporal variation in SLA was biggest with the highest cutting frequency and with the lowest cutting height. Further, the model predicts that just after cutting a large fraction of leaf growth comes from the substrate pool in the stubble. However, this fraction declined very fast in a few days after cutting as current assimilation of new leaves provided an increasing proportion of the required substrate for leaf growth.

3.1 INTRODUCTION

Leaf expansion of perennial ryegrass (*Lolium perenne* L.) after cutting depends on both substrate supply (source size) and demand for substrate (sink size). Substrate supply to expanding leaves of grass depends both on the current assimilation rate and the availability of substrate from the stubble. Sink size of leaves of perennial ryegrass depends on maximum growth rate per tiller and the tiller density or, for spaced plants, the number of tillers per plant. Source size will be limiting for leaf expansion after cutting, when the substrate demand of leaves larger than the availability. This occurs, for example, just after cutting when the leaf area and the amount of substrate in the stubble is small. Then, either the leaf expansion rate will be reduced and/or the specific leaf area (SLA) will increase. Sink size limits leaf area

expansion when substrate availability to leaves is higher than demand. Evaluations of the relative importance of source or sink limitations during regrowth are scarce, but are important for a better understanding of the regrowth of grass. Johnson and Thornley (1983) presented a model for the regrowth of grass in which leaf area expansion does not depend directly on the current assimilation rate, but is influenced by the level of an assumed storage pool. However, in that model, it is impossible to evaluate the influence of tiller density and tiller morphology on sink/source-relations. Dayan, Van Keulen and Dovrat (1981) presented a model (TILDYN) for tillering and regrowth of Rhodes grass. However, TILDYN is not suitable for simulating the effect of cutting height, since it does not include a substrate pool. Therefore, regrowth from a stubble with no residual leaf area after cutting cannot be simulated with TILDYN. In this paper we present a model for the regrowth after defoliation of perennial ryegrass including the effects of cutting height and frequency on tillering, leaf area dynamics and regrowth rates.

Sink size factors incorporated in the model are tillering parameters and the maximum growth rate per tiller. Source size factors are the current assimilation rate, the weight of the substrate pool and the weight of unemerged leaves in the stubble. The tillering parameters are i) leaf appearance rate, ii) potential site filling, i.e. the maximum relative increase in tiller number per leaf appearance interval (Neuteboom and Lantinga, 1989) and iii) the minimum substrate requirement for the production of one new tiller. A test of the model was carried out by comparing results from simulations and of a cutting experiment with widely spaced, vegetative plants of perennial ryegrass (Chapter 2).

3.2 MODEL DESCRIPTION

Model structure

The model is a dynamic, explanatory model for the regrowth of spaced vegetative perennial ryegrass plants. Regrowth, tillering and leaf area expansion after defoliation, at the plant level, are explained from processes at the plant organ level (tillers). The structure of the model is shown in three relational diagrams: dry matter accumulation (Fig. 3.1), leaf area expansion (Fig. 3.2) and tillering (Fig. 3.3). Environmental factors are supposed to be constant. Temperature, water and mineral nutrients are supposed to be non-limiting for growth.

Four types of state variables occur in the model: weights, leaf area, and tiller and leaf numbers. Plant weight is divided into weights of unemerged, emerged and dead leaves, structural sheath material, non-structural material in the sheaths and unemerged leaves ('substrate') and roots. Leaf area is calculated for each of ten tiller age classes. Four leaf age classes per tiller age class are distinguished.

The following steps are taken in the calculation of the total growth rate, the leaf expansion rate and allocation of dry matter to roots, sheath, substrate in sheaths, unemerged and expanding leaves:

- 1) Calculation of the source size for leaf growth.
 - a) Calculation of total growth rate (G_{new}), eqns. (1)-(2).
 - b) Calculation of the availability of new dry matter for leaf growth = source size, eqn. (3). The source size for leaf growth is the sum of the maximum rate of usage of substrate in sheaths for leaf growth, the flow of material from unemerged to emerged leaves and the part of the total growth rate not allocated to roots or structural material in sheaths and unemerged leaves.
- 2) Calculation of the sink size of leaves, i.e. the demand of leaves for substrate, D , based on the potential leaf expansion rates of leaves, eqn. (4)
- 3) Calculation of actual growth rate of leaves, which is the minimum of the source size and sink size, eqn. (5).
- 4) Calculation of the actual leaf expansion rates, eqn. (6).
- 5) Calculation of flows from and to the substrate pool in sheaths, eqn. (7).
- 6) Calculation of growth rates of leaves, unemerged leaves, root, structural sheath material, rate of leaf death and total growth rate, eqns. (8-9).
- 7) Calculation of the "leaf age", t , in leaf appearance intervals, tiller bud formation rate and tillering rate, eqns. (10)-(11).
- 8) Changes in weights at cutting dates, eqns. (12)-(14).

These steps are explained below in more detail. The equations of the model and the explanation of the symbols are listed in the Appendix (3.5.1 and 3.5.2).

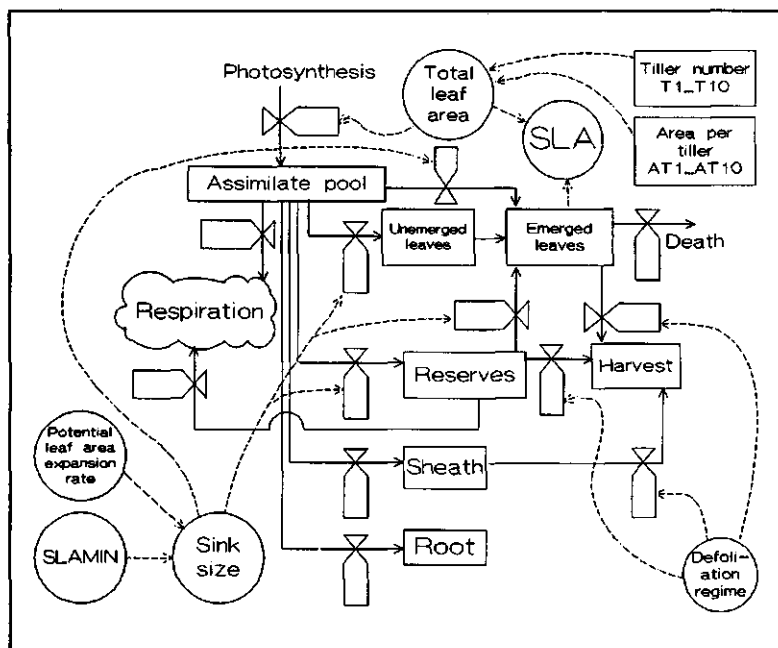


Fig. 3.1. A relational diagram of the regrowth model: dry matter accumulation. Rectangles are state variables, valves are rates and circles are driving variables. A dashed line indicates that one variable influences another variable. Solid lines indicate flows into and out of state variables. For explanation of symbols and acronyms: see Appendix (3.5.2).

Total growth rate

Since growth of spaced plants under constant light conditions is simulated with the model, CO_2 -assimilation is modelled using a constant gross leaf CO_2 -assimilation rate. The daily gross plant assimilation rate is the product of leaf area per plant, assimilation rate per leaf area and the daylength (eqn. 1). For more complex situations, with variable irradiance levels or for crop situations, eqn. (10) should be replaced by an appropriate light interception and assimilation module. In eqn. (2), the total growth rate is calculated from assimilation, respiration and conversion of assimilates into structural dry matter as in SUCROS87 (Spitters *et al.*, 1989).

Distribution of newly formed material

It is assumed that a fixed fraction of newly formed dry matter is distributed to roots (f_{rt}). Also, fixed fractions of dry matter are allocated to structural material of sheaths (f_{sh}) and unemerged leaves (f_{unlf}). The remaining fraction $((1 - f_{sh} - f_{unlf})(1 - f_{rt}))$ of newly formed dry matter is available for growing leaves. Source size is the sum of the rate at which substrate from current assimilation is available for leaves, the rate at which unemerged leaves emerge out of the sheath and the maximum rate of substrate use for leaf growth from the substrate pool in the sheaths (eqn. 3). The source size determines the potential growth rate of expanding leaves. How much is really used for leaf growth depends on the sink size of expanding leaves (eqns. (4a)-(4d) and eqn. (5)).

Structural material and the substrate pool in sheath and unemerged leaves

Dynamics of the weight of the substrate pool in the sheaths and unemerged leaves are described by eqns. (7a) - (7d). Material will accumulate in this substrate pool, when the source size is higher than the sink size of emerged, growing leaves. Material from the substrate pool is available for growth of leaves, when sink size is higher than source size. Reallocation from the substrate pool to leaves is assumed to occur at a maximum daily rate of 10 % of the amount of substrate in the sheaths ($\tau_{res} = 10$ d). Curry *et al.* (1989) showed that the decline of the concentration of soluble carbohydrates occurred with a time constant of about 5 d, but this decline was caused by use of substrate for both leaf growth and maintenance respiration. Material from the substrate pool may also be used for maintenance respiration. Therefore, the daily maintenance respiration, R_m , may exceed the daily assimilation rate (eqn. 7d).

Unemerged leaves are transferred to emerged leaves as a function of the leaf appearance rate and the mean residence time (λ) of unemerged leaves in the sheath (see second term in eqn. (9b)). This procedure is similar to the one of Sheehy, Cobby and Ryle (1979). However, their class of unemerged leaves consist of both structural and non-structural material. In our model, the non-structural material in unemerged leaves is included in the same substrate pool as the non-structural material in sheaths. The class of unemerged leaves in our model therefore consists of structural material only.

Sink size of leaves

Sink size of leaves is determined by the potential leaf area expansion rate (eqns. 4a-4c), which is divided by a minimum specific leaf area (SLA_{min}) to arrive at the substrate demand of leaves, in dry matter equivalents (eqn. 4d).

The potential leaf expansion rate per plant is the sum of the potential expansion rates of all expanding leaves. The potential elongation rates of leaves were derived from the measured relation between final leaf length and leaf number counted from tiller base up, found in spaced plants cut to 7.5 cm at three-weekly intervals (Fig. 4). The potential elongation rate of a leaf is calculated in eqn. 4b as the potential final length ($L_{max}(ij)$) divided by the leaf extension duration. The leaf extension duration is more or less constant in terms of leaf appearance intervals, since the number of expanding leaves per tiller is approximately constant (about 1.3 expanding leaves per tiller, unpublished results). Therefore, the leaf extension duration is τ_{leaf} / L_A . Leaf width in the model is a function of potential final leaf length (eqn. 4c based on data from Chapter 2).

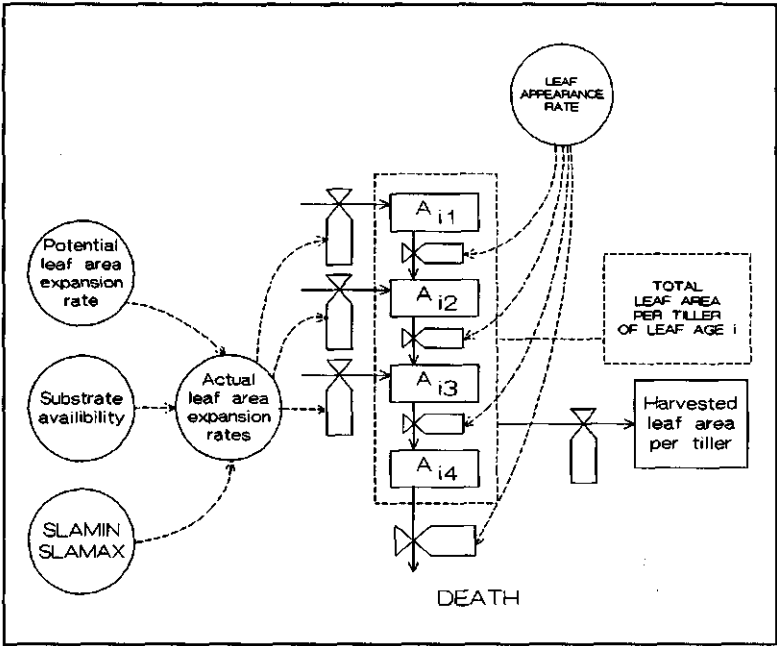


Fig. 3.2. A relation diagram of the regrowth model (continued): leaf area expansion of a tiller age class.

Actual leaf area expansion rate

Potential leaf expansion rates can be maintained as long as source size is higher than sink size (sink limited growth). When source size is less than sink size, two extreme situations can occur:

- 1) SLA remains the same and leaf expansion rates are reduced to the limit set by the source size times SLA.
- 2) SLA rises as leaf expansion rates are maintained at their potential level. In the model, the

actual leaf expansion rate will be equal to the potential until a specified maximum SLA (for new leaf area) is reached. When the maximum SLA has been reached, the actual leaf expansion rate is the product of the maximum SLA and the leaf growth rate (source limited growth, eqns. (6a) and (6b))

Tiller age classes

Difference in final leaf size and expansion rates between tillers differing in age make the introduction of tiller age classes in the model necessary. Final size of leaves shows a progressive increase with position along the tiller (Fig. 3.4). This relationship is more or less the same for all tillers (Chapter 2). Therefore, leaves on younger tillers reach a smaller final size than simultaneously expanding leaves on older tillers. The leaf extension duration varies only little with tiller age. Therefore, the lower final leaf size of the youngest fully expanded leaves on younger tillers is caused by a lower area expansion rate. The number of tillers in each of ten tiller age classes is simulated by use of the escalator box car train (Goudriaan and Van Roermund, 1989). Tiller age is expressed in leaf appearance intervals. In the model, all tillers and leaves in an age class are transported to the next age class, each time a new leaf appears (no dispersion in tiller appearance). The moment of leaf appearance is defined as the appearance of a new leaf tip on all tillers. At this time, new tillers are added to the first tiller age class. The last tiller class consists of tillers on which ten or more leaves have appeared.

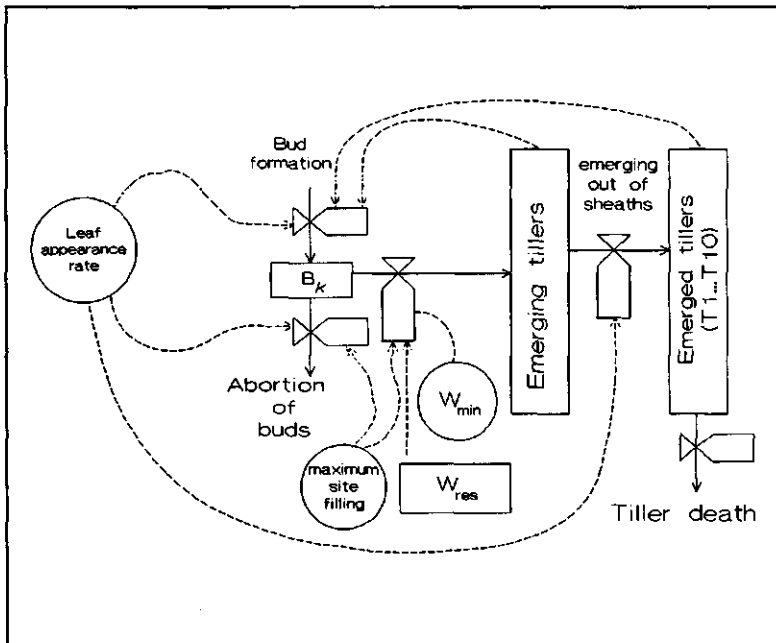


Fig. 3.3. A relation diagram of the regrowth model (continued): tillering.

Tillering

When a new leaf appears on a tiller, also a tiller bud has been formed in the axil of the new leaf. Also in the axil of prophylls (the first sheath-like leaf on a new tiller) tiller buds are

formed (Neuteboom and Lantinga, 1989). Therefore, the number of new tiller buds produced in one leaf appearance interval is the number of tillers present at the end of the leaf appearance interval. It is assumed that all tiller buds start to develop into tillers immediately after the bud has been formed. With maximum tillering, a tiller bud appears as a visible tiller one leaf appearance interval after the appearance of the leaf in the axil of which the tiller bud was formed; then tiller number doubles in one leaf appearance interval (Neuteboom and Lantinga, 1989). When new leaves appear in the model, the potential number of tiller buds that may develop into tillers is determined by the number of buds formed in the preceding leaf appearance interval (B_k). Whether or not all these tiller buds appear as new tillers one leaf appearance interval later, depends on the size of the substrate pool and on the minimum amount of substrate needed by a developing tiller bud before it emerges as a tiller (W_{min} , eqn. 11). This procedure is similar to the one used by Dayan *et al.* (1981) in their model TILDYN for Rhodes grass. In the present model, it was assumed that all dry matter needed for the formation of the first root, sheath and first real leaf should already be available for a tiller bud when it starts to grow into a tiller. W_{min} was calculated from the measured area of the first real leaf on the main tiller, an SLA of $500 \text{ cm}^2.\text{g}^{-1}$ and from an assumed leaf weight ratio of 0.7. Tiller buds are aborted, when no substrate is available at the moment their development into tillers should start. Therefore, no pool of dormant tiller buds will be formed. This is in accordance with both Porter (1985) and Skinner and Nelson (1992) who assume that a tiller bud only has a short "window of opportunity" during which it may successfully develop into a tiller. In the model, actual site filling, i.e. the relative tiller appearance rate divided by the leaf appearance rate is calculated from the simulated tillering pattern and the specified leaf appearance rate (eqn. 11e).

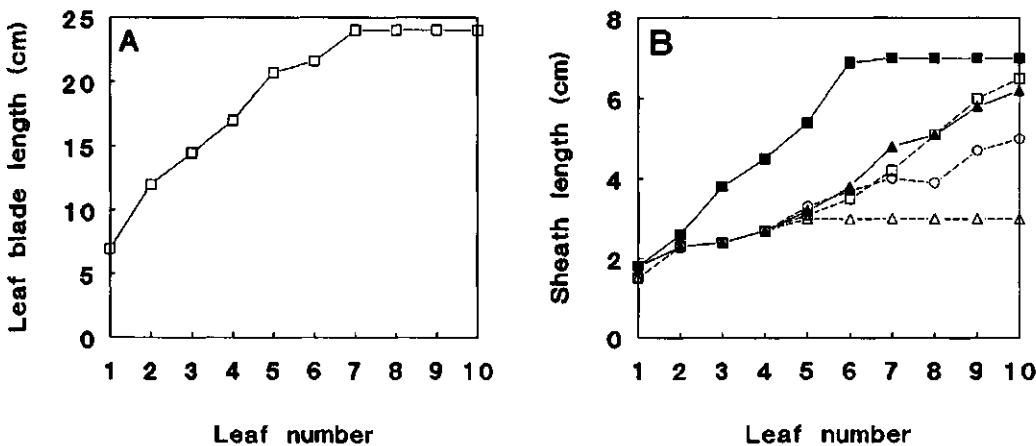


Fig. 3.4. The relationship between potential leaf blade length, sheath length and leaf number (counted from the tiller base) at different cutting regimes. A. Potential leaf blade length (independent of defoliation regime). B. Sheath length: weekly cuts: Δ 2.5; \circ 5; \square 7.5 cm cutting height; 3-weekly cuts: \blacktriangle 2.5 cm, \blacksquare 5 and 7.5 cm cutting height. Values of cultivar Wendy of the main and the third tiller (based on data from Chapter 2).

Leaf dynamics

A leaf goes through several stages of development, from leaf primordium initiation to leaf death. It takes several leaf appearance intervals from initiation of the leaf primordium until appearance of the tip of the leaf. From the moment of appearance of the leaf tip, it takes a constant number (τ_{leaf}) of leaf appearance intervals until the final length is reached. After reaching the final length, it is assumed that a leaf stays green during another $3 - \tau_{leaf}$ leaf appearance intervals. Thereafter, the green area is assumed to decline to zero with a relative rate of 0.2 d^{-1} (eqn. 6b). This life history of leaves results in a maximum number of live leaves per tiller between three and four, which is also found in reality (Davies, 1977). It is assumed that 50 % of the weight of dying leaves is restituted to the substrate pool (eqn. 7c), as suggested by Sheehy, Cobby and Ryle (1980) and Penning de Vries *et al.* (1989).

Cutting and leaf area remaining in the stubble

The fraction of leaf and sheath harvested and of leaf area remaining in the stubble after cutting and the stubble weight are determined by the leaf morphology (sheath length and leaf blade length and width) of the different tiller age classes (eqn. 12-14). The relation between sheath length and leaf number used in the model was determined experimentally for each of the six cutting treatments (Fig. 4B).

In practical crop situations, cutting is at a fixed height above soil level and then tillers and leaves are cut to different lengths depending on tiller and leaf angles. This is not simulated in this model, since the model was primarily used for comparison with the results of the cutting experiment in which each tiller was cut to the same length, irrespective of its angle, and in which leaves were held upright. Length of leaf blade remaining after cutting is therefore simply the cutting height minus the sheath length (if positive). The sheath length after cutting is simply the minimum of sheath length and cutting height. The harvest index of leaves is calculated as leaf area removed divided by leaf area before harvest (eqn. 13). The harvested fraction of structural sheaths and unemerged leaves and substrate pool in the sheaths is calculated per tiller class as the mean sheath length after cutting divided by the mean sheath length before cutting (eqn. 14). Weights of harvested and of remaining material are calculated in eqn. 12.

Initial values

Initial values were obtained from the cutting experiment for the situation after the first cut 28 days after sowing (Table 3.1). The initial stubble weight depended on the cutting height, because of differences in the amount of leaf blades in the stubble. The weight of structural material in sheaths, unemerged leaves and non-structural material in the substrate pool was the same for the different cutting heights.

Table 3.1. Initial values and cutting height dependent parameters used in the simulation model, 28 days after sowing.

Cutting height (cm)	2.5	5	7.5
Initial number of			
Tillers per plant	4.5	4.5	4.5
Tiller buds per plant	4.5	4.5	4.5
Appeared leaves (ligule visible) on main tiller	4	4	4
Initial weights (mg per plant):			
Roots	34	34	34
Stubble:			
Structural sheath	5	5	5
Structural unemerged leaves	10	10	10
Reserves	10	10	10
Emerged leaves	2.4	16	33
Leaf appearance rate per tiller (d^{-1})	0.148	0.150	0.156
SLA ($\text{cm}^2 \text{g DM}^{-1}$)	300	300	300
f_{rt}	0.17	0.20	0.20

Parameter values

Parameter values for the relation between potential leaf size and leaf number were obtained from the cutting experiment (Fig. 3.4A). Also, the minimum SLA and the fraction of dry matter allocated to roots was taken from the experiment in Chapter 2 (Table 3.1). The total daily radiation was fairly low (about 4 MJ m^{-2} or $18.8 \mu\text{mol m}^{-2}$, photosynthetic active radiation) with an average intensity of 75 W m^{-1} or $350 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Therefore, the leaf gross CO_2 -assimilation rate was set at $25 \text{ mg dm}^{-2} \text{ h}^{-1}$ (based on Woledge, 1973). Information on these and other parameter values is given in Table 3.1 and the Appendix (3.5.2).

3.3 SIMULATION RESULTS

Comparison of simulation results with experimental results

Generally, a good correspondence between simulated and measured yields was found (only small deviations from the 1:1-line, Fig. 3.5A). However, measured yields of the first cut of the three-weekly cuts exceeded simulated yields by 20-50 %. The simulated fractions of dry matter accumulated above 2.5 cm were close to the experimental results as can be seen from the small deviations from the 1:1-line (Fig. 3.5B). The fraction dry matter accumulated above 2.5 cm was highest with weekly cuts at the lowest cutting height (2.5 cm). In the experiment the fraction above 2.5 cm was determined instead of the fraction above the cutting height, because a comparison of the allocation of material instead of a comparison of the harvest index was to be made. The differences in fraction dry matter accumulated above

2.5 cm between cutting treatments may be explained from differences in sink and source size between cutting treatments. With lower cutting heights and with more frequent cuts less leaf area and less substrate remains in the stubble and therefore just after cutting sink size is larger than source size. In the model, this leads to relatively greater use of newly formed assimilates for leaf growth (all of which occurs above 2.5 cm) and to a relatively smaller accumulation of substrate in the sheath (of which at least 50 % occurs below 2.5 cm, depending on sheath length). With three-weekly cuts at 7.5 cm, source size is often higher than sink size, leading to a relatively lower use of new assimilates for leaf growth and to accumulation of substrate. The small deviations from the 1:1-relationship between simulated and experimental result give support to the hypothesized mechanisms.

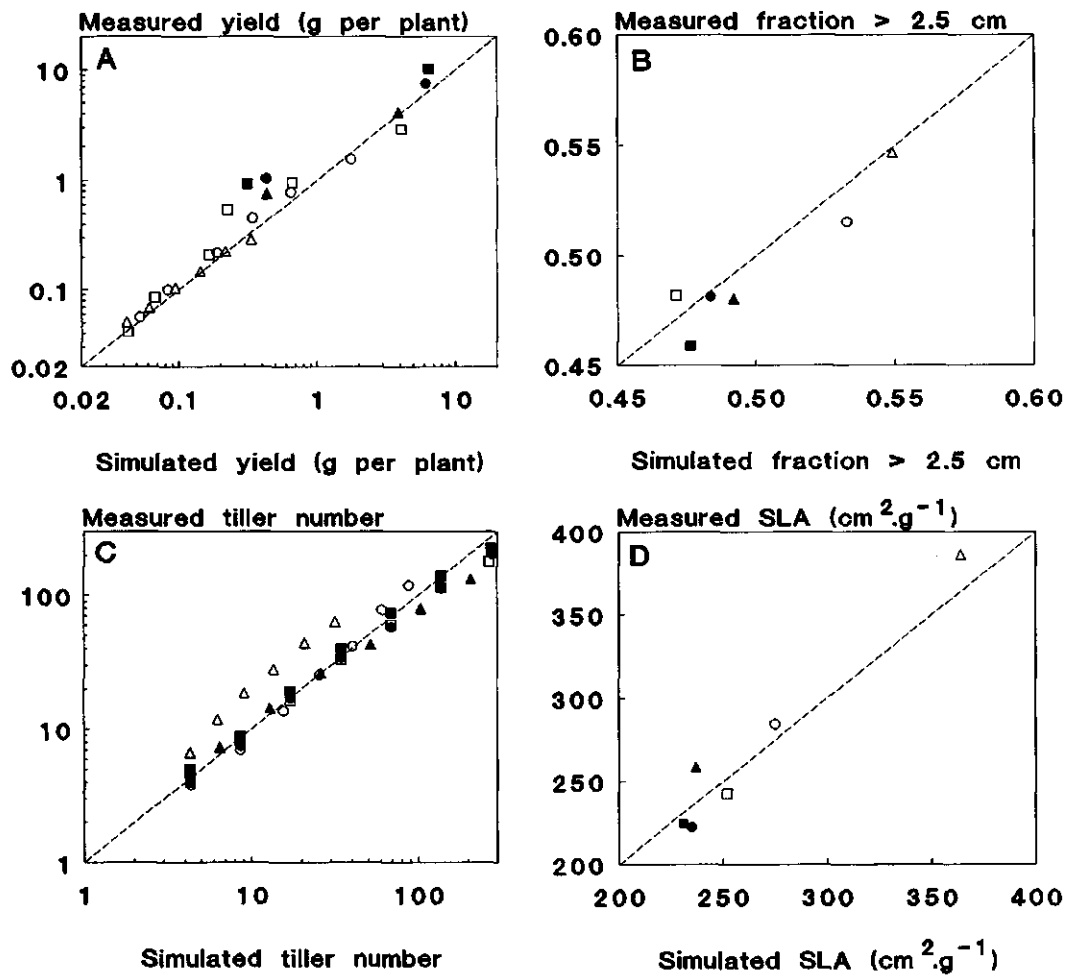


Fig. 3.5. Comparison of simulated and measured results of the cutting experiment. A) DM-yield of successive cuts. B) DM-accumulation above 2.5 cm stubble as a fraction of total DM accumulation. C) Number of tillers per plant at successive dates. D) Specific leaf area at final cut (day 70). Weekly cuts: Δ 2.5; \circ 5; \square 7.5 cm cutting height; 3-weekly cuts: Δ 2.5; \bullet 5; \blacksquare 7.5 cutting height. The dashed lines indicate the 1:1 relation.

No large systematic differences were found between simulated number of tillers per plant and measured number for most cutting treatments (Fig. 3.5C). However, with weekly cuts at 2.5 cm the model underestimated tillering and with three-weekly cuts at 5 and 7.5 the model overestimated tillering somewhat in the end (when high numbers of tillers per plant occurred).

In the regrowth model, SLA varied, because leaf area expansion was within limits independent of the level of substrate supply to leaves. Simulated SLA at the final cut (70 days after sowing) correlated well with measured values (Fig. 3.5D). This means that the proposed mechanisms that cause the changes in SLA can explain the effects of cutting treatments on SLA.

Simulated dynamics of regrowth

The simulated relative rate of increase of leaf weight was highest just after cutting and decreased with time after cutting (Fig. 3.6A). The relative rate of increase of leaf weight was higher with lower cutting heights. The absolute increase of simulated leaf weight was higher with higher cutting heights, because the amount of leaves remaining after cutting was larger. The simulated pattern of tillering showed a nearly exponential increase of plant tiller number (Fig. 3.6B). The potential tiller number (i.e. the number that would be reached when site filling is at its maximum of 0.693) on day 70 was 297 tillers per plant for cuts at 2.5 cm, 315 for cuts at 5 cm and 397 tillers per plant for cuts at 7.5 cm, based on the differences in leaf appearance rate. Even with three-weekly cuts at 7.5 cm, this potential was not reached both in the simulations and in the experiment). Differences between cutting treatments in actual tiller number were greater than in potential tiller number, because with decreasing cutting height and increasing cutting frequency progressively more tiller buds were aborted, because not enough substrate was present in the sheaths.

The simulations showed large fluctuations of SLA with weekly cuts or low cutting heights: a sharp increase of SLA just after cutting and a decrease of SLA in the following days and weeks (Fig. 3.6D). The rise in SLA was due to a higher demand of leaves for substrate than could be supplied from the substrate pool in the sheath and current assimilation.

The simulated leaf area per plant followed a time course that was similar to that of leaf weight, but the relative increase of leaf area was higher than that of leaf weight, just after cutting (compare the slopes of the lines in Fig. 3.6A and D). However, during the second phase of the regrowth period, the relative increase of leaf area decreased faster. This was most apparent for weekly cuts and for three-weekly cuts at 2.5 cm. It was caused by the large fluctuations of SLA with these cutting regimes (high SLA just after cutting and later on decreasing values of SLA, Fig.6C).

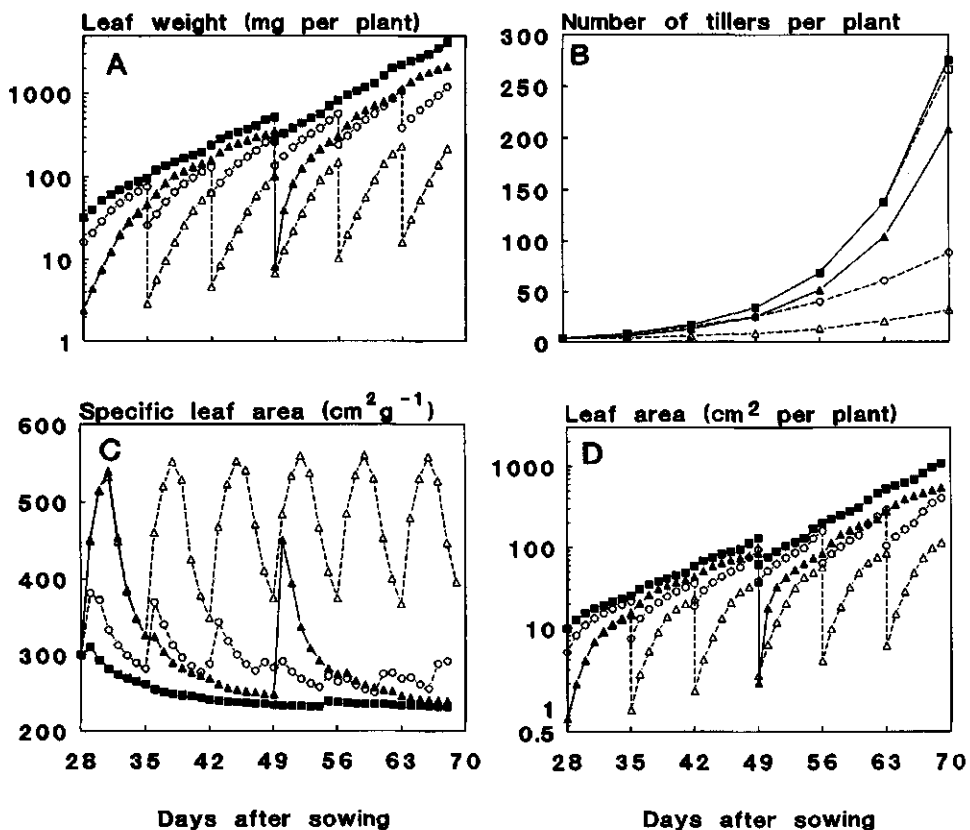


Fig. 3.6. Simulated dynamics of regrowth. A) Weight of green leaves. B) Number of tillers per plant. C) Specific leaf area. D) Mean tiller weight. E) Leaf area per plant. F) Fraction of reserves (fraction of weight of sheaths and unemerged leaves). Cuts were simulated on day 28, 35 and so on for weekly cuts, and on day 28, 49 and 70 for three-weekly cuts. Weekly cuts: Δ 2.5; \circ 5; \square 7.5 cm cutting height; 3-weekly cuts: Δ 2.5; \bullet 5; \blacksquare 7.5. In A, C and E three-weekly cuts at 5 cm and weekly cuts at 7.5 cm are not shown. In B, simulated tiller number with three-weekly cuts at 5 and 7.5 cm was the same.

3.4 DISCUSSION AND CONCLUSIONS

Comparison of experiment and simulation

Results of the simulations were generally in good accordance with experimental results for final tiller number and tillering pattern, yields, harvestable fraction and final SLA. This could be seen from the generally small systematic deviations between simulated and observed values. Simulated yield for weekly cuts showed a better agreement with experimental results than for three-weekly cuts, where simulated values of the second cut (first cut after the start of the simulations on 28 days after sowing) were too low. Because of the large positive feedback between assimilation, tillering and leaf area

increase, a minor error in one of the growth parameters can easily cause a large deviation between simulated and measured yields.

Generally, the model predicted tiller number well, except for weekly cuts at 2.5 cm. The underestimation of tillering with weekly cuts at 2.5 cm may be a consequence of an overestimation of the minimum amount of substrate needed for the production of a new tiller (W_{\min}). W_{\min} was derived from the size of the first leaf on the main tiller. It was assumed that the first leaf on any tiller would have this size. Possibly, however, the first leaf of higher order tillers (tertiary and higher) is smaller than of the main tiller and of secondary tillers with weekly cuts at 2.5 cm. Another possible explanation for the underestimated tillering rate with weekly cuts at 2.5 cm may be the short "window of opportunity" for tiller buds to start development into a visible tiller and the synchronized appearance of tiller buds. In the model, tiller buds must start to grow into a tiller on the day of the appearance of the leaf in the axil of which they are formed. With weekly cuts at 2.5 cm, the leaf appearance rate was only just higher than one leaf per week. Since the first cut was just prior to leaf appearance, with weekly cuts tiller bud formation occurred just after each defoliation. Then, all available substrate in the sheaths was used for leaf growth on existing tillers and not much substrate was available for tiller buds to grow into tillers.

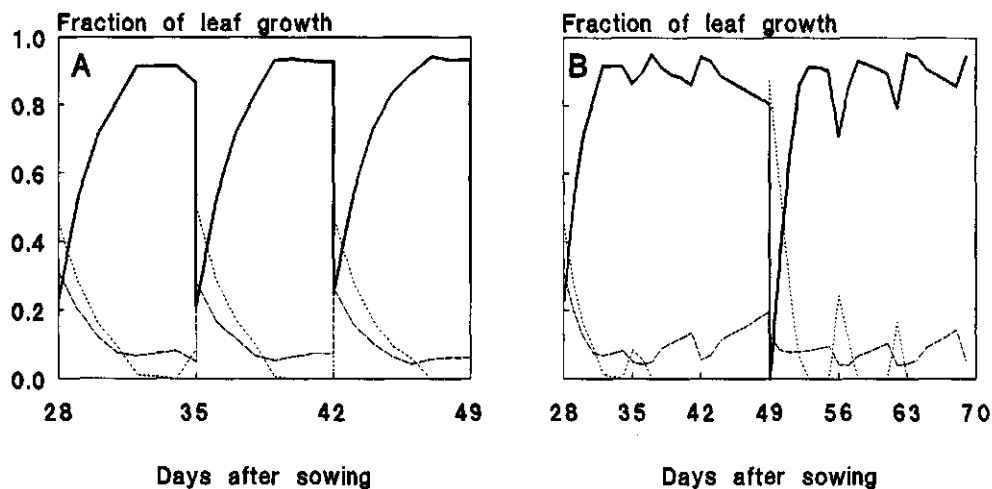


Fig. 3.7. Simulated relative contribution to leaf growth from reserves in the stubble, structural unemerged leaves and current assimilation. A) Weekly cuts at 2.5 cm. B) Three-weekly cuts at 2.5 cm. Fraction leaf growth from current assimilation (—); from unemerged leaves (---); from reserves in the stubble (.....).

Substrate pool in sheaths and unemerged leaves

The supply of substrate in the stubble to expanding leaves is generally thought to

contribute positively to regrowth of leaves (e.g. effects of water soluble carbohydrates on regrowth: Alberda, 1966; Davies, 1965; Hume, 1990). In the simulations, just after cutting indeed a high fraction of leaf growth came from the substrate pool in the stubble, but also structural material in unemerged leaves contributed much to leaf growth. Within one week after defoliation, current assimilation provided more than 90 % of the substrate needed for leaf growth, also with the most severe cutting treatment of weekly cuts at 2.5 cm (Fig. 3.7A). At three-weekly cuts, some substrate usage was also simulated after the first week of regrowth, each time tillers and leaves appeared (Fig. 3.7B). The observation of Davies (1965) that the amount of water soluble carbohydrates present in the stubble after defoliation was not enough to account for all regrowth is confirmed by the result of our model that also material from the pool of unemerged leaves contribute substantially to the initial regrowth of leaves after defoliation.

In our model, substrate availability and leaf expansion rates are independent when the SLA of new leaf material is below the specified maximum. Experimentally, Davies, Evans and Pollock (1989) have shown for perennial ryegrass that leaf extension rates may be independent of the water soluble carbohydrate concentration. They showed in an experiment with different durations of low temperature pretreatments that leaf extension rates after transfer to 15 °C did not differ, although considerable differences in water soluble carbohydrate concentrations were found.

Also, regrowth rates do not always depend on substrate availability: Davies *et al.* (1972) showed that regrowth rates of plants containing either 15.2 % or 20.4 % WSC in the stubble did not differ, while regrowth at 6.5 % WSC was lower than at 12.4 % WSC. This proves that substrate availability (source size) only limits regrowth when it is low. Davies (1988) stated that not enough data are available to establish levels of carbohydrate needed for maximum regrowth of leaves. In our model, this level is determined by the level at which substrate availability (source size) becomes greater than the substrate demand of leaves (sink size). This critical substrate concentration will therefore depend on the size of the substrate pool, the current rate of assimilation and the potential leaf area expansion rate.

Specific leaf area

Johnson and Thornley (1983) predicted that SLA will rise, when the relative substrate concentration in the leaf declines. Since it is generally found that substrate concentrations sharply decline after cutting and are restored later (Alberda, 1960; Davies, 1965; Hume 1990), it may be expected that SLA values just after cutting are high and decline when the substrate concentration rises. Sheehy *et al.* (1980) showed experimentally that this pattern of SLA really occurs: SLA values were high just after cutting and declined thereafter. However, their model showed the reverse pattern for simulated SLA: low values of SLA just after defoliation and an increase of SLA

thereafter. This simulated pattern was caused by the assumption that the SLA of new leaves decreases with increasing irradiance level reaching the developing leaf. Sheehy *et al.* (1980) assumed that the developing leaf resides at soil level. The irradiance level at soil level decreases with increasing leaf area index (LAI). Therefore, Sheehy *et al.* (1980) simulated low values of SLA just after defoliation and an ongoing increase of SLA, since LAI increased after defoliation. With our model, high values of SLA were simulated just after a cut when substrate availability was low and SLA decreased thereafter. Also, higher values of SLA were simulated with lower cutting heights and higher cutting frequencies, which is in accordance with the results of Grant, Barthram and Torvell (1981) and of Chapter 2 of this thesis.

3.5 APPENDIX

3.5.1 Equations

Dry matter accumulation

$$P_{plant} = AhP_{leaf} \quad (1)$$

$$G_{new} = \begin{cases} \alpha(\beta P_{plant} - R_m), & \text{if } \beta P_{plant} > R_m \\ 0, & \text{otherwise} \end{cases} \quad (2a)$$

$$R_m = r_{lf} W_{lf} + \sum_x r_x W_x \quad (2b)$$

for x = sheath, root, substrate in sheaths and unemerged leaves

Source size for leaf growth

$$F_{lf}(pot) = (1 - f_{sh} - f_{unlf})(1 - f_{rt})G_{new} + \frac{W_{unlf}}{\lambda} + \frac{W_{res}}{\tau_{res}} \quad (3)$$

Potential leaf area expansion rate and sink size

$$A'_{pot} = \left(\frac{dA}{dt}\right)_{pot} = \sum_i \sum_j T_i w_{ij} \frac{dL_{pot}(ij)}{dt} \quad (4a)$$

$$\frac{dL_{pot}(ij)}{dt} = \begin{cases} \frac{L_A}{\tau_{leaf}} L_{max}(ij), & \text{if } L_{pot}(ij) < L_{max}(ij) \wedge i < \tau_{leaf} \\ 0, & \text{otherwise} \end{cases} \quad (4b)$$

$$w_{ij} = 0.185 + 0.0105L_{max}(ij) \quad (4c)$$

$$S = \frac{A'_{pot}}{SLA_{min}} \quad (4d)$$

Actual growth rate of expanding leaves

$$F_{lv} = \min(S, F_{lv}(pot)) \quad (5)$$

Actual leaf area expansion and leaf extension

$$A' = \frac{dA}{dt} = \min(A'_{pot}, F_{lv} \cdot SLA_{max}) \quad (6a)$$

$$\frac{dL_{ij}}{dt} = \begin{cases} \frac{A'}{A'_{pot}} \cdot \frac{dL_{pot}(ij)}{dt}, & \text{if } i \leq 3 \\ -0.2L_{ij}, & \text{if } i > 3 \end{cases} \quad (6b)$$

Dynamics of the substrate pool in sheaths

$$\frac{dW_{res}}{dt} = F_{dying\ leaves \rightarrow res} - F_{res \rightarrow lf} - F_{res \rightarrow maint} \quad (7a)$$

$$F_{res \rightarrow lf} = \frac{W_{res}}{T_{res}} - (F_{lf(pot)} - F_{lf}) \quad (7b)$$

$$F_{dying\ leaves \rightarrow res} = 0.5M \quad (7c)$$

$$F_{res \rightarrow maint} = \begin{cases} 0, & \text{if } \beta P_{plant} > R_m \\ \alpha(R_m - \beta P_{plant}), & \text{otherwise} \end{cases} \quad (7d)$$

Leaf death

$$M = 0.2 \sum_i \frac{A_{i4}}{SLA} \quad (8)$$

Growth rates

$$\frac{dW_{lf}}{dt} = F_{lf} - M \quad (9a)$$

$$\frac{dW_{unlf}}{dt} = f_{unlf}(1 - f_{rt})G_{new} - \frac{W_{unlf}}{\lambda} \quad (9b)$$

$$\frac{dW_{rt}}{dt} = f_{rt}G_{new} \quad (9c)$$

$$\frac{dW_{sh}}{dt} = f_{sh}(1 - f_{rt})G_{new} \quad (9d)$$

$$\frac{dW_{dead}}{dt} = 0.5M \quad (9e)$$

$$\frac{dW_{tot}}{dt} = \frac{dW_{res}}{dt} + \frac{dW_{lf}}{dt} + \frac{dW_{dead}}{dt} + \frac{dW_{unlf}}{dt} + \frac{dW_{sh}}{dt} + \frac{dW_{rt}}{dt} \quad (9f)$$

Tillering dynamics and events at dates on which leaves appear

$$\frac{dl}{dt} = L_A \quad (10a)$$

$$k = \text{int}(l) \quad (10b)$$

Eqns. (11a-d) are recurrent difference equations that are evaluated each

time k becomes one higher (i.e. each time a leaf appears on the main tiller):

$$B_k = T_{tot}(k) \quad (11a)$$

$$T_{tot}(k+1) = T_{tot}(k) + \min(B_k, \frac{W_{res}(k)}{W_{min}}) \quad (11b)$$

$$T_i(k+1) = T_{i-1}(k) \quad (11c)$$

$$T_1(k+1) = \min(B_k, \frac{W_{res}(k)}{W_{min}}) \quad (11d)$$

$$F_s(k) = \ln(\frac{T_{tot}(k+1)}{T_{tot}(k)}) = \min(\ln(2), \ln(1 + \frac{W_{res}}{T_{tot}(k) W_{min}})) \quad (11e)$$

Events at cutting dates

$$W_{lf} \text{ (after cut)} = (1 - HI_{leaf}) W_{lf} \text{ (before cut)} \quad (12a)$$

$$W_{sh} \text{ (after cut)} = (1 - HI_{sh}) W_{sh} \text{ (before cut)} \quad (12b)$$

$$W_{res} \text{ (after cut)} = (1 - HI_{sh}) W_{res} \text{ (before cut)} \quad (12c)$$

$$W_{unlf} \text{ (after cut)} = (1 - HI_{sh}) W_{unlf} \text{ (before cut)} \quad (12d)$$

$$HI_{leaf} = 1 - \frac{\sum_{i=1}^{10} \sum_{j=1}^4 A_{ij}^*}{\sum_{i=1}^{10} \sum_{j=1}^4 A_{ij}} \quad (13a)$$

$$A_{ij}^* = w_{ij} L_{ij} (1 - [\frac{L_{ij} - L_{ij}^*}{L_{ij}}]^2) \quad (13b)$$

$$L_{ij}^* = \max(0, H - S_{ij}) \quad (13c)$$

$$HI_{stubble} = 1 - \frac{\sum_{i=1}^{10} \sum_{j=1}^4 S_{ij}^*}{\sum_{i=1}^{10} \sum_{j=1}^4 S_{ij}} \quad (14a)$$

$$S_{ij}^* = \min(S_{ij}, H) \quad (14b)$$

3.5.2 List of variables and parameters

γ	conversion efficiency for growth	$0.7 \text{ g CH}_2\text{O}(\text{g DM})^{-1}$
β	conversion factor from CO_2 to CH_2O	30/44
τ	mean residence time of unemerged leaves in the sheath	10 d
τ_{leaf}	leaf expansion duration	1.3 leaf appearance intervals
τ_{res}	time coefficient for reallocation of substrate	10 d
A	leaf area per plant	cm^2
A_{ij}	area of leaves j on tillers of age class i	cm^2
A_{ij}^*	area remaining after cut, of leaves j on tiller i	cm^2
k	number of buds per plant at start of leaf appearance interval k	
$A' = \frac{dA}{dt}$	actual plant leaf area expansion rate	$\text{cm}^2 \text{ d}^{-1}$
$A'_{\text{pot}} = \left(\frac{dA}{dt}\right)_{\text{pot}}$	potential plant leaf area expansion rate	$\text{cm}^2 \text{ d}^{-1}$
$f_{\text{f}}(\text{pot})$	potential flow of available substrate to leaves, per plant	g DM d^{-1}
$f_{\text{f}}(\text{new})$	actual growth rate of expanding leaves, per plant	g DM d^{-1}
$f_{\text{res} \rightarrow \text{lf}}$	flow from substrate in the sheaths to leaves or vice versa, per plant	g DM d^{-1}
$f_{\text{res} \rightarrow \text{maint}}$	rate of use of substrate for maintenance respiration, per plant	g DM d^{-1}
s	site filling	
t	fraction of current assimilation used for root growth	0.17-0.2
h	fraction of current assimilation going to shoot used for growth of <i>structural</i> sheath	0.2
n_{lf}	fraction of current assimilation going to shoot that is	

	used for <i>structural</i> material in unemerged leaves	0.1
G_{new}	rate of production of new dry matter, per plant	g DM d^{-1}
h	daylength	15 h
H	cutting height	cm
HI_{leaf}	harvest index of leaves	-
HI_{sh}	harvest index of sheath, unemerged leaves and substrate	-
k	number of leaves appeared on the main tiller	-
l	leaf age of the plant	-
L_{ij}	actual leaf blade length of leaf j on tiller i	cm
L_{ij}^*	length of leaf blade after cut	cm
$L_{pot}(ij)$	potential leaf blade length of leaf j on tiller i	cm
$L_{max}(ij)$	potential mature leaf blade length of leaf j on tiller i	cm
L_A	leaf appearance rate per tiller	d^{-1}
M	rate of leaf death, per plant	g DM d^{-1}
P_{leaf}	gross leaf CO_2 -assimilation rate	$0.25 \text{ mg CO}_2 \text{ cm}^{-2} \text{ h}^{-1}$
P_{plant}	gross plant CO_2 -assimilation rate	$\text{g CO}_2 \text{ d}^{-1}$
R_m	maintenance respiration rate, per plant	$\text{g CH}_2\text{O d}^{-1}$
r_{lf}	maintenance respiration coefficient for leaves	0.02 $\text{g CH}_2\text{O (g DM)}^{-1} \text{ d}^{-1}$
r	maintenance respiration coefficient for sheaths,	0.01
S	substrate in the sheaths, unemerged leaves and roots	$\text{g CH}_2\text{O (g DM)}^{-1} \text{ d}^{-1}$
S_{ij}	sink size of leaves, per plant	g DM d^{-1}
	sheath length of leaves j on tiller i	cm
S_{ij}^*	sheath length after cut	cm
SLA_{max}	maximum specific leaf area	$600 \text{ cm}^2 (\text{g DM})^{-1}$
SLA_{min}	minimum specific leaf area	$230 \text{ cm}^2 (\text{g DM})^{-1}$
$T_j(k)$	number of tillers per plant in age class i at start of leaf	

	appearance interval k	-
N_{ij}	mean leaf width of leaf j on tiller i	cm
N_{dead}	weight of dead leaves, per plant	g DM
N_{lf}	dry weight of emerged leaves, per plant	g DM
N_{rt}	dry weight of root, per plant	g DM
N_{sh}	dry weight of structural sheath, per plant	g DM
N_{res}	dry weight of substrate in sheaths, per plant	g DM
N_{unlf}	dry weight of structural unemerged leaves, per plant	g DM
N_{min}	minimum amount of substrate in sheaths needed for growth of tiller bud into visible tiller	$6.2 \cdot 10^{-3}$ g DM
N_{tot}	total weight per plant (including dead parts)	g DM

PART II

Limitations by a low waterpotential

Chapter 4

Tillering, leaf expansion and growth of plants of two cultivars of perennial ryegrass grown on hydroponics at two water potentials

ABSTRACT

Tillering and growth parameters of perennial ryegrass cultivars Wendy (diploid) and Condesa (tetraploid) were determined in a glasshouse experiment on hydroponics at low (-1.3 MPa) and normal water potential (0 MPa). At -1.3 MPa, leaf extension rate was reduced by 36 %. Final plant tiller number was 20 % lower, at -1.3 MPa, due to a 12 % reduction of the leaf appearance rate at -1.3 MPa in the first weeks after the start of the treatments. Site filling, the relative increase of tiller number per leaf appearance interval, was high (0.61) - but still lower than theoretically possible - and was only slightly affected by water potential. Site filling was shown to be strictly related to the number of inhibited plus unemerged tiller buds. Dry matter production was 64 % lower at -1.3 MPa. Relative growth rate (RGR) was 17 % lower at -1.3 MPa, on average, but was more reduced just after the treatments started. Also, NAR was more reduced by low water potential just after the start of the treatments. Specific leaf area (SLA) was 13 % lower at -1.3 MPa for Wendy, but not significantly reduced for Condesa. Contrary to expectations based on the theory of the functional balance between root and shoot, leaf weight ratio was slightly higher at -1.3 MPa. From comparison of results of this study with literature data, it is concluded that effects of drought in the field on tillering cannot be attributed to low water potential only.

4.1 INTRODUCTION

Leaf area dynamics of a sward or a plant are the combined result of tiller dynamics, leaf appearance and leaf death and area expansion per leaf. Tiller dynamics are determined by three processes: 1) tiller bud formation, which is related to leaf appearance, because in the axil of each leaf one tiller bud is formed; 2) development and growth of tiller buds into visible tillers; 3) tiller death. The development and growth of tiller buds into visible tillers can be quantified in terms of the delay time from tiller bud formation until the appearance of the tiller. If this delay time is constant, the result is a constant number of inhibited or delayed tiller buds per tiller. Often not the number of inhibited plus unemerged buds is presented, but a related parameter, site filling, which is the relative increase of number of tillers per leaf appearance interval (Davies, 1974; Neuteboom and Lantinga, 1989). Site filling and number of inhibited plus unemerged tiller buds per tiller are strictly related (see Appendix).

In this study the effects of low water potential on tiller and leaf area dynamics have been investigated during the primary growth of individual plants of perennial ryegrass. Most of the previously performed studies in which the effect of drought on tiller dynamics of perennial

ryegrass was studied, were carried out in the field with established swards (e.g. Norris, 1982; Barker, Chu and Korte, 1985). In these studies, tillering and leaf appearance rate were reduced in dry conditions. However, from these experiments no conclusions can be drawn about the effect of a low water potential on *potential* tillering rates, because tillering rates were much lower than maximal, even when ample water was available.

Drought may influence tillering and leaf area expansion through a direct effect of a low water potential through effects on turgor (Jones, 1988), through a change in cell wall extensibility (Davies, Mansfield and Hetherington, 1990) or through an effect on assimilate availability, possibly as a consequence of a lower assimilate availability to the shoot (Brouwer, 1962). However, effects recorded during drought in the field may also be caused by other factors associated with drought like supra-optimal temperatures (Davies and Thomas, 1983) or a reduced mineral availability (Gales, 1979).

The first aim of this study was to quantify the effect on the rate of leaf and tiller appearance of perennial ryegrass of water potential without any of the side effects possibly associated with drought in the field. Therefore, tillering and leaf appearance rates have been measured on plants grown on hydroponics with a water potential of -1.3 MPa and 0 MPa. The use of the hydroponics eliminated any effect of a low water potential on nutrient availability though not necessarily on nutrient uptake. The second aim was to investigate the effect of a low water potential on the relationship between, on the one hand, tiller number, leaf number and leaf morphology (form), and on the other hand, dry matter production and distribution (function).

4.2 MATERIAL AND METHODS

In a glasshouse, seeds of two cultivars of perennial ryegrass (*Lolium perenne* L.), Wendy (diploid) and Condesa (tetraploid), were sown in sand on 15 September 1988 (day 1).

Pretreatment period: Until day 22 no treatments were applied. During this period plants were transplanted from the sand to containers with 14 L of nutrient solution on day 14 (60 plants on a container). On day 22, plants were placed on eight containers (30 plants on each container with 40 L of nutrient solution). To avoid light competition between plants and to avoid differences between treatments in light quality, the spacing of plants was wide and increased as part of the plants were removed for assessing growth rates.

Environmental conditions: Day and night temperatures were 20 and 15 °C. Natural light was admitted from 8.00 h until 20.00 h and additional artificial light (Philips SON-T, 400 W, giving a photon flux density of $230 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ (PAR=400-700 nm)) was used from 6.00 h till 21.00 h. During the experiment, mean total daily radiation (PAR) in the glasshouse declined from 4.9 to 4.1 $\text{MJ.m}^{-2}.\text{d}^{-1}$ (or 23 to 19 $\text{mol photons.m}^{-2}.\text{d}^{-1}$). The pH of the aerated nutrient solution (Steiner, 1984) was kept between 5.0 and 6.0.

Water potential treatments: From day 22 onwards, two treatments were applied (4 containers per treatment):

1) Low water potential (treated) : a nutrient solution was applied with 250 g polyethylene glycol (PEG, mol. wt. 20000) per 1000 g nutrient solution, resulting in a water potential of -1.3 MPa.

2) Control: no polyethylene glycol added (0 MPa).

Plants growing at -1.3 MPa wilted partly for some hours just after the start of the treatments, but regained turgor during the following night.

The water potential of the PEG containing nutrient solution was determined at the Centre for Agrobiological Research (CABO-DLO, Wageningen, The Netherlands) using a WESCOR 5500 osmometer using a vapour pressure deficit method. The result was slightly higher than the value according to Steuter, Mozafar and Goodin (1981) for PEG 20,000 at 250 g PEG per 1000 g H₂O, which is -1.4 MPa.

Nutrient solution was added daily in an amount equal to the loss of water from the containers due to transpiration and evaporation. In addition, the nutrient solution of the 0 MPa treatment was completely replenished on day 32, 42 and 49. The nutrient solution of the PEG treatment did not need to be replenished completely, since expected growth and mineral nutrient uptake were much less than in the control treatments.

Growth analysis: Plants were harvested on days 14, 22, 29, 36, 43, 50 and 57 and were dissected into roots, sheaths and leaf blades. Sixty plants per cultivar were harvested on day 14 and 24 on day 22. At later harvest dates 12 plants were harvested per treatment-cultivar combination. On days 22 and 29 total leaf area per plant was determined from measurements of the length of all leaves and the width at 3 positions. Area per leaf was calculated as:

$$A = 0.25L(1.5W_1 + W_2 + W_3) \quad (1)$$

in which A is area per leaf (cm²), L is leaf length (cm) and W_1 , W_2 and W_3 are the leaf widths (cm) at 25, 50 and 75 % of the leaf length. At later dates, a sample of leaves was taken of which leaf area and dry weight was determined. Plant material was dried at 70 °C for 24 h and weighed.

Tillering, leaf appearance and leaf extension: At weekly intervals - always one day before the harvest for the growth analysis - tiller number, number of newly appeared leaves (ligule emerged) on the main tiller and number of not yet emerged tiller buds on the main tiller were counted of all plants, and lengths were measured of all leaves on the first and second tiller of 12 plants per treatment-cultivar combination. Since tiller mortality occurred, the relative tiller appearance rate was the same as the relative tillering rate. Site filling (F_s , dimensionless) was calculated as:

$$F_s = \frac{R_{tar}}{L_A} = \frac{\ln T_2 - \ln T_1}{l_2 - l_1} = \frac{\ln T_2 - \ln T_1}{\Delta t} \cdot \frac{\Delta t}{l_2 - l_1} \quad (2)$$

in which L_A is the leaf appearance rate (new leaves per tiller per time interval, d^{-1}), R_{tar} is the relative tiller appearance rate (d^{-1}), and T_1 and T_2 are plant tiller number and I_1 and I_2 are the number of leaves appeared on the main tiller at time t_1 and t_2 , respectively.

Chemical analysis: The concentration of water soluble carbohydrates (WSC) of plant parts harvested on day 36, was determined - after extraction of WSC in hot water - with an automatic analyser (which performs hydrolysis of water soluble polysaccharides into monosaccharides after which concentration of reducing monosaccharides was determined colourmetrically using ferricyanide). Total nitrogen concentration (Kjeldahl) was determined of material harvested on day 43 and 57.

Statistical design: Four blocks were formed, each consisting of two containers. The water potential treatments were randomized per block over the two containers per block. Within each container the harvest dates and cultivars were randomized (split-plot block design).

4.3 RESULTS

Tiller number, leaf appearance and site filling

On day 14, all plants had one tiller and one leaf. Final tiller number per plant was lower at -1.3 MPa than at 0 MPa (-20 % on average, Table 4.1). The lower relative tillering rate at -1.3 MPa may be explained by differences in either site filling or leaf appearance rate (Table 4.2). Site filling is shown graphically as the slope of the graph of \ln (tiller number) versus leaf number (Fig. 4.1A). Site filling was only slightly reduced at -1.3 MPa and was 0.61 on average. This is significantly lower than the theoretical maximum of 0.693, but still much higher than the 0.481 that would be maximally expected when no tillers were produced from buds in the axils of prophylls (Neuteboom and Lantinga, 1989).

Leaf appearance rate was 12 % lower at -1.3 MPa compared to 0 MPa, during the first two weeks after the start of the water potential treatments. However, the leaf appearance rate declined more with time at 0 MPa than at -1.3 MPa and after day 35 no further effect of low water potential on leaf appearance rate was found (Table 4.2).

Leaf extension rate

At -1.3 MPa, the leaf length extension rate was 36 % lower than at 0 MPa (Table 4.3). Leaf area expansion rate was more affected (-45 %) than leaf length extension rate, because also leaf widths were smaller, at the low water potential. As a result of smaller leaf extension rates at -1.3 MPa the final leaf length was considerably smaller (Fig. 4.1B), since leaf extension duration was not affected. Final leaf lengths increased with leaf number in all treatments.

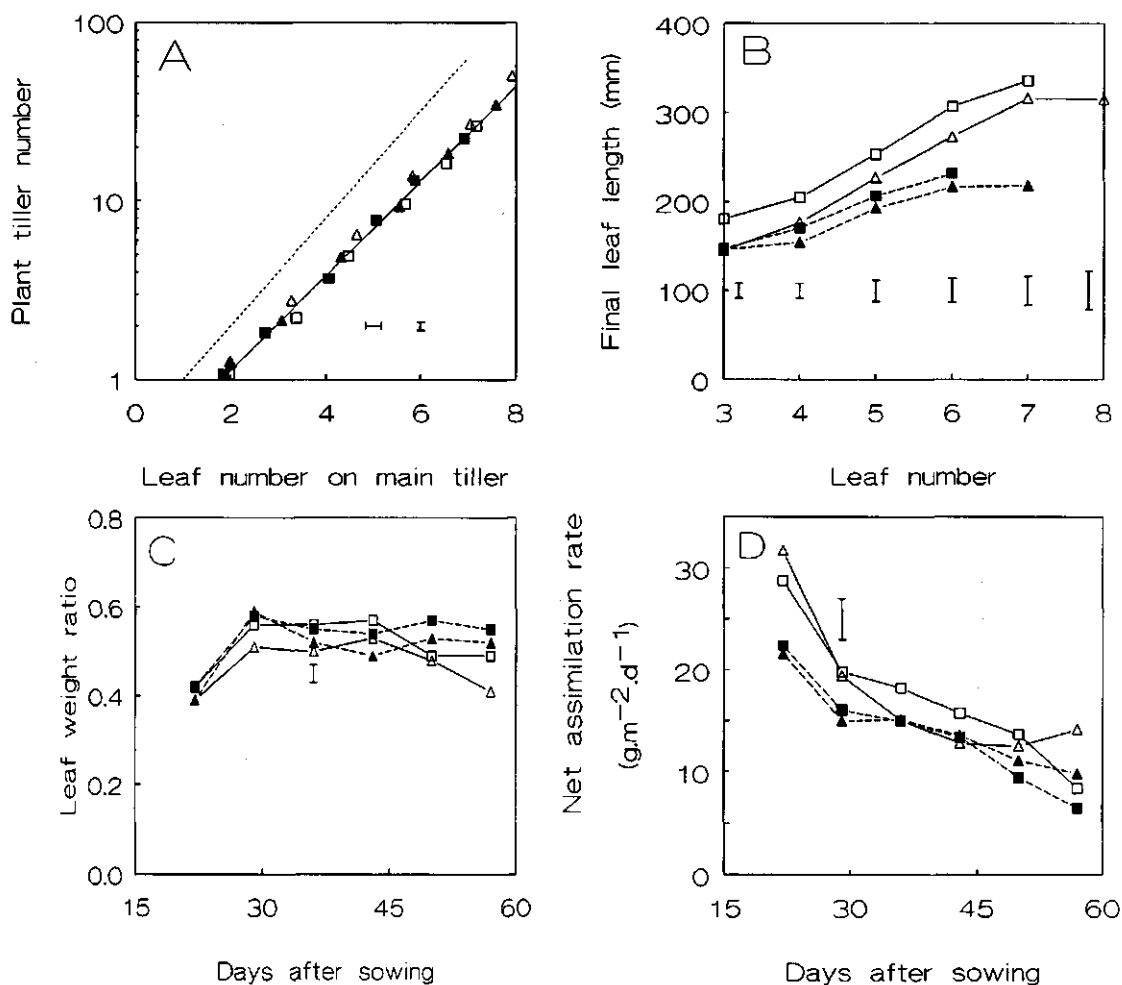


Fig. 4.1. Effect of water potential on tillering, final leaf length, dry matter distribution and net assimilation rate of two cultivars. A. Relation between \ln tiller number and number of fully emerged leaves on main tiller. The slope of the solid line equals the mean site filling for all treatments (0.61); the slope of the dotted line is the maximum site filling (0.693). B. Final leaf length (sheath + blade) of successive leaves on the main tiller. C. Leaf weight ratio. D. Net assimilation rate (calculated from RGR, LWR and SLA). Δ Wendy, 0 MPa; \blacktriangle Wendy, -1.3 MPa; \square Condesa, 0 MPa; \blacksquare Condesa, -1.3 MPa. Lines for -1.3 MPa are dashed. Data are means based on at least 12 plants. Error bars denote LSDs ($p = 0.05$).

Table 4.1. Final dry weight per plant, final number of tillers per plant and final number of fully emerged leaves on the main tiller. Means (of 12 plants) with different letters are significantly different ($p < 0.05$).

Cultivar	Water potential	Plant dry weight g per plant	Number of Tillers	Leaves
Wendy	0 MPa	6.9 ^a	50.1 ^d	7.9 ^d
	-1.3 MPa	2.3 ^a	34.8 ^c	7.6 ^c
Condesa	0 MPa	4.9 ^b	26.3 ^b	7.2 ^a
	-1.3 MPa	1.9 ^a	22.5 ^a	6.9 ^a
CV%		37 %	28 %	
LSD				0.24

RGR and physiological components of RGR

Final plant dry weight was reduced by 64 % at -1.3 MPa (Table 4.1). The mean RGR for the whole experimental period was 0.175 d^{-1} at 0 MPa and only 0.146 d^{-1} at -1.3 MPa. The effect of the low water potential on RGR decreased with time, because at 0 MPa, RGR dropped faster with time than at -1.3 MPa. Also, the relative growth rate of the mean tiller weight (RGR_{ti}) was reduced at -1.3 MPa during the first weeks after the start of the treatments (Table 4.2). Loss of plant weight due to leaf death was not important in this study, since only the first three small leaves on the main tiller died and the first two leaves on the first and second axillary tiller.

No important differences in leaf weight ratio (LWR) between the water potential treatments or cultivars were found. Until day 29, LWR increased and afterwards remained almost constant (about 0.56, Fig. 4.1C). The initial increase in LWR was probably due to a lower nutrient availability during the pretreatment than during the treatment period. After day 35, the ratio of relative growth rate of root and shoot was nearly one at 0 MPa, but was lower than one at -1.3 MPa, indicating that root growth was more reduced by low water potential than shoot growth (Table 4.4). The specific leaf area (SLA) of Wendy was lower at -1.3 MPa than at 0 MPa (Table 4.5). For Condesa, the difference between water potential treatments in SLA was not statistically significant.

At -1.3 MPa, net assimilation rate (NAR, calculated from fitted curve for dry weight, LWR and SLA) was $9 \text{ g m}^{-2} \text{ d}^{-1}$ lower than at 0 MPa just after the start of the treatments (Fig. 4.1D). The difference was smaller at the end of the experiment, because NAR declined faster with time at 0 MPa than at -1.3 MPa. With the daylength used (15 h), a NAR of $30 \text{ g m}^{-2} \text{ d}^{-1}$ corresponds with a rate of gross leaf photosynthesis (P) of about $4 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ($\text{NAR} = 0.5 * 15 * \text{P}$ - maintenance respiration: conversion from CO_2 to structural dry matter is about 0.5 g CO_2 per g structural dry matter; with a maintenance respiration coefficient of 0.015 g CO_2 per gram of biomass, maintenance respiration rate is about $0.07 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, on a leaf area basis; coefficients derived from Penning de Vries *et al.*, 1989).

Table 4.2. Relative growth rate (RGR, d^{-1}), relative growth rate of the mean tiller weight (RGR_{tij} , d^{-1}), relative tiller appearance rate (R_{tar} , d^{-1}), leaf appearance rate (L_A , leaves per tiller per day, d^{-1}) and site filling (F_s). Mean values for day 22 until 36 and for day 36 until 57. RGR and RGR_{tij} are based on means of 12 plants per date for both periods; R_{tar} , L_A and F_s are based on means of 36 plants per date for day 22 until 36 and on 12 plants per date for day 36 until 57. Note that $RGR = RGR_{tij} + R_{tar}$ and $R_{tar} = L_A * F_s$.

		day 22-36				
		RGR	RGR_{tij}	R_{tar}	L_A	F_s
Cultivar	Water potential					
Wendy	0 MPa	0.245	0.121	0.124	0.204	0.61
	-1.3 MPa	0.184 *	0.078 *	0.106 *	0.180 *	0.59
Condesa	0 MPa	0.213	0.096	0.117	0.193	0.61
	-1.3 MPa	0.168 *	0.073 *	0.095 *	0.170 *	0.56 *
LSD ($p=0.05$)		0.019	0.024	0.008	0.022	0.05
		day 36-57				
		RGR	RGR_{tij}	R_{tar}	L_A	F_s
Cultivar	Water potential					
Wendy	0 MPa	0.146	0.049	0.097	0.155	0.63
	-1.3 MPa	0.136	0.042	0.094	0.157	0.60
Condesa	0 MPa	0.139	0.059	0.080	0.130	0.62
	-1.3 MPa	0.125	0.039 *	0.086	0.135	0.64
LSD ($p=0.05$)		0.018	0.016	0.008	0.024	0.10

* significantly different from 0 MPa ($P < 0.05$)

Table 4.3. Leaf extension rate of leaves of the main and first axillary tiller between 41 and 49 days after sowing

		Leaf extension rate ($mm\ d^{-1}$)	
Cultivar	Water potential	main tiller	1 st axillary tiller
Wendy	0 MPa	28.4	27.0
	-1.3 MPa	18.4 *	18.2 *
Condesa	0 MPa	27.0	26.9
	-1.3 MPa	18.3 *	19.7 *
LSD ($p=0.05$)		8.6	8.0

* significantly different from 0 MPa ($p < 0.05$)

Cultivar differences

Relative growth rate of Wendy was about 10 % higher than of Condesa due to a higher SLA (Table 4.2 and 4.5). Leaf appearance rate of Wendy was about 10 % higher than that of Condesa and since site filling was almost the same, relative tillering rate of Wendy was also 10 % higher and therefore final tiller number of Wendy was on average 74 % higher than of Condesa (Table 4.1 and 4.2). Although site filling of Wendy was only slightly lower at -1.3 MPa than at 0 MPa, it can be calculated (using $T = T_0 e^{F_s L A^t}$, Neuteboom and Lantinga, 1989) that the difference caused a reduction of final tiller number of 11 %. Therefore, final tiller number of Wendy was more reduced (-30 %) than of Condesa (-15 %) at -1.3 MPa. Leaf extension rates were the same for both cultivars, but Condesa had leaves about 10 % longer than did Wendy (cf. Fig. 4.1B and Table 4.3). This means that leaf extension duration was 10 % longer for Condesa than for Wendy, when expressed in days, but was constant when expressed in leaf appearance intervals.

Table 4.4. Allometric relations between plant dry weight (W) and number of tillers per plant and between root (W_r) and shoot dry weight (W_s) per plant. Based on data from day 36 until 57 (48 plants per regression). Weights are in mg per plant. W_0 is the weight of a plant with one tiller.

Cultivar	Water potential	$\ln W = \ln W_0 + c \ln T$			$\ln W_r = a + b \ln W_s$		
		$\ln W_0$	$c = \frac{RGR}{R_{tar}}$	R^2_{adj}	a	$b = \frac{RGR_r}{RGR_s}$	R^2_{adj}
Wendy	0 MPa	3.0	1.46	0.92	-0.9	0.98	0.84
	-1.3 MPa	2.9	1.36	0.94	-0.7	0.81*	0.74
Condesa	0 MPa	3.2 ^a	1.61*	0.91	-1.3	1.00	0.92
	-1.3 MPa	3.0	1.41	0.91	-0.5	0.77*	0.75
LSD		0.14	0.14		0.8	0.13	

* significantly different from 0 MPa ($P < 0.05$)

^a significantly different from Wendy ($P < 0.05$)

Table 4.5. Specific leaf area (SLA), concentration of water soluble carbohydrates (WSC) on day 35 and leaf nitrogen concentration (means for day 43 and day 57). SLA and WSC are means of 12 plants and nitrogen concentrations are means of 8 plants.

Cultivar	Water Potential	SLA $\text{cm}^2 \text{g}^{-1}$	WSC mg g^{-1}	Nitrogen concentration mg g^{-1}
Wendy	0 MPa	234	169	40.9
	-1.3 MPa	204*	222*	41.5
Condesa	0 Mpa	191	249	42.7
	-1.3 MPa	184*	281*	43.7
LSD ($p = 0.05$)		24	6	3.4

* significantly different from 0 MPa ($p < 0.05$)

4.4 DISCUSSION

Tillering

The low water potential reduced tillering rates considerably by temporarily reducing the leaf appearance rate to about 85 % of the control. The reduction of leaf appearance rate of perennial ryegrass with drought is well recorded in field experiments (Jones *et al.*, 1980; Norris, 1982; Barker *et al.*, 1985; Barker, Chu and Korte, 1989). However, since in this study the reduction of leaf appearance at -1.3 MPa was only temporary, the reduction of leaf appearance with drought in the field may not be completely attributed to a low water potential. Also supra-optimal temperatures may play a role in reducing leaf appearance rate with drought in the field (Davies and Thomas, 1983). Contrary to these field experiments, here, site filling was only slightly affected by water potential. In the 1976 data of Norris (1982), the leaf appearance rate in covered plots (that received no rainfall) was 75 % compared to irrigated plots, but the relative tillering rate was only 27 %, so site filling was reduced to only 36 % on covered plots (0.44 and 0.16 for the irrigated and covered plots). In the experiment of Barker *et al.* (1985) site filling was 0.14 and 0.05 for irrigated and plots receiving no additional water. These values of site filling are much lower than those found here with spaced plants. The lower site filling in swards is probably due to the higher leaf area index in swards (Simon and Lemaire, 1987), although also Davies and Thomas (1983) found lower values of site filling with spaced plants when tiller number per plant increased. Since, here, site filling was only slightly reduced by a low water potential, the effect of drought on site filling in the field must be due to indirect effects of drought on other factors like mineral nutrient availability (Troughton, 1968; Gales, 1979). A low availability of nitrogen reduces site filling (Neuteboom *et al.*, 1988). In the present experiment, indirect effects of low water potential on mineral nutrient availability were eliminated by frequently replenishing the hydroponics. Table 4.5 shows that significant differences in total nitrogen concentration between treatments and cultivars were absent.

Number of unemerged tiller buds and site filling

From the measured site filling (about 0.61 for treatments) the mean number (n) of inhibited plus unemerged tiller buds per tiller can be estimated to be 1.28 (from eqn. A.1, see Appendix), on the assumption that in the axil of the prophyll also a tiller bud is formed that produces a tiller (which assumption was verified by observation). This estimate was close to the counted number of axils of fully emerged leaves (including the prophyll) with unemerged tiller buds, which was 1.30. From the good agreement between the expected and observed value for n , it may be concluded that site filling may be estimated from an experimentally determined n by numerically solving eqn. A.1.

Effects on growth

The most important effect of a low water potential was a smaller leaf area expansion rate. Four factors contributed to the decrease in leaf area expansion: tillering, leaf appearance, extension rate per leaf and SLA. All of these were smaller at the lower water potential. The

lower leaf extension rate at the lower water potential may be caused by either a lower turgor pressure of the leaf due to only partial osmotic adaptation (Jones, 1988) or by changes in cell wall characteristics (extensibility or threshold yielding level), possibly in response to root signalling (Davies, Mansfield and Hetherington, 1990). Dry matter production was even more reduced than the leaf area expansion rate, because also NAR was lower. Schapendonk, Spitters and de Vos (1989) measured photosynthesis of perennial ryegrass at low water potential and showed that only leaves that were present before the start of the water potential treatment had a lower maximum rate of photosynthesis; leaves formed after the start of the water potential treatment did not have lower photosynthetic rates. This may explain why the difference in NAR was smaller at the end of the experiment. The general decline of NAR, may be due to the decrease in daily radiation from 4.9 to 4.1 MJ.m⁻² (PAR) or to the increase in mean age of leaves, since both radiation intensity and leaf age affect the maximum rate of leaf photosynthesis (Woledge and Leafe, 1976; Woledge, 1979).

Dry matter distribution

The theory of the functional balance predicts that plants will react to a limited water availability with a relative increase in the flow of assimilates to the root (Brouwer, 1962). Also Van Keulen (1982) suggested that with water stress a shift occurs in the distribution of assimilates. This phenomenon was not found in this experiment. On the contrary, at the end of the experiment, LWR was even slightly larger at the lower water potential and RGR of the root was more reduced than RGR of the shoot. By combining data on above ground dry matter production and assimilation rates, Lantinga (1985) concluded that LWR was not lowered by water stress, which was also found experimentally by Gales (1979). Gales (1979) explained the lower LWR with water stress found by some authors as an effect of a reduced phosphorus availability.

Specific leaf area and water soluble carbohydrates

Differences in SLA between treatments and cultivars were highly correlated with differences in water soluble carbohydrates: a positive linear correlation between the concentration of water soluble carbohydrates (WSC) and the inverse of SLA (i.e. SLW) was found. The regression line is given by:

$$\text{SLW} = 25.1 + 0.107 \cdot \text{WSC}$$

in which WSC is in mg/g and SLW is in g m⁻²; $r = 0.9$. This confirms the idea of Johnson and Thornley (1983) that SLA depends on the ratio between reserve and structural material.

The lower SLA found at the low water potential is in agreement with data of Norris (1982), who reported a lower SLA during drought (according to his paper 34.5 and 32.2 cm².g⁻¹ for irrigated swards and covered swards (receiving no rainfall), respectively; probably his units were incorrect because the values should be about a factor 10 higher). Also Jones et al. (1980) found lower SLA values with drought (214 and 188 cm² g⁻¹ for irrigated swards and

swards receiving no additional water, respectively).

Relationship between tillering, leaf extension and dry matter production

Troughton (1968) suggested the use of the allometric relationship between plant weight and tiller number (logarithm of weight versus logarithm of tiller number) as a method of eliminating any effects of size on the comparison of growth rates. The slope of this allometric relationship between dry weight and tiller number, which is the ratio of relative growth rate (RGR) and relative tiller appearance rate (R_{tar}), was only slightly affected by water potential, but larger for Condesa than for Wendy (Table 4.4), because Condesa produced less but heavier tillers. RGR was about 1.5 times R_{tar} . This can only be the case, when the mean tiller weight (W_{til}) increases. This becomes clear when RGR is split into two components R_{tar} and the relative growth rate of the mean tiller weight, RGR_{til} :

$$\begin{aligned}
 RGR &= \frac{1}{W} \frac{dW}{dt} = \frac{1}{W_{til} \times T} \frac{d(W_{til} \times T)}{dt} = \frac{T}{W_{til} \times T} \frac{dW_{til}}{dt} + \frac{W_{til}}{W_{til} \times T} \frac{dT}{dt} \\
 &= \frac{1}{T} \frac{dT}{dt} + \frac{1}{W_{til}} \frac{dW_{til}}{dt} = R_{tar} + RGR_{til}
 \end{aligned} \tag{3}$$

RGR_{til} declined with time (Table 4.2), which can be explained by the decline of the leaf appearance rate, the linear relation between leaf length and leaf number and by the increase of the proportion of small tillers with time. Even for an individual tiller, the relative growth rate cannot be constant, even when the rate of leaf formation is constant, because the length of successive leaves increases only linearly. After prolonged growth, also leaf death would occur, limiting the number of green leaves per tiller to three to five and this would further reduce the relative growth rate of the tiller.

4.5 APPENDIX

Relation between number of inhibited and unemerged buds per tiller and site filling

Site filling is defined as the relative increase of tiller number per leaf appearance interval (Neuteboom and Lantinga, 1989). It is a measure of the proportion of laid down tiller buds that have developed into tillers. In each leaf axil, including the axil of the prophyll, a tiller bud is formed, but some of these buds may not develop into tillers or show a delayed appearance as a tiller. It will be shown below that the following relationship exists between the number of inhibited buds plus not yet emerged tiller buds per tiller (n) and site filling (F_s):

$$n = 1 - \frac{\ln(e^{F_s} - 1)}{F_s} \quad (\text{A.1})$$

Relation A.1 will be derived for a constant, integer number of inhibited plus unemerged buds per tiller. At the time of appearance (at the end of leaf appearance interval a) of a certain tiller A, a tiller bud has already been formed in the axil of the prophyll of A. The first axillary tiller on A will appear, when this prophyll tiller bud becomes visible as a tiller, which is in leaf appearance interval $a + n$. On all tillers the same age or older than A - so on all tillers already present at leaf appearance interval a ($= k - n + 1$) - a tiller will emerge from leaf appearance interval $a + n - 1$ ($= k$) to $a + n$ ($= k + 1$). The same result is obtained, when the first $n - 1$ tiller buds on a tiller do not produce a tiller, but then subsequent tiller buds must produce a tiller one leaf appearance interval after the buds were formed. Since tiller mortality is assumed to be absent, the recurrence equation for tiller number T (per plant or per area) becomes:

$$T_{k+1} = T_k + T_{k-n+1} \quad (\text{A.2})$$

in which the subscript k denotes the number of leaves appeared on the main tiller since an arbitrary starting point ($k = 0$) and n is the number of inhibited plus unemerged buds per tiller.

Site filling is here (cf. eqn. (1) in the main text):

$$F_s = \ln\left(\frac{T_{k+1}}{T_k}\right) \quad (\text{A.3})$$

For k to infinity, site filling approaches a constant value. From A.3, $\lambda = e^{F_s}$ is calculated, for k to infinity:

$$\begin{aligned} \lambda &= \lim_{k \rightarrow \infty} \frac{T_{k+1}}{T_k} = 1 + \lim_{k \rightarrow \infty} \frac{T_{k-n+1}}{T_k} \\ &= 1 + \lim_{k \rightarrow \infty} \frac{T_{k-1}}{T_k} \cdot \frac{T_{k-2}}{T_{k-1}} \cdot \frac{T_{k-3}}{T_{k-2}} \cdot \dots \cdot \frac{T_{k-n+2}}{T_{k-n+3}} \cdot \frac{T_{k-n+1}}{T_{k-n+2}} \\ &= 1 + \lambda^{1-n} \end{aligned} \quad (\text{A.4})$$

In the second line of eqn. A.4, the product after the limit sign contains $n-1$ factors, which all approach to λ^{-1} for $k \rightarrow \infty$. Therefore, the limit of this product is $(\lambda^{-1})^{n-1} = \lambda^{1-n}$. Now, n can be expressed in λ :

$$n = 1 - \frac{\ln(\lambda-1)}{\ln \lambda} \tag{A.5}$$

Finally, A.1 can be found by substituting λ by e^{F_s} in A.5; the relation is graphically shown in Fig. 4.2. Checking for $n=2$ results in an analytical solution for $F_s = \ln(\frac{1+\sqrt{5}}{2}) \approx 0.481$, which was numerically obtained by Davies (1974) for the situation of $n=2$.

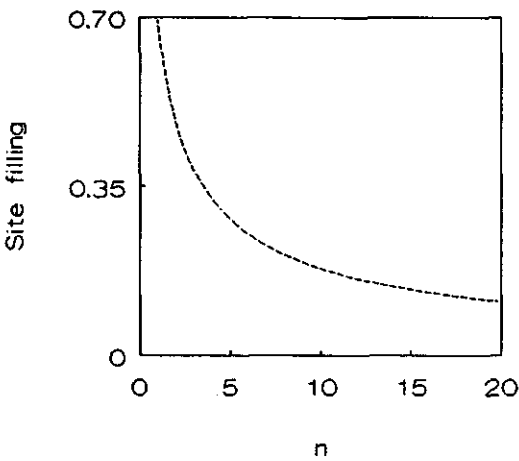


Fig. 4.2. The relation between site filling and n , the number of inhibited plus unemerged tiller buds per tiller, according to eqn. A.1.

PART III

Limitations by low nitrogen supply

CHAPTER 5

Effects of nitrogen supply on tillering dynamics and regrowth of perennial ryegrass populations

with A.H.C.M. Schapendonk and A.L.F. de Vos

ABSTRACT

In a glasshouse, three diploid and three tetraploid populations of *Lolium perenne* L. were grown to evaluate population differences in tillering, leaf extension and assimilate distribution between root and shoot. Three subsequent conditions were used: 1) two steady-state plant nitrogen concentrations (0.027 and $0.042 \text{ g N g DM}^{-1}$), 2) changing nitrogen availability to zero, 3) changing nitrogen availability to a supra-optimal level. Populations were grown in monocultures and mixtures to investigate the correlation between monoculture and mixture performance. The highest yielding population yielded 40 % more cut herbage than the lowest yielding population. Its nitrogen uptake was 29 % higher and its organic nitrogen concentration was between 5 and 8.5 % lower. Population differences in mixtures were larger, but population order remained the same. It was therefore concluded that selection of populations for regrowth characteristics can best be done in mixtures. Stopping nitrogen application reduced tiller density and assimilate allocation to shoots. Both were restored to more than the original values after changing to a supra-optimal nitrogen availability. Analysis of tillering in terms of leaf appearance and site filling showed a negative genetic correlation between the two factors. A simulation model was developed to evaluate the relative importance of tiller density, leaf expansion and assimilate partitioning for regrowth.

5.1 INTRODUCTION

For a high growth rate of perennial ryegrass after defoliation a high leaf area expansion rate is needed. High nitrogen fertilizer application rates are used to stimulate leaf area expansion after defoliation. However, since removal of nitrogen in animal products (milk, meat) is only about 16 % of applied nitrogen in intensive grassland systems ('t Mannetje & Jarvis, 1990), excess nitrogen is causing an increasing environmental problem. Therefore, much attention has been given to improve grassland management and to achieve a higher recovery of fertilizer nitrogen in the grass crop and in animal production (Van der Meer & Van Uum-Loohuyzen, 1986). The best way of improving nitrogen recovery in animal products would be to decrease the nitrogen concentration in the herbage. From a viewpoint of nutritive value, a concentration of about $0.02 \text{ g N (g DM)}^{-1}$ in the diet would be sufficient for dairy cows (Van Vuuren, 1991). However, dry matter yield of existing cultivars of perennial ryegrass is too low at such a low nitrogen concentrations. Possibly, through plant breeding, cultivars can be created capable of higher growth rates at lower nitrogen concentrations. In this study, differences between populations of perennial ryegrass under various levels of nitrogen availability are determined.

In studies with steady-state plant nitrogen concentrations of populations grown in mixtures, Schapendonk *et al.* (1990) showed that differences between populations of perennial ryegrass in growth rate were not caused by differences in *nitrogen use efficiency* (dry matter production per nitrogen built into organic substances), but by differences in *nitrogen uptake* caused by differences in leaf area expansion rates after defoliation. Differences in tiller density between nitrogen treatments and populations caused differences in leaf area expansion after defoliation. However, since tillering pattern was not studied in great detail, it was not clear what had caused the differences in tiller density. Schapendonk *et al.* (1990) used nitrogen application rates ensuring a steady state plant nitrogen concentration and did not answer the question whether genetic differences in nitrogen use efficiency could be found with large fluctuations in nitrogen availability. Furthermore, their studies were carried out with mixtures of populations and it is not certain that performance of populations in monocultures will be the same as in mixtures.

Although there have been many studies on the response of populations of perennial ryegrass to nitrogen in tillering and leaf expansion characteristics (Wilman & Wright, 1983; Pearse & Wilman, 1984; Ryle, 1964), few attempts have been made to show the relative importance of such population characteristics for regrowth potential and yield formation after defoliation. In addition, many of these experiments have been carried out in the field, where it is hardly possible to maintain a constant plant nitrogen concentration. If plant nitrogen concentrations vary, it is difficult to quantify the relation between physiological parameters and plant nitrogen concentration accurately. Therefore, genotypic differences in these relations have to be studied under steady state nitrogen conditions (Schapendonk *et al.*, 1989).

This leads to the following aims of this study: 1) to evaluate the importance of tiller density and tillering rates, leaf extension rates and dry matter distribution for grass growth rate after defoliation at several levels of nitrogen availability; 2) to determine whether genetic variation exists in the response of these plant characteristics to nitrogen supply; 3) to quantify the ability of populations to adapt to changing nitrogen conditions; 4) to determine whether populations have the same performance in mixtures as in monocultures.

5.2 MATERIAL AND METHODS

Plant material: Seeds of six populations (diploid cultivars Splendor and Wendy, one family of R₃-Splendor and tetraploid cultivars Condesa, Modus and Citadel) were germinated on 23 December 1988. R₃-Splendor is a selection from Splendor obtained from a recurrent selection program aimed at improving persistency (Deri Nijs & Winkelhorst, 1989). On 2 January 1989, plants were transplanted to containers with nutrient solution (24 plants per container with an area of 0.145 m². Edge effects were eliminated by using vertical aluminium screens around the containers. On 12 January 1989, plants were cut to a height of 6 cm to increase uniformity in plant size. On this date also the nitrogen treatments were started. Dates will be referred to in days from the start of the nitrogen treatments (12 January = day 1). Plants

were not vernalized and therefore remained vegetative during the experiment.

Light and temperature: Mean photosynthetically active radiation (PAR) in the glasshouse increased from 1.2 to 2.1 MJ.m⁻².d⁻¹, during the first four growth periods. During the last two growth periods radiation was not measured, but from radiation data of the weather station Wageningen and the transmission coefficient of the glasshouse mean daily radiation was estimated to be c. 4 MJ.m⁻².d⁻¹. Mean daily radiation in the glasshouse remained so low, because sunblinds had to be used to prevent high temperatures. Average air temperatures were 13.7/19.4 °C (night/day) and nutrient solution temperatures were 17.8/17.9 °C (night/day).

Cutting regime: Every four weeks herbage was cut to 2 cm stubble height and roots were trimmed to 3 cm length. Roots were excised to assess dry matter distribution to shoot and root.

Sward types: Three simulated sward types were used: 1) containers with monocultures of each population; 2) containers with mixtures of three diploid populations (eight plants per population per container); 3) containers with mixtures of three tetraploid populations (eight plants per population per container). For each monoculture-nitrogen treatment combination three replications were used and each mixture-nitrogen treatment combination was replicated four times.

Nitrogen application system: The nitrogen application system is the same as described in Schapendonk *et al.* (1990). Four nitrogen dosage units were used (two for each steady state nitrogen level). Each nitrogen dosage unit regulated the nitrogen supply to a nutrient solution storage container (150 L). The flow of nutrient solution from the storage container to each of 15 containers (volume per container 20 L) was 2 L.min⁻¹. Flow out of the containers was fed back to the storage container. Nitrogen (as nitrate) was added continuously at a rate that was calculated by use of a simple simulation model described below. The model needs an initial value for leaf area index at the beginning of each (re)growth period. Leaf area index some days after cutting was based on measured leaf widths and length and tiller density. The pH of the nutrient solution was automatically adjusted to 5.6.

Nitrogen treatments: Three subsequent conditions of nitrogen availability were applied: 1) steady state (2 levels); 2) no nitrogen; 3) supra-optimal nitrogen availability. From day 1 to day 112 (4 growth periods of 4 weeks each), two steady state nitrogen treatments were used:

N_{opt}: an optimal (just enough for maximum growth) application rate aiming at a total nitrogen concentration in shoot dry matter of 0.045 g N.g DM⁻¹.

N_{lim}: a suboptimal application rate aiming at a total nitrogen concentration of 0.0225 g N.g DM⁻¹. According to Ingestad (1980), this leads to a relative growth rate of 50 % of that at N_{opt}.

Growth periods 1 to 4 will be referred to as the steady state nitrogen period. After the fourth cut, the nutrient solution was replaced by a nitrogen free nutrient solution (0 N period). After

the fifth cut, nitrogen availability was restored again by replacing the nutrient solution by a full Steiner's nutrient solution (supra-optimal N; Steiner, 1984).

Table 5.1. Parameters and initial value of leaf area index used in the simulation model for the nitrogen dosage system at two levels of plant nitrogen concentration (PNC).

Parameter	Unit	Value at PNC of 0.0225 0.045 g N g DM ⁻¹		Reference
Extinction coefficient	-	0.55	0.55	Schapendonk <i>et al.</i> 1990
Reflection coefficient	-	0.08	0.08	"
Radiation use efficiency	g DM MJ ⁻¹ (PAR)	3.9	5.8	"
Shoot weight ratio*	-	0.58	0.78	"
Specific leaf area	cm ² g ⁻¹			
diploid populations		475	525	"
tetraploid populations		445	485	"
Initial leaf area index	m ² m ⁻²			
(means for all populations)				
at start of period 1		0.40	0.40	"
(five days after cutting)				
period 2		0.10	0.14	estimated from measured
3		0.25	0.65	tiller density, leaf
4		0.22	0.62	length and width

* Harvested shoots/(harvested roots + shoots), equivalent to incremental leaf weight ratio, since harvestable shoot consists mainly of leaf blades and no leaf blades remain in the stubble after cutting.

Measurements: At weekly intervals, tiller number and number of newly appeared leaves on marked tillers of three plants per container, for monocultures only, were counted. Five days after harvest 1, 2 and 3, tiller number and the length of the youngest expanding leaf on the main tiller of all plants in the monocultures were determined. Leaf width on the main tiller was measured of one plant per population in one of the replicates, in order to obtain a mean width per nitrogen treatment, which was needed for the nitrogen dosage system. Harvested material of root and shoot was dried at 70 °C for 24 h and weighed per container for monocultures and per population for mixtures. Total nitrogen concentration was determined of harvested roots and shoots (Kjeldahl) and concentration of nitrate was determined colorimetrically.

Analysis: From tiller numbers and number of appeared leaves, relative tillering rate (R_{til}), leaf appearance rate (L_A) and site filling (F_s) were calculated. Site filling (F_s , unitless) is the relative appearance rate of tillers per leaf appearance interval (Davies, 1974; Neuteboom & Lantinga, 1989):

$$F_s = \frac{R_{tar}}{L_A} = \frac{\ln T_2 - \ln T_1}{I_2 - I_1} = \frac{\ln T_2 - \ln T_1}{\Delta t} \cdot \frac{\Delta t}{I_2 - I_1} \quad (1)$$

in which L_A is the leaf appearance rate (new leaves per tiller per time interval, d^{-1}), R_{tar} is the relative tillering rate (d^{-1}), and T_1 and T_2 are plant tiller number and I_1 and I_2 are number of leaves appeared on the main tiller, at time t_1 and t_2 respectively. Site filling is related to the number of tiller buds per tiller not yet emerged as secondary tillers (Chapter 4).

The fraction of assimilates allocated to harvestable shoot - called shoot weight ratio here - was calculated for each growth period as dry weight of cut herbage (shoot above the cutting height) divided by total dry matter harvested (root + shoot). After the first cut, this shoot weight ratio may be regarded as the incremental leaf weight ratio, since no harvestable shoot or root remained after a cut and therefore all shoot and root material harvested at the next cut was formed during that growth period.

Simulation model:

The simulation model used, has been described by Schapendonk *et al.* (1990). This model calculates dry matter accumulation as the product of intercepted radiation and the radiation use efficiency. Initial regrowth during the first days after cutting can not be simulated correctly with this model, since it does not simulate leaf growth from reserves in the stubble. Therefore, simulation was started five days after cutting. Leaf area index five days after defoliation was initiated as the product of tiller density and leaf width and length of the expanding leaf on the main tiller, at that moment.

The model was used for three purposes:

1. Regulation of the nitrogen application rates during the steady state nitrogen period. Nitrogen application rate was based on the predicted mean growth of all populations and sward types. Initial and parameter values are given in Table 5.1.
2. Validation of the model: comparison of simulated and measured herbage yields of the different populations, with population specific input (tiller density, leaf area expansion rate, incremental shoot weight ratio and specific leaf area).
3. Sensitivity analysis of initial leaf area index five days after defoliation and assimilate partitioning to shoot and roots.

5.3 RESULTS

Plant nitrogen concentrations

During the period with steady state nitrogen concentrations, in the monocultures, mean total nitrogen concentration of harvested shoots was $0.027 \text{ g N g DM}^{-1}$ (no nitrate) at N_{lim} and $0.042 \text{ g N g DM}^{-1}$ at N_{opt} . At N_{lim} , no nitrate was found, but at N_{opt} the nitrate concentration was $0.007 \text{ g N g DM}^{-1}$. Differences in organic nitrogen concentration between populations were generally small (Table 5.2). R_3 -Splendor, however, showed a 5 % lower organic nitrogen concentration relative to Splendor during steady state nitrogen conditions. After transition to 0 N, the nitrogen content of R_3 -Splendor in the N_{lim} -treatment was even 8.5 % lower. In the mixtures of diploids, organic nitrogen concentrations were somewhat lower than

in the monocultures.

Table 5.2. Organic nitrogen concentrations (g N (100 g DM)⁻¹) of harvested shoots. Values for steady-state period are means for cut 1 to 4. Values for 0 N are of fifth cut. Values are means of 3 or 4 replications (a replication is a container with 24 plants for monocultures and consists of 8 plants for mixtures).

Nitrogen treatment (during 'steady- state' period)	N _{opt}		N _{lim}					
	Monoculture		Mixture		Monoculture		Mixture	
Sward type	Steady state	0 N	Steady state	0 N	Steady state	0 N	Steady state	0 N
Period	Steady state	0 N	Steady state	0 N	Steady state	0 N	Steady state	0 N
Populations								
Splendor	3.61	1.19	3.46	1.41	2.80	1.29	2.57	1.30
R ₃ -Splendor	3.43	1.18	3.28	1.23	2.65	1.18	2.43	1.24
Wendy	3.53	1.27	3.37	1.28	2.85	1.20	2.70	1.30
Condesa	3.54	1.21	3.39	1.29	2.63	1.33	2.41	1.23
Modus	3.52	1.32	3.47	1.33	2.79	1.25	2.78	1.31
Citadel	3.43	1.31	3.36	1.24	2.73	1.25	2.66	1.31
LSD (p=0.05)	0.08	0.14	0.08	0.18	0.04	0.08	0.04	0.10

Dry matter and nitrogen yield

Simulated leaf area index of successive cuts increased from cut 2 to 4 (Fig. 5.1). At N_{opt}, cut herbage yield of successive cuts also increased during the steady state nitrogen period (Fig. 5.2). At N_{lim}, the first harvest gave a higher yield than the second, but thereafter herbage yields increased again. Cut herbage yield of N_{lim} was on average only 40 % of that of N_{opt} (sum of the first four growth periods). Modus had the highest herbage yield of the tetraploid populations, while R₃-Splendor outyielded all populations (Table 5.3). Ranking of populations according to herbage yield was the same at N_{lim} and N_{opt}. Herbage yield of mixtures was higher than that of monocultures at N_{opt}, but this was not found at N_{lim}. At N_{opt}, population differences in mixtures were larger than in monocultures, both absolutely and relatively, but population ranking remained the same.

Mean nitrogen yield (in shoots plus roots) of growth period 1 to 5 was equal to the sum of nitrogen applied and amount of nitrogen initially present in plants (Table 5.4). However, nitrogen yield of individual containers differed significantly. Mixtures showed a larger nitrogen yield than monocultures at N_{opt}. In mixtures, large differences between populations occurred in nitrogen yield (Table 5.5). In monocultures, differences in nitrogen yield between populations were less. The highest yielding populations (R₃-Splendor and Modus) showed the highest nitrogen yield both in monocultures and mixtures.

Table 5.3. Total herbage harvested (dry matter, g per plant) of cut 1 to 4. Means of 3 replications in monocultures and 4 in mixtures. Within sward types, population differences of more than 22 % and differences between nitrogen treatments of more than 12 % are statistically significant ($p < 0.05$).

	N_{opt}		N_{lim}	
	Monoculture	Mixture	Monoculture	Mixture
Population				
Splendor	1.6	1.8	0.66	0.52
R ₃ -Splendor	2.3	3.5	0.91	1.12
Wendy	1.8	2.0	0.81	0.67
Condesa	2.0	2.1	0.62	0.65
Modus	2.2	2.7	0.87	0.99
Citadel	1.9	2.2	0.79	0.71
Mean	2.0	2.4	0.78	0.79
Coefficient of variation (%)	17	21	17	21

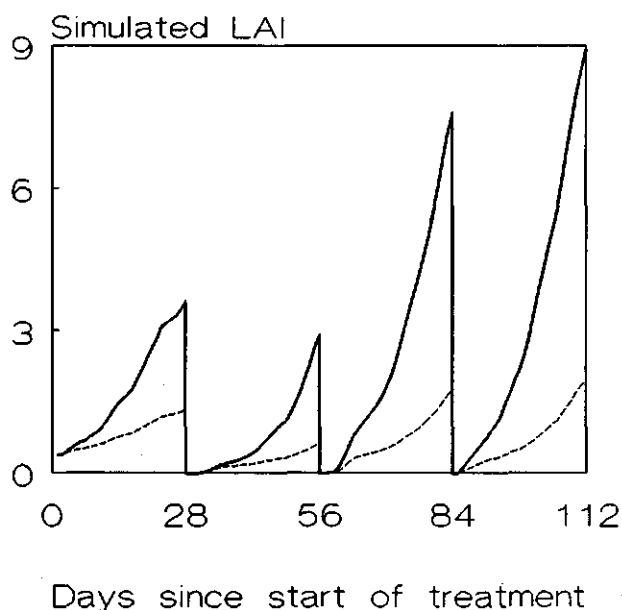


Fig. 5.1. Time course of simulated leaf area index during the first four growth periods (steady state nitrogen concentrations). Dashed line: N_{lim} ; solid line: N_{opt} .

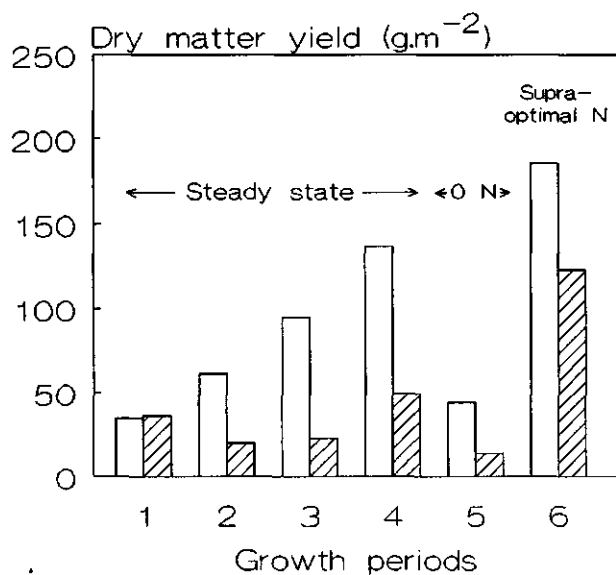


Fig. 5.2. Herbage dry matter yield (g m^{-2}) of consecutive cuts. Means for all populations. Left bar = N_{opt} ; right bar = N_{lim} .

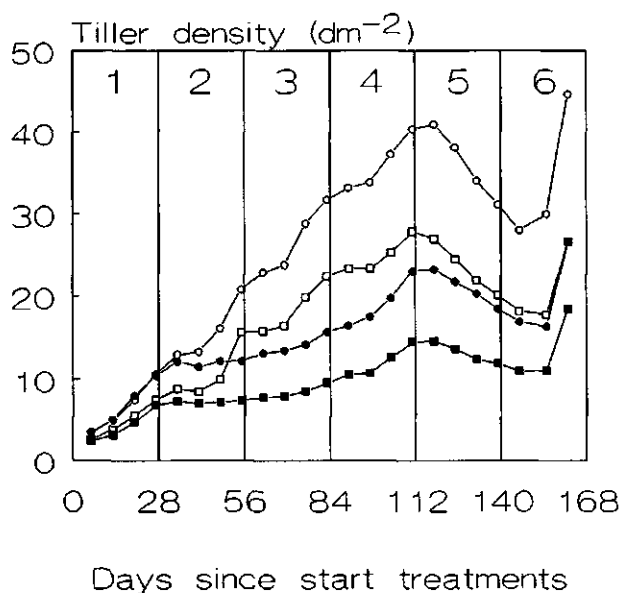


Fig. 5.3. Time course of the tiller density in monocultures.
 N_{opt} : ○ diploids; □ tetraploids;
 N_{lim} : ● diploids; ■ tetraploids.
 Vertical lines represent harvest dates. Steady state plant nitrogen concentrations: periods 1-4; Zero nitrogen: period 5; Supra-optimal nitrogen: period 6. Points are means of 9 containers.

Table 5.4. Nitrogen present in plants at the start of the experiment, total nitrogen application of period 1-4 and total nitrogen harvested of growth period 1-5.

Nitrogen treatment	N applied	N initially present in the plants	Total N available	Mean total N harvested in plants
N_{opt}	18.7	0.7	19.4	18.4
N_{lim}	4.5	0.7	5.2	5.4

Table 5.5. Nitrogen harvested in roots and shoots (g N m^{-2}). The coefficient of variation of N-uptake is 12 % in monocultures and 18 % in mixtures. In both sward types, population differences of more than 16 % are statistically significant ($p < 0.05$). - = not available since roots of populations within a mixture could not be separated.

Populations	Monocultures			Mixtures of diploids or tetraploids		
	shoot	root	total	shoot	root	total
<i>Nitrogen treatment: N_{opt}</i>						
Splendor	11.8	3.6	15.1	12.7	-	-
R ₃ -Splendor	16.4	3.3	19.7	23.1	-	-
Wendy	13.9	3.3	16.9	14.7	-	-
mean of diploids	14.0	3.4	17.2	16.8	3.7	20.5
Condesa	14.9	3.3	18.2	14.4	-	-
Modus	16.4	3.5	19.9	20.2	-	-
Citadel	13.9	3.3	17.2	15.4	-	-
mean of tetraploids	15.4	3.3	18.4	16.7	3.8	20.5
<i>Nitrogen treatment: N_{lim}</i>						
Splendor	3.3	1.6	4.9	2.4	-	-
R ₃ -Splendor	4.3	1.8	6.1	4.8	-	-
Wendy	4.1	1.7	5.8	3.2	-	-
mean of diploids	3.9	1.7	5.6	3.5	1.6	5.1
Condesa	2.9	1.3	4.2	2.8	-	-
Modus	4.3	1.9	6.2	4.8	-	-
Citadel	3.8	1.8	5.6	3.3	-	-
mean of tetraploids	3.7	1.7	5.3	3.6	1.7	5.3

Dry matter distribution between roots and shoots

Shoot weight ratio was 23 % lower at N_{lim} than at N_{opt} . Shoot weight ratio of R3-Splendor was highest and of Splendor was lowest (Table 5.6). Changing from steady state nitrogen application rate to zero N reduced the shoot weight ratio by 21 and 12 %, for N_{opt} and N_{lim} , respectively. After changing to supra-optimal nitrogen availability, shoot weight ratio was raised again to values even higher than before the zero N period. The ranking of populations in shoot weight ratio changed.

Leaf extension rate

Leaf extension rate was reduced by 26 % at N_{lim} (Table 5.7). Hardly any genotypic differences were found. Only Splendor showed a lower leaf extension rate at N_{opt} , and Modus showed a higher leaf extension rate at N_{lim} , compared to the other populations. Leaf width of tetraploids was 17 % larger than of diploids. Thus, the leaf area expansion rate of tetraploids (per leaf and thus per tiller) was higher than of diploids.

Table 5.6. Shoot weight ratio with steady state nitrogen conditions and adaptation to changed nitrogen availability. Values are means of 3 replications.

Populations	N_{opt}			N_{lim}		
	Steady state	0 N	Supra-optimal	Steady state	0 N	Supra-optimal
Splendor	0.71	0.60	0.96	0.56	0.55	0.85
R ₃ -Splendor	0.79	0.61	0.95	0.61	0.54	0.88
Wendy	0.76	0.60	0.94	0.59	0.53	0.88
Condesa	0.77	0.62	0.95	0.61	0.47	0.88
Modus	0.78	0.59	0.90	0.58	0.54	0.86
Citadel	0.77	0.60	0.91	0.60	0.49	0.87
LSD (P=0.05)	0.022	0.056	0.028	0.022	0.056	0.028

Tillering and leaf appearance

Tillering was only studied in monocultures. Mean tiller density for all populations increased rapidly during the first four growth periods (Fig. 5.3). Differences between N_{opt} and N_{lim} in tiller density and in tillering rates were not yet apparent during the first growth period. During the second growth period the relative tillering rate of N_{lim} was on average 84 % lower than that of N_{opt} . However, in the fourth growth period, the relative tillering rate of N_{lim} was 71 % higher than that of N_{opt} , although the absolute tillering rates were similar ($0.5 \text{ tiller dm}^{-2} \text{ d}^{-1}$). Differences in relative tillering rate can be attributed either to differences in leaf appearance rate or differences in site filling. As with tillering rates, the effect of the nitrogen treatments on leaf appearance rate also became only apparent after the first cut. During the second until fourth growth period the leaf appearance rate was constant. The highest leaf appearance rate

occurred in a diploid population (Wendy) and the lowest in a tetraploid (Condesa), but on average no difference in leaf appearance rate between ploidy levels was found (Table 5.7). There was no significant overall interaction between population and nitrogen treatments for leaf appearance rate, although the leaf appearance rate of Condesa was less reduced by the low nitrogen treatment than that of the other populations.

Table 5.7. Tillering characteristics: tiller density (T_1 =initial, T_5, T_6 =at start of fifth and sixth growth period, T_f =final tiller density, dm^{-2}), relative tillering rate ($R_{\text{til}}, \text{d}^{-1}$) and site filling (F_s) of period 2, leaf appearance rate of growth period 2, 3 and 4 (L_A, d^{-1}) and leaf length extension rate of the main tiller ($\text{cm} \cdot \text{d}^{-1}$) during the first five days of regrowth. Means of 3 containers per population (monocultures). Leaf appearance rate remained constant in period 2 till 4.

Nitrogen treatment	Population	T_1	T_5	T_6	T_f	R_{til}	L_A	F_s	Leaf extension rate
			tillers dm^{-2}			d^{-1}	d^{-1}		$\text{cm} \cdot \text{d}^{-1}$
N_{opt}	Splendor	3.1	32	21	38	0.025	0.099	0.25	1.48
	R_3 -Splendor	4.2	52	38	57	0.025	0.094	0.27	1.64
	Wendy	3.0	39	25	39	0.021	0.111	0.19	1.67
	Condesa	2.1	31	19	28	0.030	0.086	0.35	1.61
	Modus	2.6	22	15	24	0.024	0.103	0.24	1.67
	Citadel	3.0	28	20	28	0.025	0.102	0.25	1.68
N_{lim}	Splendor	3.0	20	15	25	0.010	0.079	0.12	1.15
	R_3 -Splendor	4.6	25	17	25	0.005	0.077	0.06	1.15
	Wendy	2.9	25	18	30	0.002	0.087	0.02	1.22
	Condesa	3.1	13	10	20	0.003	0.081	0.04	1.10
	Modus	2.6	13	10	17	0.004	0.083	0.05	1.29
	Citadel	2.5	17	13	19	0.001	0.086	0.01	1.20
LSD Population		0.4	8	8	10	0.010	0.020	0.12	0.08
LSD Nitrogen		0.2	4	4	4	0.006	0.012	0.06	0.04

Since during the first four growth periods no tiller mortality occurred, site filling could be calculated from relative tillering rates and leaf appearance rates. During the first growth period no difference between nitrogen treatments existed in site filling and mean site filling was high (0.486), although the theoretical maximum of 0.693 (Neuteboom & Lantinga, 1989) was not reached. The time course of the relative tillering rate is shown in Fig. 5.4. The time course of site filling shows the same pattern, since site filling is the relative tiller appearance rate divided by the leaf appearance rate and the leaf appearance rate was almost constant. Mean site filling per growth period declined steadily, but large fluctuations occurred in relative tillering rate and site filling *within* growth periods. This pattern for site filling and relative tillering rate was similar for all populations. Both population differences in relative tillering rate and differences in tiller density at the start of the treatments caused the

population differences in tiller density (Table 5.7). Population differences in relative tillering rate were smaller than differences in site filling and leaf appearance due to a negative genetic correlation between site filling and leaf appearance, at N_{opt} .

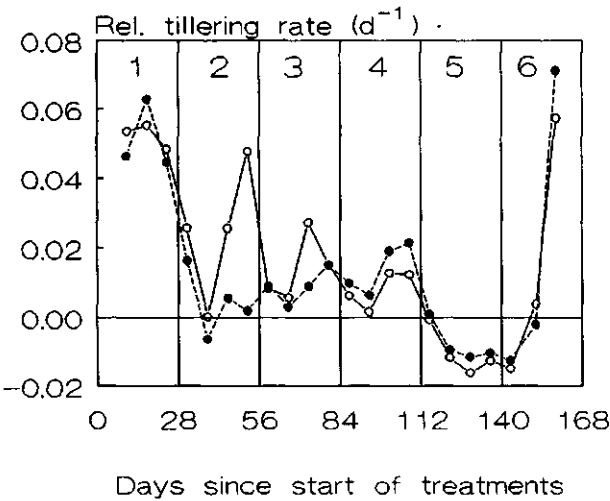


Fig. 5.4. Relative tillering rate. N_{opt} ○; N_{lim} ●. Vertical lines represent harvest dates. Steady state plant nitrogen concentrations: periods 1-4; Zero nitrogen: period 5; Supra-optimal nitrogen: period 6. Points are means for all populations (6 populations replicated 3 times).

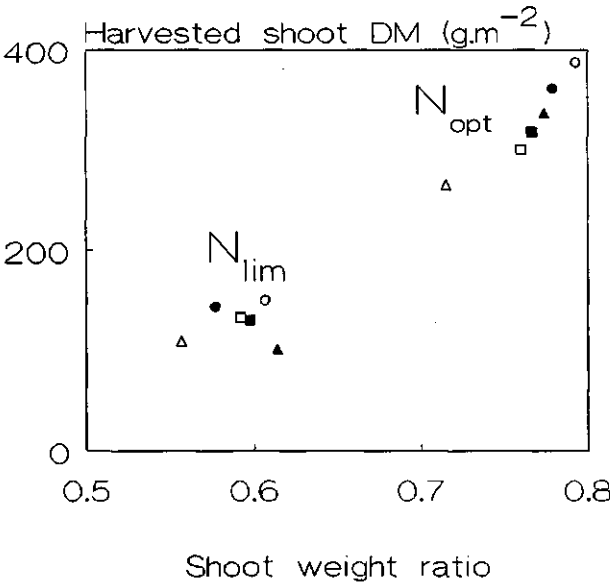


Fig. 5.5. Genetic correlation between total dry weight of herbage harvested per container of cuts 1 to 4 ($g\ DM\ per\ m^2$) and shoot weight ratio = harvested shoot/(harvested root + shoot). Diploids: ▲ Splendor; ○ R_3 -Splendor; □ Wendy. Tetraploids: ▲ Condesa; ● Modus; ■ Citadel. Points are means of 3 replications. Points in left lower corner are for N_{lim} and points in right higher corner for N_{opt} .

Changing nitrogen availability

During the 0 N period, associated with a reduction of the shoot weight ratio, tiller density declined. Absolutely, tiller mortality was higher in the N_{opt} treatment, but relative tiller mortality was on average not significantly higher than in the former N_{lim} treatment (Fig. 5.4). The recovery of tiller density during the supra-optimal nitrogen period was not immediate. This was due to a low survival of old tiller buds that could produce new tillers. Only R3-Splendor was able to produce new tillers from old tiller buds, but the other populations first had to form new tiller buds in the axils of new leaves before new tillers could appear.

Relation between growth and dry matter distribution

The difference in herbage production between the two nitrogen treatments were highly determined by the difference in fraction of dry matter production allocated to herbage above the cutting height (shoot weight ratio, Fig. 5.5). At N_{opt} , a strong positive genetic correlation was found between herbage production and shoot weight ratio. Such a genetic correlation was not found at N_{lim} .

Validation of the simulation model

Fig. 5.6 shows comparisons of simulated and measured herbage yields of the monocultures of cut 2, 3 and 4. In Fig. 5.6A, the initial leaf area index (five days after defoliation) was calculated as the product of tiller density, leaf width and leaf length measured on the main tiller, for each individual container. A high correlation ($R^2 = 0.94$) between simulated and measured yields was found. The residual standard deviation was even lower than found in the ANOVA for populations and nitrogen treatments, because differences between containers with the same population could be accounted for, in part, by differences in initial leaf area index or incremental shoot weight ratio. Only simulations for N_{opt} for the fourth growth period resulted in higher simulated than measured herbage yields, presumably due to an overestimation of the initial leaf area index.

This overestimation may be due to the high fraction of young tillers (age 1 to 5 weeks) with N_{opt} in the fourth growth period. At N_{opt} the fraction of young tillers was much higher than at N_{lim} (Fig. 5.7). Young tillers normally have smaller leaves than older ones. The estimate of leaf area based on measurements on the main tiller was therefore too high at N_{opt} . After correcting for effects of tiller age distribution on mean leaf size, simulated yields also corresponded well with measured yields for N_{opt} for the fourth growth period (Fig. 5.6B).

The importance of population differences in tiller density and leaf extension (resulting in differences in the initial leaf area index after defoliation) was demonstrated in Fig. 5.6C. Here, differences in initial leaf area index between populations were ignored. As a consequence the residual standard deviation of the relation between simulated and measured herbage yield increased. This means that differences in initial leaf area index between populations were indeed partly responsible for differences in herbage yields *within* growth periods and nitrogen treatments.

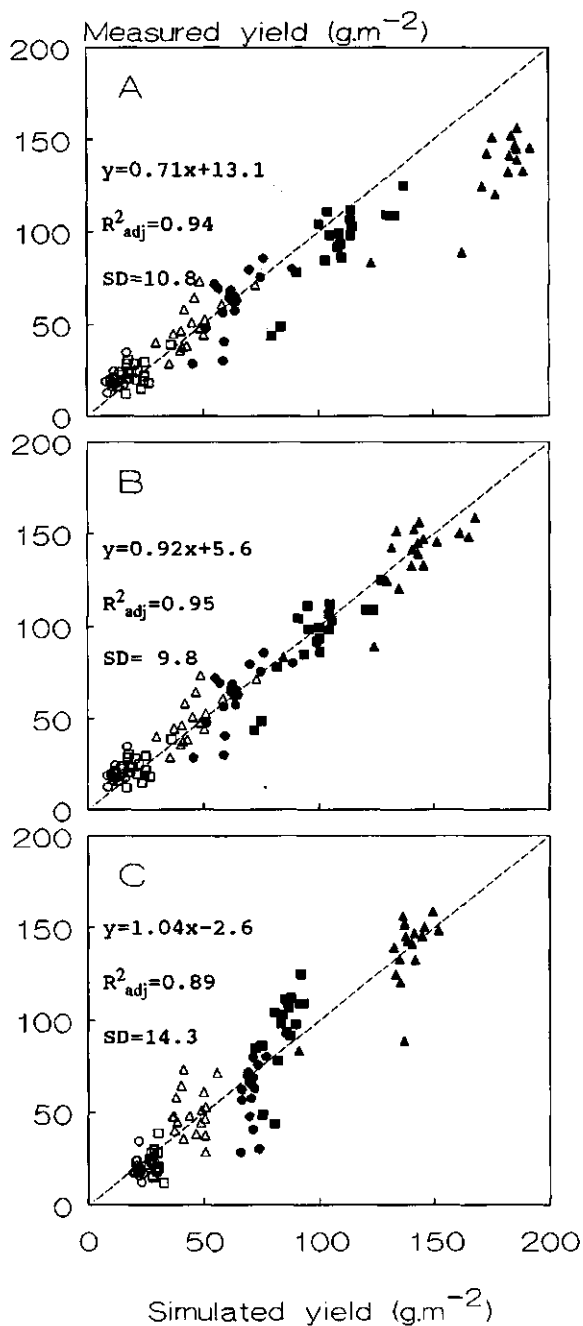


Fig. 5.6. Comparison of simulated and measured herbage dry matter yields of harvests 2 to 4. A) LAI 5 days after cutting = leaf area (main tiller) * tiller density (per container). B) As with A) but with a multiplication factor, F , from leaf area of main tiller to mean area per tiller: $F = 1$ except for the third ($F = 0.8$) and fourth ($F = 0.4$) growth period of N_{opt} . C) As with B) but instead of an individual value of LAI 5 days after cutting for each container and each cut, one average initial value was used for all treatments, populations and periods. Symbols: N_{lim} : ● period 2; ■ period 3; ▲ period 4. N_{opt} : ○ period 2; □ period 3; △ period 4. Each point represents one experimental unit (container).

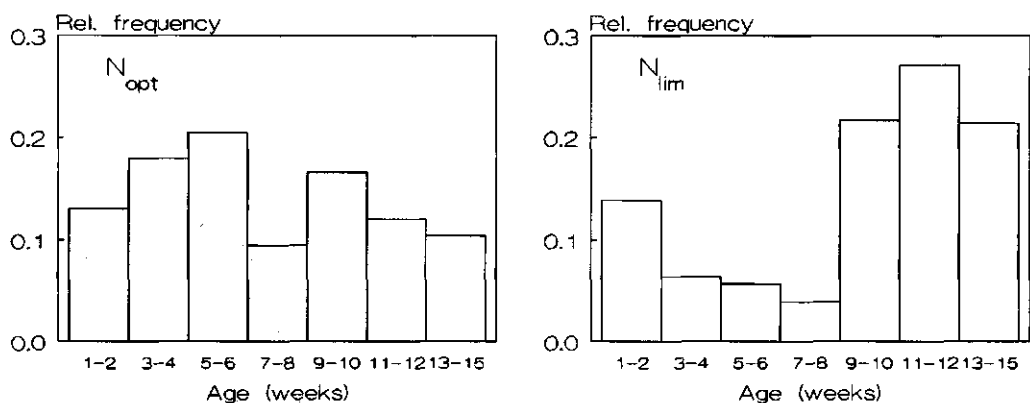


Fig. 5.7. Tiller age distribution at the start of the fourth growth period. Left: N_{opt} ; Right: N_{lim} .

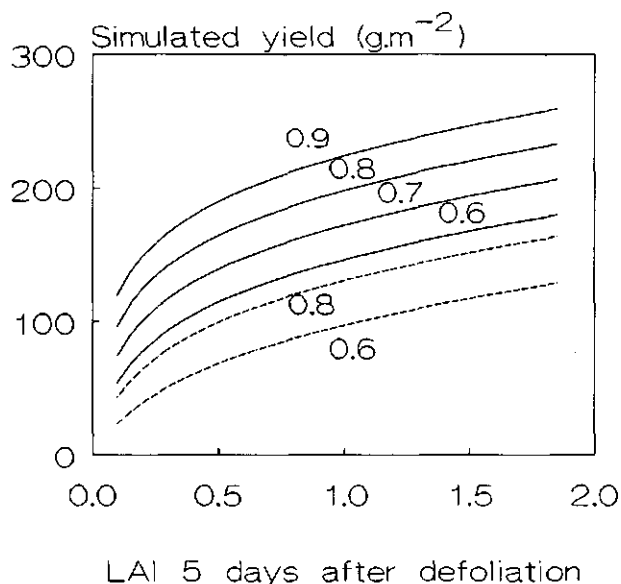


Fig. 5.8. The relation between leaf area index 5 days after defoliation and simulated herbage dry matter yield of a four week regrowth period for several values of the incremental shoot weight ratio. Simulations for total nitrogen concentration of 4.5 (solid lines) and 2.25 g N (100 g DM)⁻¹ (dashed lines). The numbers in the figure indicate the values of the shoot weight ratio. Parameters used for the simulations: specific leaf area: 500 cm² g⁻¹, light use efficiency: 5.8 and 3.9 g DM (MJ intercepted PAR)⁻¹ for nitrogen concentration of 4.5 and 2.25 g N (100 g DM)⁻¹, respectively. Radiation data of the fourth growth period were used.

Sensitivity analysis

The simulation model was used to quantify the relative contribution of tiller density, leaf extension and incremental shoot weight ratio to yield formation at different plant nitrogen concentrations. Since in the model tiller density and leaf expansion rate both affect the initial leaf area index in the same way, the effect of initial leaf area index on herbage yield is shown (Fig. 5.8). Due to the positive feedback between leaf area and growth rate, an increase of the incremental shoot weight ratio results in a proportionally larger increase in simulated herbage yield. In reality, the effect of the incremental shoot weight ratio on herbage yield seems to be even higher than expected from the simulations (Fig. 5.5), but this is due to a positive genetic correlation between the incremental shoot weight ratio and tiller density (compare Table 5.6 and 5.7). Comparing simulated herbage yields at total nitrogen concentrations of 0.045 and 0.0225 g N (g DM)⁻¹ at the same shoot weight ratio shows that at 0.045 g N (g DM)⁻¹ still a higher herbage yield is reached, due to a higher radiation use efficiency for dry matter production.

5.4 DISCUSSION

Population differences in nitrogen uptake and concentration

In previous experiments with the same nitrogen application system, only mixtures were used with the same set of populations on each container (Schapendonk *et al.*, 1990). In that case, mean growth was the same for each container and nitrogen yield per container (of roots and shoots) was equal to the mean nitrogen application per container. Different populations (within a container) did not all have the same nitrogen yield as a consequence of competition for both light and nitrogen. In the present study, with monocultures and mixtures, growth rate was not the same for each container.

Since flow of nutrient solution and nitrate through each container was the same, either one of two situations could be expected: 1) nitrogen yield equals mean nitrogen application for each container and outflow from the container does not contain nitrate; containers with less dry matter production will show higher total nitrogen concentrations; 2) nitrogen yield is not the same for each container; some containers take up less nitrogen than applied to the container. The second situation will occur when nitrogen uptake is proportional to the growth rate, for example because the nitrogen concentration of new plant material cannot exceed a certain maximum. In the second situation, plants on a container are not able to take up all applied nitrogen, when the actual growth rate for a container is lower than the mean expected growth rate and some outflow of nitrogen occurs out of the container back to the storage container. From the storage container, this nitrogen is again equally redistributed to all containers.

In the present study, situation 1 did not take place: large differences in nitrogen uptake and small differences in (organic) nitrogen concentration were found, especially at N_{opt}. Further, nitrogen uptake of some monocultures of populations and the mean nitrogen uptake of

mixtures was higher than the mean nitrogen application per container. An explanation is offered by situation 2. The absence of large differences in (organic) nitrogen concentration indicates that the proportionality between growth rate and nitrogen uptake rate was more or less the same for all populations. Only R₃-Splendor had between 5 and 8.5 % lower organic nitrogen concentrations than Splendor, which indicates that R₃-Splendor had a better nitrogen use efficiency than Splendor.

Light conditions

Although daily radiation levels remained relatively low (compared to field conditions in summer), herbage production during the last growth period (c. 1900 kg DM.ha⁻¹) reached a level comparable to herbage production of a four week growth period in the field. However, specific leaf area values will have been higher in this study than normally found in the field, due to the low radiation level.

Fluctuations in site filling and relative tillering rate

The gradual decline in relative tillering rates at N_{opt} coincided with an increase in mean leaf area index. This was presumably caused by the effect of the red/farred ratio on site filling (Casal *et al.*, 1990), since the red/farred ratio underneath a canopy decreases with leaf area index. Simon & Lemaire (1987) have also shown that above a leaf area index of 3, tillering rates become very low. Although this can explain the general decline of the relative tillering rate with time, it cannot easily explain the high values of site filling at the end of growth periods and low values just after cutting, found in this study. A possible explanation for this pattern within growth periods is a positive effect of assimilate or nitrogen availability on tillering rates. Assimilation rates immediately after cutting were low, because of the low leaf area index and because regrowth of tillers that were already present immediately after cutting already consumed much of the available reserves. This normally results in a characteristic decrease of levels of water soluble carbohydrates in the week after cutting that has been described by many authors (Alberda, 1960; Del Pozo, 1963; Davies, 1966; Hume, 1990). Ourry *et al.* (1988) showed a similar pattern for organic nitrogen contents of roots and stubble. Low tillering rates just after cutting could therefore be caused by a low availability of assimilate or nitrogen to developing tillers. However, tiller bud inhibition by a low red/farred ratio at the end of a growth period cannot be excluded. It would result in a reduction of the number of new tillers that become visible during the first week after cutting, because it takes at least one leaf appearance interval for a tiller bud to grow out of the encompassing sheath tube when site filling is maximal (Neuteboom & Lantinga, 1989). For the same reason, the breaking of tiller bud inhibition by a high red/farred ratio at the beginning of a growth period would lead to a higher tillering rate, but at least one week after defoliation.

At N_{lim}, after the first cut, the relative tillering rates gradually increased with time, despite an overall increase in mean leaf area index. The lower site filling during growth period 2 and 3 at N_{lim} as compared to N_{opt}, has to be ascribed to a direct effect of a lower nitrogen availability. However, in the fourth growth period, when relative tillering rate and site filling were higher at N_{lim} than N_{opt}, it was the higher leaf area index that limited site filling at N_{opt}.

Neuteboom *et al.* (1988) measured site filling only during the first growth period, when leaf area index was still too low to find such limiting effects of leaf area index on tillering.

Negative genetic correlation between site filling and leaf appearance rate

When site filling is lower than maximal, not all tiller buds develop into tillers. Then, tiller bud formation (and therefore leaf appearance) is not a limiting factor for tillering. In this study, tiller bud formation was not strictly limiting any more after the first cut and this resulted in a negative correlation between population means of site filling and leaf appearance (see Table 5.7). This means that selecting populations for a higher leaf appearance rate does not necessarily lead to populations with a higher tillering rate.

Adaptation of the shoot weight ratio to changing nitrogen availability

Shoot weight ratios are generally very strongly positively related to plant nitrogen concentration (Schapendonk *et al.*, 1990). In the present study, it was demonstrated that perennial ryegrass can lower the fraction of assimilates allocated to the shoot very rapidly when the application of nitrogen to the nutrient solution is stopped. Restoration of the incremental shoot weight ratio at supra-optimal nitrogen availability was also very fast. Such fast changes of assimilate distribution between root and shoot after termination of the nitrogen supply have also been shown by Jarvis & Macduff (1989). R₃-Splendor showed a larger plasticity of the shoot weight ratio than the cultivar Splendor, after stopping the nitrogen application and restoring the nitrogen availability to a supra-optimal level again.

Resource complementarity in mixtures

At N_{opt}, yields of mixtures were higher than of monocultures. This means that populations were not fully competing for the same resources in mixtures. This could for example be due to differences in growth form between populations (prostrate/erect), which would cause a higher light interception in mixtures during the first week after defoliation when the fraction of light intercepted is still very low, or to differences in the pattern of regrowth between populations, which would lead to a better matching of nitrogen application and nitrogen uptake in mixtures.

5.5 CONCLUSIONS

In this study, both experimentally and by use of a simulation model, the relative importance of tiller density, area expansion per leaf and shoot weight ratio for the regrowth of grass after defoliation was demonstrated, at various plant nitrogen concentrations. Differences between populations in tillering rate and final tiller number were due to both differences in leaf appearance rate and site filling. Selection for higher leaf appearance rate, however, will not necessarily lead to populations with higher tillering rates, since a negative correlation between leaf appearance rate and site filling was found with optimal nitrogen supply.

No important genetic variation in leaf length extension rate was found. As a result of the

larger leaf width of the tetraploid populations, total leaf area expansion rates of tetraploid populations were not lower than of diploid populations, despite lower tiller densities.

The best yielding population, R₃-Splendor, had the highest tiller density, the highest shoot weight ratio and also a 5 to 8.5 % lower organic nitrogen concentration than Splendor. This indicates that genetic variability towards a higher nitrogen use efficiency exists in perennial ryegrass.

A high plasticity of the shoot weight ratio was generally found with changing nitrogen conditions and also genotypic differences in plasticity of the shoot weight ratio were found.

Selection of populations on characteristics important for regrowth can best be done in mixtures of populations, since populations with a higher regrowth rate will have a competitive advantage in mixtures. Therefore, yield differences between populations with differences in regrowth characteristics will be largest in mixtures, as was found in the experiment.

CHAPTER 6

Regrowth of perennial ryegrass: effects of nitrogen supply and plant density on tillering, leaf area expansion, light interception and growth

with A. Milimonka and J.G. Huisman

ABSTRACT

In a phytotron, we studied tillering, leaf area expansion, light interception and (re)growth of perennial ryegrass at various plant densities and levels of nitrogen supply. Substantial compensation of low plant densities occurred by increased tillering rates and increased leaf area expansion and growth per tiller. However, even 98 d after sowing, tiller density and yield were still lowest at the lowest plant density. At low nitrogen supply, the effect of tiller density on the increase of leaf area index (LAI) and fraction of light intercepted by the swards (%LI) was less than at high nitrogen supply. This was because at low nitrogen supply, compensation of a low density by an increased leaf area expansion rate per tiller was relatively greater than at high nitrogen supply. A negative correlation was found between site filling (F_s , relative tiller appearance rate per leaf appearance interval) and %LI. At low nitrogen supply, F_s decreased faster as %LI increased than at high nitrogen supply. The observed low F_s after defoliation was presumably caused by a low level of reserves in the stubble. Radiation-use efficiency was high (1.2 g OM.mol⁻¹ photons intercepted) and hardly affected by treatments. Differences in leaf growth between nitrogen and plant density treatments could be explained from differences in increase of LAI and %LI that were related to differences in tiller density. Leaf area expansion was not strictly related to leaf growth, since the specific leaf area increased after defoliation and declined again later.

6.1 INTRODUCTION

Yield of perennial ryegrass swards with large open spaces or low plant densities is much lower than of dense swards (Van Loo, 1991; Neuteboom, Lantinga and Van Loo, 1992). Recovery of open swards can be very slow, also at high nitrogen (N) application rates, because of low water soluble carbohydrate levels after defoliation with high N-supply (Alberda, 1960; Davies, 1965). The aim of this study was to examine the extent to which perennial ryegrass can compensate for a lower plant density by increasing the tiller number per plant and/or the growth rate per tiller at different levels of N-supply. At high densities, the so-called 3/2-thinning law applies for the relationship between plant density and plant size (Yoda *et al.*, 1963) and also for tiller density and tiller size (Kays and Harper, 1979; Simons, Davies and Troughton, 1973). However, not much is known about the dynamics towards this relationship and these dynamics are important during the recovery of swards from low densities.

Tiller number of spaced plants grown in a constant environment increases exponentially with a constant relative tiller appearance rate (Neuteboom and Lantinga, 1989). The relative tiller

appearance rate is the product of the leaf appearance rate and site filling (a measure related to the proportion of tiller buds per tiller that have developed into tillers; Davies, 1974; Van Loo, 1992). Simon and Lemaire (1987) have shown that at higher leaf area index (LAI) site filling declines. The exact form of this relationship determines how fast tiller density moves towards an equilibrium density of the 3/2-thinning law. Tiller dynamics are highly influenced by the N-supply to the grass sward (Wilman and Pearse, 1984; Neuteboom, Lantinga and Wind, 1988; Schapendonk, Spitters and de Vos, 1990; Pilbeam and Robson, 1992; Van Loo, Schapendonk and de Vos, 1992). So, it may be expected that the relation between site filling and leaf area index depends on N-supply.

Removal of leaf area through defoliation will influence the recovery from low densities, because site filling depends on the leaf area index. Just after defoliation, tillering may not be limited by the leaf area index, but a low supply of substrate to developing tillers may hamper their outgrowth. Tillering after defoliation will therefore depend on the regrowth rate. Regrowth after defoliation depends on the residual leaf area index, the increase of leaf area, on CO₂-assimilation rate by old and new leaves and on the amount of substrate available in the stubble. The regrowth rate further depends on the assimilate distribution to root, sheaths and leaves. The leaf weight ratio declines with decreasing N-supply (for perennial ryegrass shown by for example Belanger, Gastal and Warembourg, 1989; Schapendonk, Spitters and de Vos, 1990; Pilbeam and Robson, 1992; see also Chapter 5). However, it is not known whether this response of leaf weight ratio to N-supply depends on the density of the sward. Since both the leaf area expansion after defoliation and the amount of substrate in the stubble depend on the tiller density, the regrowth rate depends on the tiller density. For a better understanding of recovery from low tiller densities in periodically defoliated swards, the interrelationships between tiller density, tillering and regrowth after defoliation should be understood.

An important aspect of the relationship between tiller density and regrowth after defoliation is whether or not leaf area expansion after defoliation is limited by the substrate pool in the stubble (sink limited or source limited growth). With sink limited growth, the leaf area expansion rate of the crop does not depend on the substrate supply to leaves, but is limited by the maximum leaf area expansion rate per tiller and the tiller density. Then the specific leaf area (SLA) depends on the supply of assimilates to leaves from current assimilation and from reserves in the stubble (Johnson and Thornley, 1983; see also Chapter 3). Conflicting experimental data have been reported in the literature on the time course of SLA after defoliation. Sheehy, Cobby and Ryle (1980) found high values of SLA just after defoliation and a decline with time, but the reverse pattern has been found by Grant, Barthram and Torvell (1981).

Therefore, four questions related to the rate of recovery from low densities were studied:

i) Does the relationship between site filling and leaf area index depend on N-supply? ii) Is leaf area expansion of a sward during the initial regrowth after defoliation limited by the

availability of assimilates (source-limited growth) or by the tiller density (sink-limited growth) and how is this reflected in the SLA? iii) How important are carbohydrate reserves in the stubble for the regrowth and continued tillering after defoliation? iv) Does dry matter and nitrogen distribution to different organs depend on the density of the sward? These questions were studied in a phytotron experiment with three initial plant densities using three levels of N-supply.

6.2 MATERIAL AND METHODS

Plant material and growing conditions

On 6 September 1990 (day 1), small swards of perennial ryegrass (*Lolium perenne* L. cultivar Wendy) were created by sowing seeds at their final positions, in containers (length*width*height=0.46*0.31*0.2 m; area = 0.143 m²) filled with river sand. Edge effects were eliminated by using vertical aluminium screens around the containers. The experiment was carried out in three climate rooms (replicates). Light was provided by Philips SON-T and HPL-lamps giving a photosynthetic photon flux density of 260 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ at plant level. The day length was 15 h. Day/night temperatures were 20/15 °C. On day 35, 56 and 77 all swards were cut to 2 cm. On day 84, 91 and 98, part of the plots were completely harvested.

Treatments

Three plant densities and three levels of N-supply were used (Table 6.1). Seeds were not randomly distributed over the area of a container, but were placed at two, eight or 32 spots (five seeds at each spot), depending on the sowing density. Combinations of plant densities and nitrogen treatments are denoted D_iN_j for plant density i and N-supply level j . Each of the nine combinations of the plant densities and N-supply level occurred three times in each climate room (81 containers in total). At D_3N_3 , the N-application rate per growth period was aimed at just achieving maximal growth. Since at D_3 , swards were initially not very dense whilst growth rates were less than maximal, the swards required less nutrients per unit area for maximal growth during the first two growth periods than later. Nutrients were supplied on day 13 and the day after each cut as Steiner's nutrient solution (Steiner, 1984), which was adapted for the lower levels of N-supply. With this nutrient solution, phosphorus was applied at 0.13, 0.5, 0.75 and 0.75 g.m⁻² and potassium at 0.94, 3.5, 5.3 and 5.3 g.m⁻², for growth periods 1, 2, 3 and 4 respectively.

Measurements

Number of tillers per plant (of the plants in two spots per plot) and the number of newly appeared leaves on main tillers (two per plot were used) were counted weekly. Site filling (F_s), was calculated as:

$$F_s = \frac{R_{tar}}{L_A} = \frac{\ln T_2 - \ln T_1}{I_2 - I_1} = \frac{\ln T_2 - \ln T_1}{\Delta t} \cdot \frac{\Delta t}{I_2 - I_1} \quad (1)$$

in which L_A is the leaf appearance rate (new leaves per tiller per time interval, d^{-1}), R_{tar} is the relative tiller appearance rate (d^{-1}), and T_1 and T_2 are plant tiller number and I_1 and I_2 are the number of leaves appeared on the main tiller at time t_1 and t_2 , respectively. Skinner and Nelson (1992) calculate site usage (SU) instead of site filling. SU and F_s are related parameters, since SU equals $F_s/F_s(max)$. $F_s(max)$ is 0.693 (Neuteboom and Lantinga, 1989).

On the main tillers of which leaf appearance rate was determined, the length of the sheath and blade of all visible leaves were measured weekly until the final length was reached. The leaf extension duration (in days) was calculated by dividing the final leaf length (cm, extension before and after cuts were summed for cut leaves) by the leaf extension rate ($cm\ d^{-1}$). The number of expanding leaves per tiller was calculated as the leaf extension duration divided by the length of the leaf appearance interval (in days).

From day 35 onwards, every 3 or 4 days light transmission was measured at seven representative spots using a $1\ cm^3$ quantum light sensor type number 63900.4 developed by TFDL-DLO (Wageningen, The Netherlands) which measures the photosynthetically active photon flux density ($\mu mol\ m^{-2}\ s^{-1}$).

On cutting dates, from the cut herbage a representative sample of five leaves was taken of which dry weight and leaf area was determined. Leaf length (L , cm) and leaf widths (cm) were measured at the base (b_1) and the top (b_5 , only larger than zero when the leaf was previously cut) and the widths at three positions along the leaf (at 25 % (b_2), 50 % (b_3) and 75 % (b_4) of the length of the leaf). The area of a leaf (A) was calculated from the leaf length and these widths assuming a trapezoidal form of the four leaf segments (from leaf base to 25 % of the leaf length and so on):

$$A = (0.5b_1 + b_2 + b_3 + b_4 + 0.5b_5) * L/4 \quad (2)$$

When swards were completely harvested (one sward per nitrogen treatment-plant density in each climate room on each date), harvested material was separated into root, stubble below 2 cm and herbage above 2 cm cutting height. A subsample of 50 tillers of the herbage above 2 cm cutting height was taken and dissected into leaf sheath (inclusive of unemerged leaves) and leaf blade. No leaf blades were present in the stubble ($< 2\ cm$). Also, a subsample of five leaves was taken of which leaf area was estimated as described before. Sand was removed as much as possible from the roots and stubble by washing. Dry weight (DM) was determined after drying at $70\ ^\circ C$ for 24 h and organic matter weight (OM) was determined as the difference between that dry weight and ash weight after ashing at $550\ ^\circ C$.

The fractions of growth allocated to leaf, root, sheath and herbage above 2 cm were calculated from organic matter weights at successive dates during the fourth growth period as $(X_2 - X_1)/(W_2 - W_1)$, in which X denotes leaf, root or sheath weight or herbage weight above

2 cm and W denotes the whole biomass of the sward (including roots); the index denotes time.

N-concentration of harvested herbage, stubble and root material of each sward was determined colorimetrically after digestion of samples in a solution of salicylic and sulphuric acid with hydrogen peroxide (Kjeldahl). Concentration of water soluble carbohydrates (WSC) was colorimetrically determined with an automatic analyser on basis of ferricyanide (Hume, 1991; Van Loo, 1992).

Table 6.1. Plant densities used and nitrogen application rate (g N m^{-2}). Nitrogen was applied at the start of each growth period.

Treatment	Plant density, seeds m^{-2}	Number of spots with 5 seeds each, per container	Distance between spots, cm		
D ₁	70	2	28		
D ₂	280	8	12		
D ₃	1120	32	6		

Nitrogen treatment	Growth period				Total
	(1)	(2)	(3)	(4)	
		g N m^{-2}			
N ₁	0.1	0.4	0.6	0.6	1.7
N ₂	0.4	1.5	2.4	2.4	6.7
N ₃	1.6	6.0	9.0	9.0	25.6

6.3 RESULTS

Total yield, herbage yield, nitrogen uptake and nitrogen yield

At D_1N_3 , cumulative biomass production and herbage harvested above 2 cm were about 40 % less than at D_3N_3 (Fig. 6.1). The difference between the lowest and highest plant density was relatively less at lower N-supply. Although the plant density of D_2 was four times lower than at D_3 , N-uptake and N-yield in herbage was the same, herbage harvested was only 10 % lower and total biomass production only 7 % lower at D_2 than at D_3 . The marginal N-recovery in total biomass (the slope of the uptake-application rate line in Fig. 6.1) was close to 100 % at D_2 and D_3 (Table 6.2). At D_1 , the marginal N-recovery in total biomass was reduced to 75 %, on average. The fraction of nitrogen allocated to herbage above 2 cm was higher at higher N-supply (Table 6.3) and therefore also the marginal N-recovery in herbage above 2 cm was higher at higher N-supply (Table 6.2). Plant density did not affect the fraction of nitrogen or organic matter allocated to herbage above 2 cm (Table 6.3). The fraction of organic matter growth allocated to herbage above 2 cm increased stronger with increasing N-supply than the fraction of nitrogen allocated to herbage above 2 cm (Table 6.3). Therefore, herbage yield increased proportionally more with increasing N-yield in herbage than total biomass increased with increasing total N-uptake (Fig. 6.1).

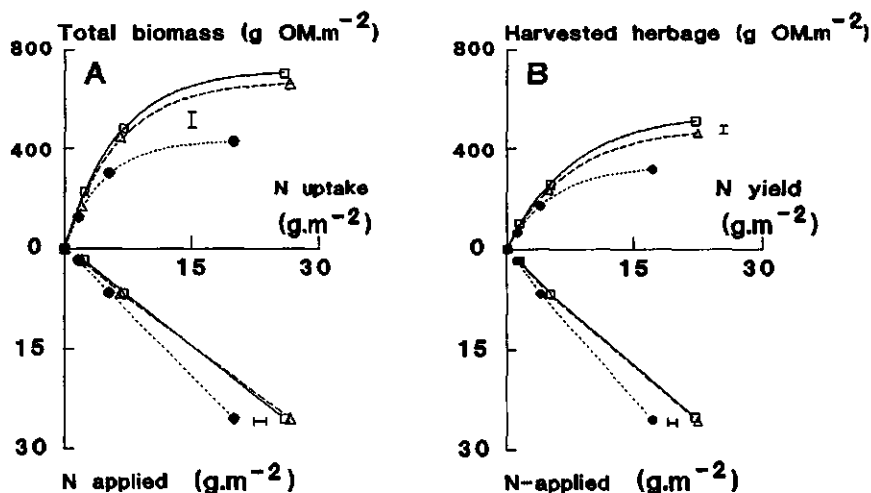


Fig. 6.1. Relationship between nitrogen application rate, nitrogen uptake and cumulative biomass production and herbage harvested above 2 cm, at three plant densities. A. Cumulative biomass production and total nitrogen uptake. B. Harvested herbage above cutting height and nitrogen yield in harvested herbage. Totals of the four cuts. ● D_1 ; △ D_2 ; □ D_3 . Curves for biomass (Y) - nitrogen uptake (X) relationships are of the form $Y = Y_{\max} * (1 - e^{-bX})$ and based on iterative regression of $\ln(Y_{\max} - Y)$ on X. In A. $Y_{\max} = 437, 673$ and 720 g OM m^{-2} and $b = 0.220, 0.160$ and 0.160 for D_1, D_2 and D_3 respectively. In B. $Y_{\max} = 335, 489$ and 545 g OM m^{-2} and $b = 0.187, 0.137$ and 0.131 for D_1, D_2 and D_3 respectively. Error bars indicate LSD ($p=0.05$) for comparing pairs of means.

Table 6.2. Marginal nitrogen recovery in total biomass and in harvested herbage (slope of the relationship between N-uptake in total biomass or harvested herbage and nitrogen application rate in Fig. 6.1. For comparing nitrogen recovery in total biomass or in harvested herbage, figures accompanied by the same lowercase letters are not significantly different ($P > 0.05$).

Range of nitrogen application rate	Plant density					
	D ₁	D ₂	D ₃	D ₃	D ₁	D ₃
	in total biomass			in harvested herbage		
N ₁ -N ₂	0.72 ^a	0.91 ^b	0.92 ^b	0.56 ^a	0.72 ^b	0.72 ^b
N ₂ -N ₃	0.78 ^a	1.06 ^b	1.01 ^b	0.70 ^b	0.92 ^b	0.91 ^b

Table 6.3. Fraction of organic matter accumulation and of total nitrogen uptake allocated to herbage above the cutting height of 2 cm from day 84 until 98. Figures accompanied by different letters are significantly different ($P < 0.05$).

Nitrogen treatment	Fraction to herbage above 2 cm					
	of organic matter accumulation			of total nitrogen uptake		
	Plant density			Plant density		
	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃
N ₁	0.48 ^a	0.53 ^a	0.42 ^a	0.70 ^b	0.65 ^a	0.63 ^a
N ₂	0.52 ^a	0.49 ^a	0.50 ^a	0.75 ^c	0.74 ^c	0.74 ^c
N ₃	0.80 ^b	0.73 ^b	0.86 ^b	0.86 ^d	0.84 ^d	0.86 ^d

Tillering

Final tiller density was lowest at D₁N₁ and increased with increasing N-supply and plant density (Fig. 6.2). The initial fourfold difference in tiller density between the plant density levels decreased to a ratio of 1.5 between D₂ and D₁ and to a ratio of 1.3 between D₃ and D₂, because the mean relative tillering rate was highest at D₁ and lowest at D₃. For the whole experimental period, the number of tillers formed per gram of nitrogen taken up by the crop was on average 3.7 times higher at N₁ than at N₃ and 2.5 times higher at N₂ than at N₃ (Table 6.4). This means that it is not valid to assume that a fixed amount of nitrogen is needed for the formation of one tiller. The mean relative tiller appearance rate was lower at N₁ than at N₃ (30 % lower at D₁, 37 % lower at D₂ and 48 % lower at D₃). The lower tillering rate at N₁ compared to N₃ was caused by a 14 % lower leaf appearance rate (Table 6.5) and a 20 to 40 % lower site filling at N₁. The leaf appearance rate did not change significantly with time, but the relative tiller appearance rate did and therefore site filling was not constant in time.

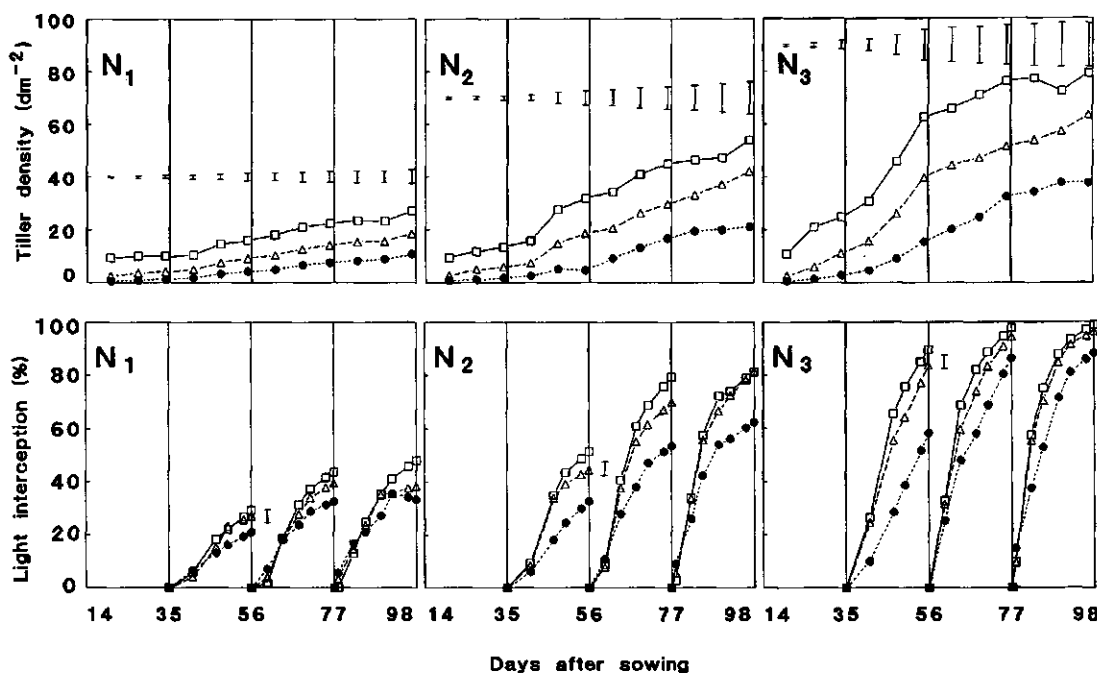


Fig. 6.2. Effect of nitrogen and plant density on the time course of tiller density and fraction of light intercepted by the sward. Plant densities: ● D_1 ; △ D_2 ; □ D_3 . Error bars indicate LSD ($p=0.05$) for comparing pairs of means. During the first growth period light interception was not measured.

Table 6.4. Total number of tillers produced per gram of total N-uptake during the whole experimental period.

Nitrogen treatment	Plant density		
	D_1	D_2	D_3
N_1	900	1260	1590
N_2	570	890	1090
N_3	260	330	430

Just after defoliation, the tillering rate was low and consequently site filling was low. In the second week after defoliation site filling increased to values slightly higher than the maximum site filling with undisturbed growth (0.693). In the third week after defoliation, site filling decreased.

Site filling related to the fraction of light intercepted by the sward

Due to the complete defoliation of the swards, the fraction of light intercepted by swards just after cutting was zero (Fig. 6.2). The increase of light interception after defoliation was faster

at both higher plant density and N-supply. As tiller density was higher in the third and fourth growth period, light interception increased much faster after defoliation than in the second growth period (Fig. 6.2). A negative correlation was found between site filling and fraction of light intercepted, for the second and third week after defoliation (Fig. 6.3). The decline of site filling with higher light interception was greater at the lower N-supply. Therefore, the lower nitrogen availability caused an extra reduction - on top of the effect of increasing light interception - of the extent to which tiller buds developed into tillers.

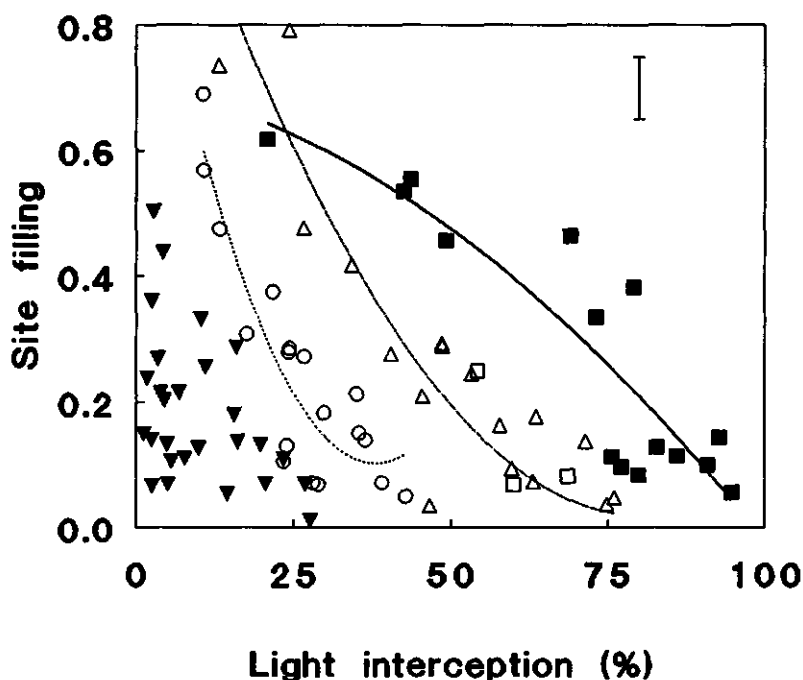


Fig. 6.3. The relationship between site filling and fraction of light intercepted by the swards during growth period 2, 3 and 4. Data for second and third week after defoliation: \circ N₁; Δ N₂; \blacksquare N₃. Data for first week after defoliation: \blacktriangledown . Regression lines: N₁: $Y = 1.06 - 5.05X + 6.66X^2$; SD = 0.089; $R^2_{adj} = 0.814$. N₂: $Y = 1.25 - 3.06X + 1.98X^2$; SD = 0.112; $R^2_{adj} = 0.839$. N₃: $Y = 0.71 - 0.21X - 0.52X^2$; SD = 0.100; $R^2_{adj} = 0.796$. For N₃, data of D₁ for the second and third week after defoliation (\square) showed an extremely clumped distribution of leaf area and were excluded from the regression. Error bar indicates LSD ($p = 0.05$) for comparing pairs of means. Fraction of light intercepted is the mean of the period for which site filling was calculated.

Table 6.5. Average site filling (from 20 to 97 days after sowing), leaf appearance rate, leaf expansion duration, number of simultaneously expanding leaves per tiller, mean leaf length extension rate and mean leaf width of leaf number 3-9 of the main tiller. Means of nine replicate swards and two plants per sward. Figures accompanied by different letters are significantly different ($P < 0.05$).

Site filling				Leaf appearance rate (d ⁻¹)			Leaf expansion duration (d)		
Nitrogen				Plant density					
Treatment	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃
N ₁	0.35 ^e	0.26 ^c	0.14 ^a	0.11 ^b	0.10 ^a	0.10 ^a	12.0 ^a	12.9 ^b	13.6 ^b
N ₂	0.35 ^e	0.33 ^d	0.23 ^b	0.13 ^d	0.11 ^b	0.10 ^a	13.0 ^b	12.4 ^a	13.3 ^b
N ₃	0.42 ^f	0.34 ^d	0.24 ^b	0.13 ^d	0.12 ^c	0.11 ^b	12.1 ^a	11.9 ^a	12.9 ^b
LSD (p = 0.05)		0.02			0.008			0.8	

Number of simultaneously expanding leaves				Leaf length extension rate (mm d ⁻¹)			Mean leaf width (mm)		
Nitrogen				Plant density					
Treatment	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃
N ₁	1.4 ^d	1.3 ^b	1.2 ^a	15.4 ^c	13.3 ^b	11.1 ^a	3.1 ^c	3.0 ^b	2.5 ^a
N ₂	1.7 ^f	1.4 ^d	1.3 ^b	18.1 ^d	17.3 ^d	13.5 ^b	3.1 ^c	2.9 ^b	2.6 ^a
N ₃	1.8 ^g	1.6 ^e	1.6 ^e	20.9 ^e	19.5 ^e	17.8 ^d	3.6 ^e	3.3 ^d	2.8 ^b
LSD (p = 0.05)		0.1			0.8			0.2	

Leaf area expansion rate per tiller

Generally, final leaf length increased with leaf number on the main tiller, but at N₁ the increase of final leaf length stopped after leaf 7. (Fig. 6.4A). Since the leaf extension duration was approximately constant with time, expansion rate per leaf also increased with leaf number. Leaf area expansion rate of the main tiller ($\text{mm}^2 \text{d}^{-1}$) was analysed as the product of four factors: i) leaf appearance rate (d^{-1}), ii) leaf extension duration (d), iii) length extension rate per leaf (mm.d^{-1}) and iv) leaf width (mm). The latter three factors were greater at higher nitrogen levels (Table 6.5). Therefore, the greater increase of the leaf area index of the swards with time at increasing N-supply was not only caused by a higher tiller density, but also by a higher mean area expansion rate per tiller. All four factors influencing the leaf area expansion per tiller were higher at lower plant density.

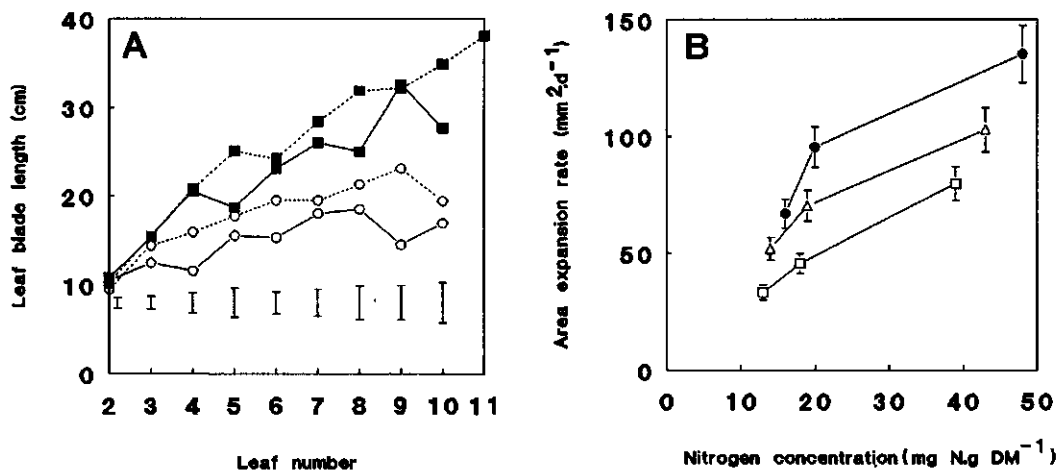


Fig. 6.4. Leaf length and leaf area expansion rate per tiller. A. Final leaf blade length as function of leaf number (for cut leaves length before the cut and length extension after the cut were summed). \circ N₁; \blacksquare N₃; D₁ dotted lines; D₃ solid lines. N₂ and D₂ were intermediate (not shown). B. Leaf area expansion rate of the main tiller (means for day 21 to 98 and for the third to the ninth leaf) as a function of the mean N-concentration in harvested herbage. \bullet D₁; Δ D₂; \square D₃.

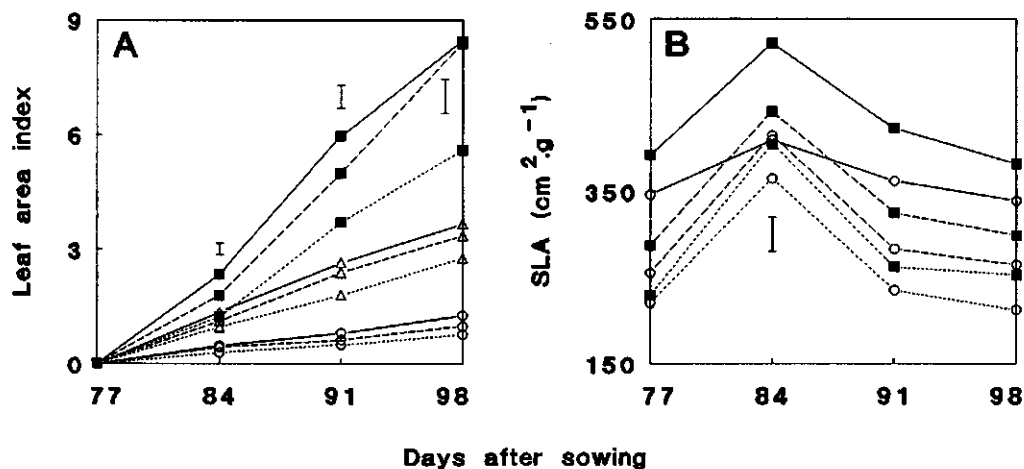


Fig. 6.5. Leaf area index and specific leaf area during the fourth growth period (cut at day 77). A. Leaf area index. B. Specific leaf area (leaf area per leaf dry weight). \circ N₁; Δ N₂; \blacksquare N₃. D₁ dotted lines; D₂ dashed lines; D₃ solid lines. N₂ was intermediate (not shown) between N₁ and N₃. SLA on day 77 is the value just before cutting. Error bars are LSDs ($p=0.05$) for comparing pairs of means. Organic matter content of leaves was 90 % of dry weight.

This shows that plants compensated for the lower plant density not only through increased tillering but also through increased leaf area expansion rates per tiller. The relationship between leaf area expansion rate per tiller and mean N-concentration of harvested herbage was not the same for the three plant densities (Fig. 6.4B).

Leaf area index and extinction coefficient

During the last growth period, leaf area index increased approximately linearly throughout the three weeks after defoliation (Fig. 6.5A). The rate of increase was larger at higher N-supply and higher plant density. A negative exponential relationship between fraction of light intercepted by swards and leaf area index was found. This relationship was characterised by the extinction coefficient (Table 6.7). The extinction coefficient was not significantly different for N_2 and N_3 or for D_2 and D_3 (on average 0.50). The extinction coefficient was quite low, because a high fraction of light was diffuse light and because of the erect growth form of the plants. With N_1 , however, the extinction coefficient was much higher at D_1 than at higher densities, probably because of the more prostrate growth form with this density.

Specific leaf area, leaf weight ratio and radiation-use efficiency

The specific leaf area (SLA) was higher at higher N-supply and plant density (Fig. 6.5B). Effects of plant density and nitrogen on SLA were approximately additive. In the week after defoliation, SLA increased by more than $150 \text{ cm}^2 \text{ g DM}^{-1}$ and later declined to the original value before defoliation (less strong for D_3N_2).

Plant density did not significantly affect the allocation of organic matter production to the different plant organs. The fraction allocated to leaves (leaf weight ratio, LWR) was larger with higher N-application rate (Table 6.6). LWR was smaller than the fraction allocated to herbage above 2 cm (Table 6.3), since the herbage above the cutting height consisted for about 10 % of sheaths and unemerged leaves. A decrease with time of LWR occurred at N_1 , no change with time at N_2 and an increase at N_3 . The fraction of biomass production allocated to growth of sheaths and unemerged leaves was constant with time (on average 0.24) and was not influenced by N-supply or plant density. Effects of nitrogen, plant density and changes with time of the fraction of organic matter allocated to roots (root weight ratio, RWR) were therefore opposite to effects on LWR (Table 6.6). Radiation-use efficiency (RUE) for total organic matter production was hardly affected by treatments; only for the lowest plant density at N_1 , RUE was significantly lower than the average (Table 6.7).

Chemical composition

N-concentration of leaves increased in the week after defoliation. At N_1 and to a smaller extent at N_2 and N_3 , N-concentration started to decrease in the second week after defoliation, since the N-supply was exhausted (Fig. 6.6). The reverse was found for the concentration of WSC: an increase in the second week and third week after defoliation. This increase was strongest at the lowest nitrogen level. This pattern with time after defoliation has been generally observed for perennial ryegrass and other grasses (e.g. Alberda, 1960; Hume, 1990).

Table 6.6. Leaf weight ratio (LWR) and root weight ratio (RWR) during the second and third week of the fourth growth period. Means for the three plant densities (plant density did not influence organic matter distribution). Means accompanied by different letters are significantly different ($p < 0.05$) within columns of LWR and RWR.

Nitrogen Treatment	Week after defoliation			
	2	3	2	3
	LWR		RWR	
N ₁	0.53 ^b	0.35 ^a	0.28 ^b	0.47 ^a
N ₂	0.42 ^a	0.39 ^a	0.24 ^b	0.41 ^a
N ₃	0.57 ^b	0.70 ^c	0.17 ^c	0.04 ^d
LSD ($p = 0.05$)	0.06	0.06	0.06	0.06

Table 6.7. Radiation-use efficiency (RUE) and extinction coefficient (k , determined from regression of $\ln(\text{transmission}) = -k \cdot \text{LAI}$). ^a significantly lower, ^b significantly higher, than the average ($p < 0.05$).

Nitrogen Treatment	Plant density					
	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃
	RUE, g OM (mol photons, 400-700 nm) ⁻¹					
N ₁	0.9 ^a	1.4	1.5	0.75 ^b	0.65 ^b	0.58
N ₂	1.2	1.4	1.3	0.48	0.54	0.52
N ₃	1.3	1.2	1.2	0.45	0.49	0.49
LSD ($p = 0.05$)		0.2			0.07	

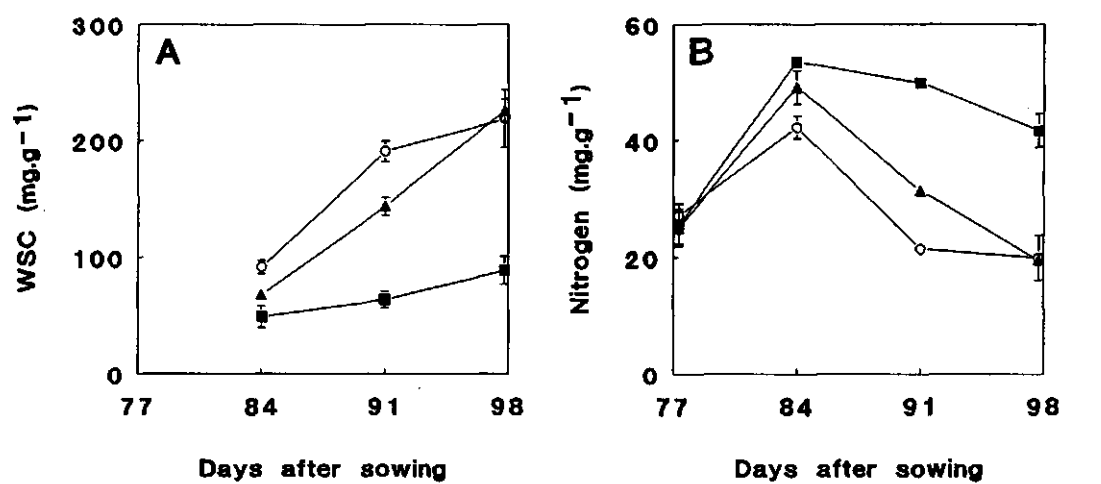


Fig. 6.6. Time course of concentration of WSC in the stubble and nitrogen in leaves during the last regrowth period. A. WSC. B. N-concentration (mg N per g DM). ○ N₁; △ N₂; ■ N₃. Error bar indicate 95 % confidence intervals (when larger than symbol size). Data are averaged over for the three plant densities.

6.4 DISCUSSION

Regrowth after defoliation

Leaf area expansion rate after defoliation was largely independent of current assimilate availability, since leaf area index increased almost linearly after defoliation, while current assimilation rate just after defoliation was zero (no leaf area remained after defoliation). Also, the variations with time in SLA that were found indicate that leaf area expansion was independent of the leaf growth rate. The very high values of SLA one week after defoliation and the decline in the second and third week after defoliation agree with the results from the detailed tillering and regrowth model (Chapter 3).

Just after defoliation, WSC and material in unemerged leaves in the stubble must have contributed to leaf growth during the first week after defoliation. During the first hours after defoliation, no assimilation occurred (all leaves were removed), but unemerged leaf parts continued to expand. However, the contribution of WSC cannot have been large, since much of the available WSC is used for maintenance respiration of the stubble and root during the first week after defoliation. Maintenance respiration of stubble and roots amounts to about 10 % of the weight of stubble and roots in one week (using a respiration coefficient of $0.015 \text{ g CH}_2\text{O (g DM)}^{-1}$). This is about 20 % of the weight of the stubble, since the fraction of growth occurring in roots and in stubble is about the same. WSC-concentration in the stubble prior to defoliation must have been between 250 and 300 mg g^{-1} to account for all maintenance respiration by the use of WSC from the stubble. WSC-concentrations prior to cutting after a three week growth period are usually not higher than these values (e.g. Hume, 1990). Also, here the WSC-concentration at the end of the last growth period was not higher than 250 mg g^{-1} . With the regrowth model of Chapter 3, it was shown that the contribution of unemerged leaves to leaf growth during the first week after defoliation usually is less than 10 % of total leaf growth. Therefore, leaf growth after defoliation depends largely on current CO_2 -assimilation.

Using the mean value of RUE and extinction coefficient (k) for the whole experimental period and the measured LAI seven days (LAI_7) after the third cut, the expected total growth (W , g OM m^{-2}) during the first week after the third cut was calculated as follows:

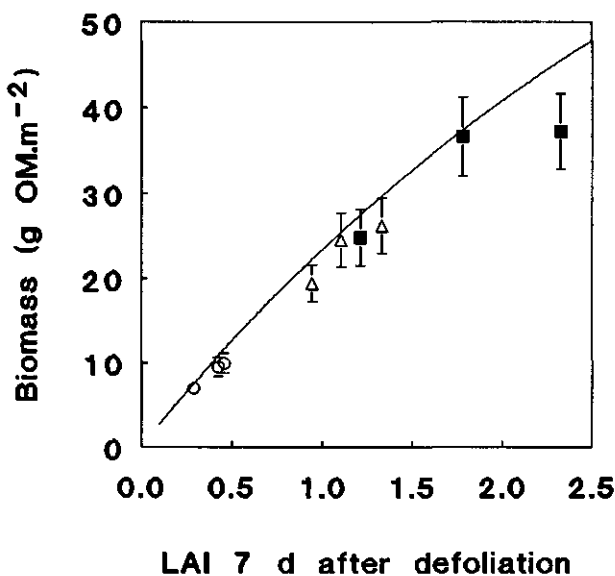


Fig. 6.7. The relationship between leaf area index 7 days after defoliation and actual leaf growth (above 2 cm stubble) and expected total growth during the first 7 days after defoliation. The expected total growth was calculated using eqn. (3) and the measured LAI 7 days after defoliation; RUE = 1.2 g organic matter per mol photons intercepted, $k=0.50$ and $I_0=14 \text{ mol m}^{-2} \text{ d}^{-1}$. ○ N_1 ; △ N_2 ; ■ N_3 . Points indicate measured herbage yield above 2 cm 7 days after defoliation. Error bars indicate 95 % confidence intervals.

$$\frac{dW}{dt} = RUE \cdot I_0 \cdot (1 - e^{-k \cdot LAI_t})$$

$$LAI_t = \frac{LAI_7}{7} \cdot t$$

$$W_t = RUE \cdot I_0 \cdot \left(t - 7 \cdot \frac{1 - e^{-k \cdot \frac{LAI_7}{7} \cdot t}}{k \cdot LAI_7} \right) \quad (3)$$

in which I_0 is the photosynthetic photon flux density above the canopy and t is expressed in days. This simple analysis is possible because i) leaf area index increases linearly (Fig. 6.4), ii) irradiance is low, so RUE is high and constant, iii) irradiance was constant.

Measured herbage growth during the first week after the third cut (above 2 cm stubble height) was about 10 % lower than the expected total growth using eqn. 3 (Fig. 6.7). This means that all herbage growth during the first week could be accounted for by new assimilates. Since this herbage growth consisted of leaf blade only, the calculated LWR during the first week after defoliation was 0.9 on average. This is much higher than the average leaf weight ratio (Table 6.6). In the first week after cutting, assimilates were produced only by expanding leaves. Such leaves do not export more than 10 % of their

assimilate production to stubble or root (Ryle and Powell, 1974). Also, Caloin, Clement and Herrmann (1990) showed (for *Dactylis glomerata* L.) that roots do not show a net increase in weight during the first six days after defoliation (at 25 °C).

Tiller density and the increase of leaf area index and light interception

Especially during the first week after defoliation, leaf area expansion rate is difficult to predict from the total growth rate as both LWR and SLA were found to be variable. An alternative approach is to predict the leaf area expansion rate from the tiller density at defoliation and the leaf area expansion rate per tiller. The relationship between leaf area index one week after defoliation and tiller density proved to be linear within each nitrogen treatment (Fig. 6.8A). The slope of the line between the origin and a point on this relationship represents the amount of leaf area produced per tiller during the first week after defoliation. At N_3 , the mean leaf area expansion rate per tiller did not depend much on tiller density, since the relationship between leaf area index and tiller density almost went through the origin. At N_1 and N_2 , that relationship had a significant, positive intercept on the Y-axis. This means that at the lower tiller densities the mean expansion rate per tiller was higher than at higher densities. This confirms the results derived for the main tiller.

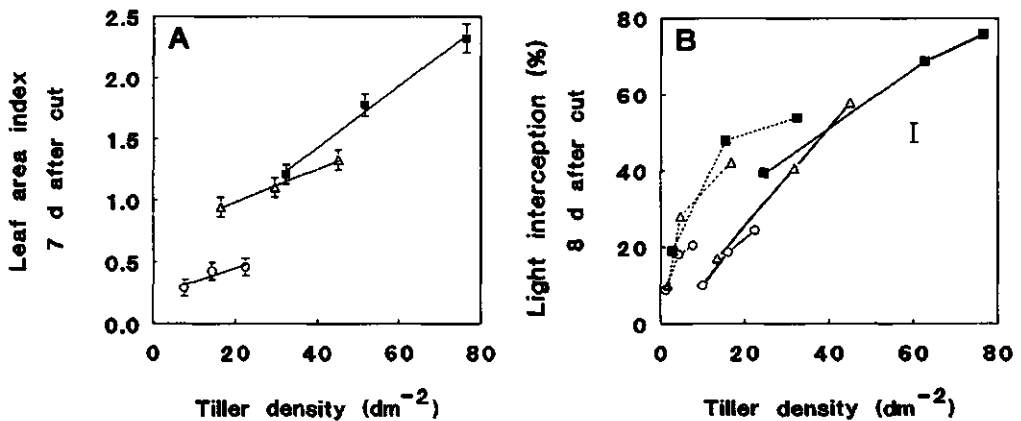


Fig. 6.8. Increase of leaf area and light interception by the swards after defoliation as a function of tiller density. A. Leaf area index seven days after the third defoliation vs. tiller density at the moment of the third defoliation. Error bars indicate 95 % confidence intervals. B. Light interception eight days after defoliation vs. tiller density at the moment of defoliation, for growth periods 2 and 4. Growth period 2: dotted lines; period 4: solid lines. Error bar indicates LSD ($p=0.05$). \circ N_1 ; Δ N_2 ; \blacksquare N_3 .

Surprisingly, the relationship between light interception and tiller density shifted to the right from growth period 2 to 4 (Fig. 6.8B). This means that the mean leaf area expansion rate per tiller declined from growth period 2 to 4. For individual tillers, the leaf area expansion rate increased with age, since the length and extension rate of successive leaves increased (shown for the main tiller in Fig. 6.5A). With time, also the proportion of younger tillers increased, since much tillering occurred and no tillers died. Younger tillers have smaller leaf area expansion rates than older tillers. Apparently, the increase of the proportion of young tillers must have been more important than the increase of leaf area expansion rates of older tillers.

Control of tillering

Tillering was influenced by N-supply both by effects on leaf appearance rate and site filling. However, tiller density and tiller production was not just proportional to N-uptake, because tiller production per gram of N-uptake depended on both N-supply and plant density (Table 6.4). Tiller production varied with time after defoliation as a consequence of variation in time of site filling. Apart from nitrogen supply, three other factors influenced site filling:

1. The maximum rate of tiller bud production. Relative tiller appearance rate equals maximum site filling times leaf appearance rate (Neuteboom and Lantinga, 1989).
2. A low carbohydrate availability limited growth of tiller buds into visible tillers (i.e. reduced site filling. A low carbohydrate status of the plant may limit tillering (Davies, 1965; Hume, 1990).
3. A morphogenetic effect of the fraction of light intercepted by the sward. This effect is mediated through an effect of the red/far-red ratio on tiller bud development. A low red/far-red ratio reduces site filling (Casal, Sanchez and Gibson, 1990). Therefore, site filling is low at a high fraction of light intercepted by the sward (thus at high leaf area index; Simon and Lemaire, 1987). The red/far-red ratio decreases with increasing leaf area index, because of a higher extinction coefficient of red than of far-red light.

The reduced tillering rate during the first week after defoliation can only be explained by the low availability of carbohydrates to developing tiller buds (low concentration of WSC). Nitrogen was amply available during the first week after defoliation (N-concentration increased from 2.5 % to more than 4 %) and light interception by the canopy was still so low that the red/far-red was not low enough to cause a large reduction of the tillering rate.

At low light interception, in the second week after defoliation, both carbohydrate availability (WSC-concentration increased) and the morphogenetic effect of the red/far-red ratio did not limit tillering. Consequently, values of site filling were found close to the theoretical maximum of 0.693. Occasionally, site filling was even slightly higher than 0.693, when site filling had been low before. This indicates that tiller buds that first were delayed in their development into tillers, could develop and grow into tillers later. However, not many of the delayed tiller buds produced tillers at a later time, because then site filling would have exceeded the

maximum for undisturbed growth much more. This supports the hypothesis of Porter (1985) and of Skinner and Nelson (1992) that each tiller bud has a short 'window of opportunity' (for example, only one phyllochron) during which it can initiate development into a tiller.

At N_3 , the decrease of site filling with increasing light interception by the swards must be ascribed to the effect of a decreasing red/far-red ratio, because N-concentration remained high and the concentration of WSC increased a little. At N_1 and N_2 , site filling declined even faster with increasing light interception. N-concentration decreased and WSC-concentration increased with increasing light interception. Therefore, a reduction by the lower N-concentration must have occurred at the lower N-supply in addition to the reduction of site filling with increasing light interception by a reduced red/far-red ratio.

Concluding, it has been shown that perennial ryegrass is capable of substantial compensation of low plant densities by increasing tillering rates and leaf area expansion and growth per tiller. Nitrogen supply had a profound effect on the increase of tiller density because of a large effect on site filling. Differences in leaf growth after defoliation could be explained from differences in increase of leaf area index and the fraction of light intercepted by the sward. These were related to differences in tiller density. Leaf area increase after defoliation could not be predicted from leaf growth, since the specific leaf area depended on plant density and was variable with time after defoliation.

PART IV

Effects of plant density and cutting in the field

CHAPTER 7

Effect of plant density and cutting regime on tillering and growth of perennial ryegrass swards

with E.A. Lantinga

ABSTRACT

Tillering, leaf appearance, leaf area expansion and growth of perennial ryegrass were studied both theoretically using a simulation model and experimentally in two field experiments with a wide range of seeding rates. The effect of leaf appearance rate and leaf size on tillering and maximum tiller density was determined of a diploid and a tetraploid cultivar differing in these leaf characteristics. Three cutting regimes were used to study the effect of light and heavy cuts on tiller density and regrowth after cutting. Seasonal variation in tiller density was caused by variation in leaf appearance rate (L_A), site filling (F_s) and tiller mortality. F_s decreased as leaf area index (LAI) increased, but just after cutting F_s was also low. L_A was positively correlated with soil temperature. At the same temperature, L_A was lower in autumn than in spring. Using the simulation model, we showed that relationships between temperature and L_A and leaf expansion rate, between LAI and F_s and a seasonal pattern for the maximum leaf assimilation rate could explain seasonal variation in tiller density and growth rates. The model showed that the lower L_A and the higher leaf expansion rate per tiller of the tetraploid contributed to its lower tiller density. Measured herbage harvested with 'light cuts' was positively correlated with tiller density. The effect was similar to that predicted by the model. With 'light cuts', herbage harvested in the first harvest year was only substantially reduced at densities less than 1 plant dm^{-2} or when tiller density in early May was less than 80 tillers dm^{-2} for the diploid and less than 60 tillers dm^{-2} for the tetraploid cultivar.

7.1 INTRODUCTION

In a grass sward, the regrowth rate is positively related with the average tiller density at the start of a growing cycle (Davies, 1988). However, different relationships between tiller density and regrowth rate may be expected when comparing different species or varieties as a result of differences in size of tillers. Immediately after cutting, tiller density generally shows little change: some tillers die, especially after a heavy cut, and only few new tillers are produced, since relatively little carbohydrates are translocated to tiller buds (Clifford and Langer, 1975). Especially after harvesting reproductive swards with elongated tillers in spring this may lead to a lag phase in herbage accumulation of up to about ten days (Behaeghe, 1979). Hereafter, tiller density may increase up to a maximum value and decrease thereafter following the $-3/2$ self-thinning law relating tiller density and tiller size (Yoda *et al.*, 1963; Simons, Davies and Troughton, 1973; Kays and Harper, 1974; Davies, 1988). This inverse relationship between tiller size and tiller density arises, because when the leaf area index increases, i) the mean tiller size increases, ii) tiller appearance rate declines (Simon and Lemaire, 1987), and iii) tiller mortality rate of small tillers increases (Ong, 1978; Ong and

Marshall, 1979). Although the relationship between tiller size and tiller density is found to follow the $-3/2$ self-thinning law in closed canopies, it cannot be used to predict the rate of recovery of swards with a low tiller density (e.g. due to decapitation of reproductive tillers). Also, the seasonal pattern in the maximum tiller density (Davies, 1988) can probably be better explained by the seasonal variation in temperature affecting the leaf appearance rate (Davies and Thomas, 1983) and by the seasonal variation in tiller mortality (Ong *et al.* 1978; Korte, Watkin and Harris, 1985; Tallowin, Williams and Kirkham, 1989) than by means of the $-3/2$ self-thinning law.

The aims of this study were to investigate the seasonal variation in tiller density and to relate tiller density and leaf area expansion rate per tiller to herbage accumulation. An increase in our understanding of these relationships may be expected from the use of mechanistic simulation models that integrate knowledge on tillering, leaf area expansion and growth physiology. Therefore, a simulation model has been formulated for the seasonal variation in tiller density, leaf area expansion and herbage accumulation. A dynamic population model was made for changes in tiller density based on tiller bud production per tiller, site filling (the relative tiller appearance rate per leaf appearance interval; Davies, 1974; Neuteboom and Lantinga, 1989) and several tiller mortality factors. For the simulation of CO_2 -assimilation and herbage accumulation, the model of Lantinga (1985, 1988) was used.

Two field studies were carried out to validate the simulated relationship between tiller density and herbage accumulation and to check assumptions in the model concerning relationships between temperature and leaf appearance rate and leaf area expansion rate per tiller, and between leaf area index and site filling. In the field studies, the seasonal variation in tiller density, leaf area expansion rate per tiller and the relationship between tiller density and herbage accumulation were investigated of two cultivars of perennial ryegrass differing in leaf appearance and leaf area expansion rate per tiller, sown at several seeding rates. Different cutting regimes were used to study the effect of light and heavy cuts on tiller density and regrowth after cutting.

7.2 MODEL DESCRIPTION

Three state variables occur in the model: i) tiller density (subdivided into vegetative and reproductive tillers), ii) leaf area index, iii) herbage mass. Tillering is assumed to depend on temperature (through the effect on leaf appearance rate) and on leaf area index (through the effect on site filling). Temperature and light affect the growth rate through effects on CO_2 -assimilation. Water and nutrients are supposed to be non-limiting for growth and tillering. The differential equations of the model are presented in Appendix 1; a list of symbols is presented in Appendix 2 (sections 7.5.1 and 7.5.2).

Leaf area index: Net increase of leaf area index (LAI) is calculated as the product of tiller density and the leaf area expansion rate per tiller minus loss of leaf area through leaf and tiller

death (eqn. 1a). In the model, the expansion rate per tiller is a function of temperature (eqn. 1b, derived from Keatinge *et al.* (1979). The leaf width is assumed to be 2 mm for vegetative tillers and 3 mm for reproductive tillers. The leaf expansion rates thus calculated are supposed to be valid for diploid cultivars. Tetraploids generally have larger leaves and a greater leaf area expansion rate per tiller. A larger leaf area expansion rate per tiller is introduced in the model by multiplying the standard leaf area expansion rate by a leaf size factor F ($F = 1$ in the standard situation). The mean life span of leaves is about three leaf appearance intervals (Anslow, 1966; Vine, 1983). Therefore, the relative mortality rate of leaves is calculated as the leaf appearance rate divided by three (eqn. 1c).

Tiller appearance: The relative tiller appearance is the product of site filling (F_s) and leaf appearance rate (eqn. 2a, see also: Davies, 1974; Neuteboom and Lantinga, 1989). Site filling is a measure of the readiness of tiller buds to develop into tillers. It is influenced by the red/far red-ratio beneath the canopy (shown for *Lolium multiflorum* by Deregibus, *et al.*, 1983; Casal *et al.*, 1985 and Casal *et al.*, 1990). Since the extinction coefficient for red light is higher than for far-red light, the red/far-red ratio decreases with increasing LAI and therefore F_s decreases with increasing LAI (shown for perennial ryegrass by Simon and Lemaire, 1987). The relationship between LAI and F_s was also determined in the field study. It appeared that the relationship depends on the time after defoliation. Therefore, different relationships are used in the model for the first and later weeks after defoliation (see Results and eqn. 2c). Only tillers showing no internode elongation are assumed to produce new tillers, since elongating tillers will show apical dominance.

Tiller mortality: Three causes of mortality are included: i) a basal level of tiller death of vegetative tillers due to a mean life expectancy of 100 days or a constant relative mortality rate of 0.01 d^{-1} (eqn. 2e), ii) death of vegetative tillers beneath a canopy with a high leaf area index, as a consequence of a negative carbon balance of those tillers (relationship derived from Exp. 1, see Results and eqn. 2f) and iii) tiller death due to decapitation of growing points on elongated reproductive tillers (eqn. 4c). It is assumed that all tillers present on 1 March are vernalised (eqn. 2g) and that no further vernalisation occurs after 1 March, because temperatures are too high and the daylength is too long for vernalisation after 1 March. After 1 March, vernalised tillers do not die due to causes i) or ii), since tillers produced before March have a much lower mortality rate in spring than tillers produced after 1 March (Ong *et al.* 1978; Tallowin *et al.*, 1989). It has been assumed that the start of internode elongation has a uniform distribution, the first tillers starting to elongate on 7 May and the last on 8 June (eqn. 2b). The start of internode elongation is defined here as the time at which the growing point is above the cutting height.

Leaf appearance rate: The leaf appearance rate (L_A) determines the rate of tiller bud production. The temperature effect found by Davies and Thomas (1983) on L_A has been used for dates between 1 March and 1 June. This relationship does not significantly differ from the one presented by Davies and Simons (1979) for that period. For the period from 1 June until

1 March the next year, a different relationship between temperature and leaf appearance is used (eqn. 2d; see also Discussion).

Herbage accumulation: Herbage accumulation above the cutting height of 5 cm (harvestable herbage accumulation) is calculated as a function of daily radiation total, leaf area index and three parameters: the maximum leaf assimilation rate, the initial light use efficiency and the efficiency of producing harvestable herbage from assimilates (following Lantinga (1985, 1988), eqns. 3a-g). A double exponential function relating absorbed radiation and leaf assimilation rate is used (eqn. 3h; Lantinga, 1988). The daily rate of crop gross CO_2 -assimilation rate is calculated as in SUCROS87 (Spitters *et al.*, 1989). Above ground maintenance respiration (eqn. 3i) is subtracted from the crop gross CO_2 -assimilation (eqn. 3g) and the remainder is converted in dry matter and allocated to harvestable herbage with an efficiency factor f between 0.30 and 0.35 (eqn. 3a, Lantinga, 1988; Appendix 3). The factor f is the product of a factor for conversion of sugars into dry matter ($0.7 \text{ g DM (g CH}_2\text{O)}^{-1}$, Penning de Vries *et al.*, 1989), the fraction of total dry matter accumulation occurring above ground (0.7; Lantinga, 1985) and the fraction of above ground dry matter accumulation occurring above the cutting height of 5 cm (0.7 in spring and 0.6 during the rest of the year; Lantinga, 1985). The maximum leaf gross CO_2 -assimilation rate at 20°C (P_m^*) was calculated as a function of the time of the year (linear interpolations between mid-monthly values given in the Table of Appendix 3, based on Parsons and Robson, 1981; Lantinga, 1985, 1988). This relationship with time of the year is due to effects on P_m^* of the developmental stage of the crop (vegetative *versus* reproductive, Parsons and Robson, 1981) and of irradiance level (Woledge, 1973). The effect of temperature on P_m is modelled as in SUCROS87 for wheat (eqn. 3d). Disappearance of herbage mass as a consequence of tiller and leaf mortality is calculated in eqn. 3b.

Harvesting: When a harvest occurs in the model, the mass of harvestable herbage is set to zero (eqn. 4b) and reproductive tillers are decapitated (eqn. 4c). For light cuts, residual leaf area after cutting (in the stubble) is set to 0.8. For heavy cuts, residual leaf area is a decreasing function of the amount harvested (eqn. 4a, derived from Lantinga, 1988).

Initial conditions and weather data: At the start of simulation the state variables were given initial values. Weather data needed are soil temperature at 10 cm depth, minimum and maximum air temperatures and daily global radiation. These were obtained from a nearby weather station.

7.3 EXPERIMENTS

Experiment 1

On 24 August 1988, 96 plots ($4 \times 5 \text{ m}$) were hand sown with two cultivars of perennial ryegrass (*Lolium perenne* L.), viz. Wendy (diploid) and Condesa (tetraploid). Condesa has a 10 % lower leaf appearance rate, reaches a 30 % lower tiller density and has broader leaves

than Wendy (Neuteboom, *et al.*, 1988).

Treatments

There were four seeding rates: 0.625, 2.5, 10 and 40 seeds dm^{-2} (1 to 64 kg ha^{-1}) and three cutting regimes:

1. cuts whenever the herbage height of the highest seeding rate was 15 cm (about 1700 kg ha^{-1} of DM, 'light cuts').
2. first cut at the time of the second cut of treatment 1; subsequent cuts at the same dates as in cutting treatment 1 ('one heavy cut').
3. first cut at the time of the second cut of treatment 1 and the second cut at the time of the fourth cut of treatment 1; subsequent cuts at the same dates as in treatment 1 ('two heavy cuts').

Plots were cut to 5 cm using an Agria motor mower. The experiment was laid out in four adjacent blocks of three rows with eight plots each. In each row, the eight cultivar*density combinations occurred once. The three rows were randomly assigned to the three cutting treatments (split-plot block design).

Experiment 2

On 21 September 1989, 32 plots (3 m * 6.88 m) were hand sown with perennial ryegrass cv. Wendy, at 8 seeding rates (between 0.15 and 10 seeds dm^{-2} , i.e. 0.24 to 16 kg ha^{-1}). Plots were cut to 5 cm with a Haldrup motor mower, whenever the herbage height at the highest seeding rate was 15 cm ('light cuts').

Site and fertiliser application rates

Both experiments were carried out on a heavy river clay soil at the experimental farm of the Department of Agronomy in Wageningen. Since the experimental fields had not been used as grassland for many years, no seedbank of grassland weeds was present. Phosphorus was applied as P_2O_5 at 90 kg P ha^{-1} per year and potassium as K_2O at 300 kg K ha^{-1} per year, in equal split applications in spring and summer. Nitrogen (as nitrolime) was applied before each growth period as indicated in Table 7.1. In the first harvest year of both Exp. 1 and 2 (1989 and 1990, respectively) one cut more was harvested than in the second harvest year.

Measurements

In Exp. 1, tiller density was assessed during two years by counting the number of tillers in four fixed rings of 0.25 dm^2 in each plot. Checks of this method were made in March 1990, November 1990 and March 1991 by determining the tiller density of each plot from 25 soil cores of 0.25 dm^2 . For the determination of tiller mortality, all tillers in one of the fixed rings of 0.25 dm^2 per plot were marked with coloured plastic rings made from drinking straws, on 8 May 1989 (cohort 1) and on 30 May 1989 (only newly appeared tillers, cohort 2). On 30 August, survival of the two cohorts was determined (for cohort 1 also on 30 May).

Table 7.1. Nitrogen application rates before each cut and total annual nitrogen application rate. In experiment 1, the first harvest year is 1989, and the second harvest year is 1990. In experiment 2 (only 'light cuts'), the first harvest year is 1990 and the second 1991.

Harvest year	Cutting treatment	Harvest number ^a									Total N applied per year
		1	2	3	4	5	6	7	8	9	
kg N ha ⁻¹											
First harvest year	'light cuts'	80	80	80	60	60	60	40	40	0	500
	'one heavy cut'	120	-	80	60	60	60	40	40	0	460
	'two heavy cuts'	120	-	100	-	60	60	40	40	0	420
Second harvest year	'light cuts'	80	80	80	60	60	60	40	0	-	460
	'one heavy cuts'	120	-	80	60	60	60	40	0	-	420
	'two heavy cuts'	120	-	100	-	60	60	40	0	-	380

^a Cuts of the 'light cuts' treatment have been numbered 1 to 9. Cuts on the same date have been given the same number, although with 'one heavy cut' and 'two heavy cuts' no harvests occurred on the date of cut 2 and/or 4 of the 'light cuts' treatment. - = no harvest on this cutting date.

Leaf appearance rate was determined between May and October 1989 by weekly marking the uppermost fully emerged leaf on one tiller in each fixed ring and by counting the number of new leaves which had appeared two weeks after marking.

At each harvest date, herbage mass above 5 cm was determined by cutting a strip of 0.88 m x 4.7 at 5 cm from each plot. Specific leaf area, total above ground dry matter, leaf dry matter and (pseudo)stem dry matter were determined in subplots (0.1m²), at several dates between cutting dates. This was done for Wendy at the lowest and highest plant density (for 'light cuts' and 'two heavy cuts'), and for Condesa at the lowest and highest plant density (for 'light cuts' only). Specific leaf area was determined from leaf area and weight as described in Chapter 6. For data of spring 1990, radiation use efficiency (RUE, g DM MJ⁻¹) for herbage accumulation above the cutting height was calculated from measured herbage accumulation and the cumulative amount of radiation intercepted by the sward. The daily amount of radiation intercepted was calculated from the daily radiation receipt (400-700 nm) and the fraction of radiation intercepted (1-exp(-k*LAI), extinction coefficient k=0.5). The extinction coefficient is rather low because of the erect growth form of the grass crop.

In Exp. 2, plant density in March 1990 was assessed using the absence frequency method of Neuteboom *et al.* (1992). Herbage mass above a cutting height of 5 cm was determined of nine cuts in 1990 and of eight cuts in 1991.

7.4 RESULTS

7.4.1 Experimental results

Relationship between tiller or plant density, dry matter and nitrogen yield

In Exp. 1, a positive correlation between tiller density on 1 May and total dry weight of herbage harvested of the first four cuts in the first harvest year was found with 'light cuts' (Fig. 7.1A). With 'two heavy cuts', maximum yields were obtained at intermediate seeding rates (Table 7.2). Condesa had a 10 % higher annual dry matter yield than Wendy (Table 7.2), while Condesa had lower tiller densities (Fig. 7.2A). Yields increased in the range 'light cuts', 'one heavy cut', 'two heavy cuts'. Differences in yield between seeding rates were no longer present after the fifth cut in 1989 (Table 7.3).

In Exp. 2, about 50 % of sown seeds were accounted for as established plants. A hyperbolic relationship between plant density and dry weight of herbage harvested was found in the first harvest year (Fig. 7.1B). When plant densities in March 1990 were higher than 1 plant per dm^2 , annual herbage dry matter yields were less than 10 % lower than at the highest plant density. In both experiments, in the second harvest year no effect of seeding rate was found any more on herbage dry matter yield (shown for Exp. 1 in Table 7.3). In the second harvest year of Exp. 2, cumulative dry weight of herbage harvested of eight cuts was $10,320(\pm 250) \text{ kg ha}^{-1}$, on average. Both in Exp. 1 and 2, annual herbage yields in the second harvest year were about 4000 kg ha^{-1} lower than in the first harvest year. The difference is partly explained by a higher growth rate in spring in the first harvest year, because no harvests occurred before the first harvest year after seeding in August or September, while in the first harvest year herbage was harvested two times from half August until October.

Nitrogen yield increased significantly with seeding rate only for Wendy at the 'light cuts' and 'one heavy cut' treatments (Table 7.4). The amount of nitrogen harvested related to the amount applied was generally very high in Exp. 1 (100-120 % of the amount applied was harvested). In Exp. 2, this percentage was 90 % at the highest seeding rates, but decreased to 54 % at the lowest seeding rates. In Exp. 2, the average nitrogen concentration in herbage increased with decreasing seeding rate (Table 7.4).

Seasonal variation in tiller density, differences between cultivars and seeding rates

Tiller density showed a clear seasonal pattern with highest tiller densities in late spring/early summer and lowest tiller densities in autumn/winter (Fig. 7.2A). Generally, Condesa had a lower tiller density than Wendy (Fig. 7.2A and Table 7.5). Despite the 64-fold difference between the lowest and highest seeding rate in Exp. 1, tiller density at the highest seeding rate on 1 May 1989 was only about 40 % higher than at the lowest seeding rate (Table 7.5). The difference did not fully disappear until the spring and early summer of 1990 (Fig. 7.2B). The rate of increase of tiller density was much higher in spring 1990 than in spring 1989. Tiller density also started to decline earlier in 1990 than in 1989.

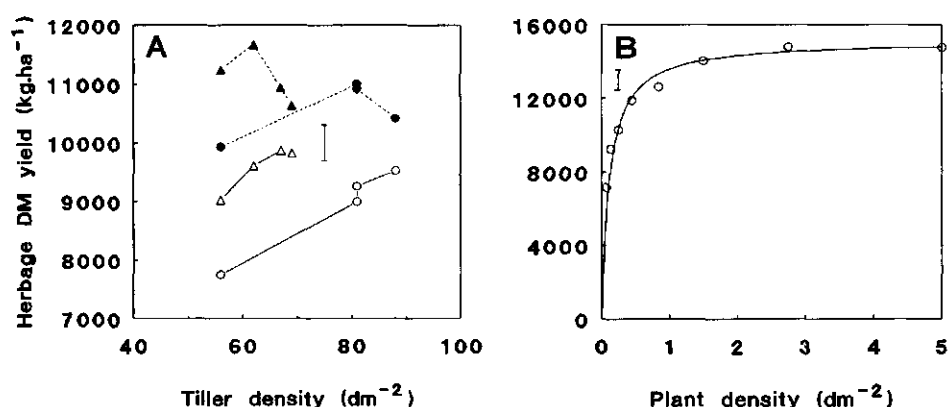


Fig. 7.1. The relationship between plant or tiller density and herbage harvested.

A. Exp. 1: Total dry weight of harvested herbage of first four cuts in 1989 versus tiller density on 1 May 1989. B. Exp. 2: Total dry weight of harvested herbage in 1990 versus plant density in March 1990; regression line based on the yield-density model of De Wit, 1962:

$y = 15144x / (0.115 + x)$, $SD = 1071$, $R^2_{adj} = 0.86$. Condesa: Δ light cuts; \blacktriangle two heavy cuts; Wendy: \circ light cuts; \bullet two heavy cuts. All points are means of four plots. Error bars indicate LSD ($P = 0.05$) for comparing pairs of means.

Table 7.2. Exp. 1. Total dry weight of herbage harvested in 1989.

Cultivar	Wendy				Condesa			
Seeding rate (seeds dm ⁻²)	0.625	2.5	10	40	0.625	2.5	10	40
Cutting treatment	x 1000 kg ha ⁻¹							
Light cuts	16.2	17.3	17.2	17.6	18.0	18.0	19.2	19.0
1 heavy cut	17.0	19.0	17.9	18.4	18.7	18.7	19.3	18.9
2 heavy cuts	17.3	19.1	19.0	18.3	19.8	20.1	19.4	18.9

LSD ($P = 0.05$) = 1.1

Table 7.3. Exp. 1. Dry weight of harvested herbage (kg ha^{-1}) of individual cuts of Wendy. Figures with different lowercase letters in a row are significantly different ($P < 0.05$).

Cutting treatment	Light cuts		One heavy cut		Two heavy cuts	
Seeding rate (seeds dm^{-2})	0.625	40	0.625	40	0.625	40
Harvest number	1989					
1 + 2	3650 ^a	4770 ^b	4520 ^b	5250 ^c	4450 ^b	5190 ^c
3	2200 ^a	2570 ^b	1580 ^c	2040 ^d	-	-
4	1900 ^a	2190 ^b	2140 ^a	2210 ^a	5480 [*]	5230 [*]
5	2750 ^a	2420 ^b	2670 ^a	2510 ^b	1980 ^c	2206 ^d
6 + 7 + 8 + 9	5700 ^a	5650 ^a	6130 ^b	6330 ^b	5340 ^a	5680 ^a
	1990					
1 + 2	5900 ^a	5100 ^b	6190 ^a	5590 ^c	5840 ^c	5650 ^c
3	2030 ^a	2140 ^a	1240 ^c	1420 ^d	-	-
4	1680 ^a	1630 ^a	1610 ^a	1660 ^a	4002 [*]	4130 [*]
5	1740 ^a	1850 ^a	1590 ^c	1660 ^c	1490 ^d	1580 ^c
6 + 7 + 8	3580 ^a	3480 ^a	3210 ^b	3050 ^b	3040 ^b	2930 ^b

* significantly higher ($P < 0.05$) than the sum of harvest number 3 and 4 of 'light cuts' and 'one heavy cut'.

Table 7.4. Total nitrogen harvested in herbage in the first harvest year of Exp. 1 and 2 and average nitrogen concentration in harvested herbage (N_c , g N (100 g DM) $^{-1}$) in Exp. 2.^a

Experiment 1:

Cultivar	Wendy		Condessa	
Seeding rate	0.625	40	625	40 seeds dm^{-2}
Cutting treatment	kg N $\text{ha}^{-1} \text{ yr}^{-1}$			
Light cuts	510 ^a	560	550	590
1 heavy cut	480 ^a	540	550	540
2 heavy cuts	410	450	490	450

Experiment 2:

Seeding rate (seeds dm^{-2})	0.15	0.27	0.50	0.91	1.7	3.0	5.5	10.0
Nitrogen yield (kg N $\text{ha}^{-1} \text{ yr}^{-1}$)	270	330	360	400	410	440	450	450
N_c	3.73	3.55	3.47	3.35	3.25	3.15	3.04	3.01

^a In both experiments: LSD ($P = 0.05$) for comparing N-yields = $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. LSD for nitrogen concentrations = $0.1 \text{ g N (100 g DM)}^{-1}$ ^b significantly lower than at 40 seeds dm^{-2}

Effect of cutting treatments on tillering

In 1989, 'two heavy cuts' resulted in lower tiller densities than 'light cuts', except at the highest seeding rate where cutting frequency did not affect tiller density (Table 7.5). The lower tiller density with heavy cuts was mainly due to decapitation of reproductive tillers. Differences in tiller density between the cutting treatments had disappeared in early March 1990 (Fig. 7.3A). With 'light cuts', tiller density after the first heavy cut was higher than with 'two heavy cuts', because after the first light cut tillering occurred in the 'light cuts', which did not occur in the heavy cut treatment in that period. Further, more tillers died because of decapitation of growing points at the first heavy cut than at the second light cut in 1990 (Fig. 7.3A). The difference in tiller density between cutting regimes had disappeared by the end of June, as a result of a fast recovery of tiller density after the heavy cut and a loss of tillers with the third light cut. The reduction of tiller density after cutting was not observed at the highest seeding rate of Condesa (Fig. 7.3B).

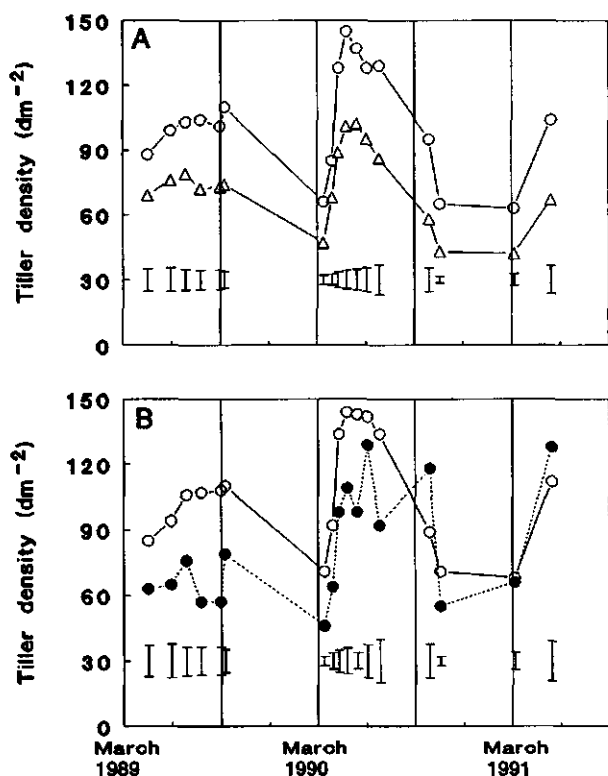


Fig. 7.2. Time course of tiller density during two years (Exp. 1). A. Cultivar differences at 40 seeds dm⁻²: Wendy (○), Condesa (Δ). Means of 12 plots per cultivar (average of cutting treatments). B. Effect of seeding rate with light cuts of Wendy: 40 (○) and 0.625 seeds dm⁻² (●). Means of 4 plots per seeding rate. Error bars indicate LSDs (P=0.05) for comparing pairs of means on the same date.

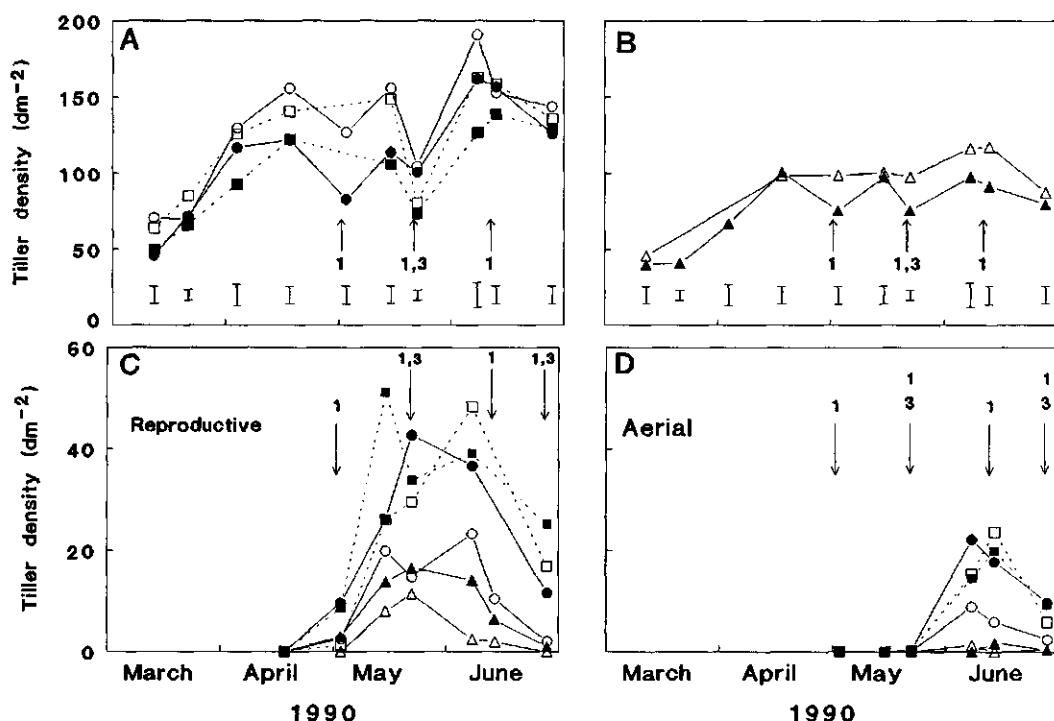


Fig. 7.3. Exp. 1. Effects of seeding rate and cutting treatment on tiller density during spring 1990. A. Total tiller density of Wendy. B. Total tiller density of Condesa. C. Density of reproductive tillers. D. Density of aerial tillers.

Wendy, 40 seeds dm^{-2} : \circ 'light cuts'; \square 'two heavy cuts'.

Wendy, 0.625 seeds dm^{-2} : \bullet 'light cuts'; \blacksquare 'two heavy cuts'.

Condesa ('light cuts' only): \triangle 40 seeds dm^{-2} ; \blacktriangle 0.625 seeds dm^{-2} .

Arrows indicate cutting dates (numbers indicate cutting treatments: 1) 'light cuts', 3) 'two heavy cuts'). Error bars indicate LSDs ($P=0.05$) for comparing pairs of means at the same date.

Seeding rate and percentage of reproductive tillers, aerial tillering and pseudostolon formation

In both harvest years of Exp. 1, the percentage of reproductive tillers was in general negatively correlated with seeding rate (Table 7.5 and Fig. 7.3C). This effect was most pronounced with 'light cuts' (Table 7.5). Both in 1989 and in 1990, the density (and percentage) of reproductive tillers was higher for Wendy than for Condesa (Table 7.5 and Fig. 7.3C). In Wendy, density of aerial tillers (tillers on nodes of elongated tillers) was higher for the lowest seeding rate with 'light cuts', but not with 'two heavy cuts'. Condesa hardly produced aerial tillers (Fig. 7.3D). Density of aerial tillers rose to 25 dm^{-2} in mid-June for Wendy.

Table 7.5. Cultivar differences and effect of seeding rate and cutting treatment on tiller density, percentage reproductive tillers and tiller mortality. Tillers in cohort 1 were marked on 8 May 1989 and tillers in cohort 2 on 30 May 1989. Figures in a row accompanied by the same lowercase letters are not significantly different ($P > 0.05$).

			Seeding rate (seeds dm ⁻²)				
			0.625	2.5	10	40	Mean
Cultivar	Cutting treatment						
<i>Tiller density on 1 May 1989 (tillers dm⁻²)</i>							
Wendy ¹			56 ^a	81 ^b	81 ^b	88 ^c	77 [*]
Condesa ¹			56 ^a	62 ^b	67 ^c	69 ^c	64
<i>Tiller density on 26 June 1989 (tillers dm⁻²)</i>							
	'light cuts' ²		82 ^a	92 ^b	89 ^b	94 ^b	89
	'1 heavy cuts' ²		67 ^a	72 ^b	85 ^c	86 ^c	78
	'2 heavy cuts' ²		60 ^a	65 ^b	68 ^b	93 ^c	72
<i>Percentage reproductive tillers on 3 June 1989</i>							
Wendy	'light cuts'		56 ^a	56 ^a	31 ^b	19 ^c	41 [*]
	'heavy cuts' ³		50 ^a	34 ^b	41 ^b	22 ^c	36 [*]
Condesa	'light cuts'		44 ^a	31 ^b	19 ^c	25 ^{bc}	30
	'heavy cuts' ³		25 ^a	38 ^b	28 ^a	6 ^b	24
<i>Percentage survival of marked tillers</i>							
<i>Cohort 1</i>							
Wendy	'light cuts'	until 30/5/89	92 ^a	92 ^a	86 ^a	98 ^a	92 [*]
	'light cuts'	until 30/8/89	6 ^a	5 ^a	49 ^b	37 ^b	29
Condesa	'light cuts'	until 30/5/89	99 ^a	97 ^a	90 ^b	90 ^b	95
	'light cuts'	until 30/8/89	15 ^a	27 ^b	42 ^c	33 ^b	29
<i>Cohort 2</i>							
Wendy	'light cuts'	until 30/8/89	38 ^b	21 ^a	53 ^c	29 ^a	35 [*]
	'2 heavy cuts'	until 30/8/89	38 ^b	34 ^b	23 ^a	34 ^b	33 [*]
Condesa	'light cuts'	until 30/8/89	55 ^b	50 ^b	40 ^a	50 ^b	49
	'2 heavy cuts'	until 30/8/89	35 ^b	59 ^c	26 ^a	32 ^{ab}	38

¹ Averages over all cutting treatments, since first cut was on 1 May 1989. ² Averages over cultivars

³ Averages over 'one heavy cut' and 'two heavy cuts', since both treatments had experienced only one heavy cut before this date. * Significantly different from Condesa ($P < 0.05$).

In Wendy, at the lower plant densities, new tillers on prostrate reproductive tillers were capable of rooting. This process of pseudostolon formation increased the density and homogeneity of the distribution of "plants" and tillers, since rooted nodes of elongated tillers were spread over a much wider area (sometimes more than 30 cm from the original plant) than vegetative tillers of the original plant.

Tiller mortality

The relative tiller mortality (d^{-1}) was calculated from the percentage survival of tillers (S ; Table 7.5) at different observation dates t_1 and t_2 , as $(\ln(S_1/S_2))/(t_2-t_1)$. In cohort 1 (marked on 8 May 1989), relative tiller mortality was $0.003 d^{-1}$ on average from 8 to 30 May. In this cohort, relative tiller mortality was much higher from 30 May to 30 August 1989 (it increased from highest to lowest seeding rate from $0.011 d^{-1}$ to $0.024 d^{-1}$). The increased mortality at the lower seeding rates was caused by a greater extent of decapitation of tillers at lower seeding rates due to the higher percentage of reproductive tillers (Table 7.5). In cohort 2 (marked on 30 May), the percentage survival was higher for Condesa than for Wendy both with light cuts and with heavy cuts (Table 7.5). In cohort 2, differences between seeding rates and cutting regimes were generally small. The mean relative tiller mortality of cohort 2 was $0.012 d^{-1}$ for Wendy and $0.009 d^{-1}$ for Condesa.

Leaf appearance rate

Condesa had a smaller L_A than Wendy, except in early September (Fig. 7.4A). Both Wendy and Condesa showed a decrease of L_A at the end of the growing season which was correlated with the decrease in temperature (Fig. 7.4B). At the low temperature in early May 1989 L_A of non-elongated (but vernalised) tillers was higher than of non-elongated, vegetative tillers in August-October at similarly low temperatures. In early June of 1989, L_A of vegetative tillers was negatively correlated with seeding rate (Table 7.6). In that period, L_A with 'light cuts' was less than with 'one heavy cut' or 'two heavy cuts'. During mid-summer, both the effect of seeding rate and of cutting treatments were much smaller.

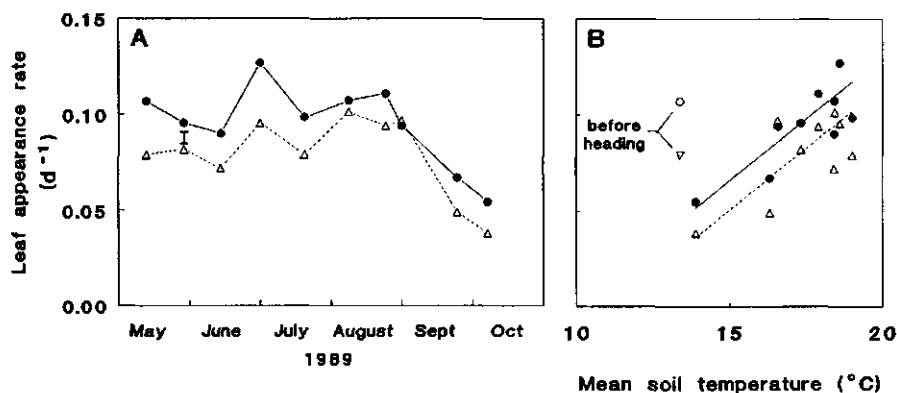


Fig. 7.4. Seasonal variation in leaf appearance rate (L_A) of two cultivars (non-elongated tillers). A. Seasonal pattern. B. Effect of soil temperature (T , °C) at 10 cm depth on leaf appearance rate. Regression formula for data after 8 June ($C=1$ for Condesa, $C=0$ for Wendy): $L_A = -0.091(\pm 0.040) + 0.0106(\pm 0.0023)T - 0.0153(\pm 0.0069)C$, $R^2_{adj}=0.64$. Wendy (●), Condesa (Δ). ○ and ▽ indicate leaf appearance rates in May before heading (for Wendy and Condesa, resp.). Error bar indicates LSD ($P=0.05$) for comparing pairs of means.

Table 7.6. Exp. 1. Effect of seeding rate and cutting regime on leaf appearance rate of vegetative tillers (average of cultivars) during two periods in 1989. Figures with different letters in the same column are significantly different ($P<0.05$). The interaction between seeding rate and cutting treatment was not significant.

Treatments	29 May-16 June	16 June-8 August
Cutting treatments		
Light cuts	0.08 ^a	0.10 ^a
One heavy cut	0.10 ^c	0.11 ^b
Two heavy cuts	0.09 ^b	0.11 ^b
Seeding rates (seeds dm ⁻²)		
0.625	0.07 ^a	0.10 ^a
2.5	0.09 ^b	0.10 ^a
10	0.11 ^c	0.11 ^b
40	0.10 ^c	0.11 ^b

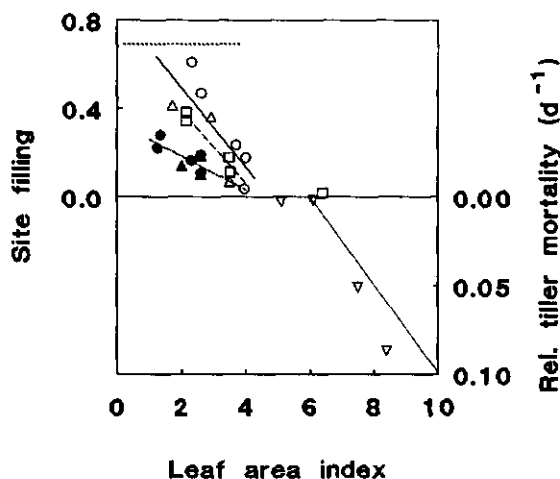


Fig. 7.5. Relation between site filling (F_s) and tiller mortality and leaf area index. *Site filling*: data for first week after defoliation: ● Wendy; ▲ Condesa. Data from second week after defoliation: ○ Wendy, 'light cuts'; □ Wendy, 'two heavy cuts'; △ Condesa 'light cuts'. Maximum site filling is 0.693 (dotted line). Regression lines: 'light cuts': first week after defoliation: $F_s = 0.335 - 0.076 \cdot LAI$, $R^2_{adj} = 0.72$; for data from second week after defoliation: $F_s = 0.867 - 0.183 \cdot LAI$, $R^2_{adj} = 0.71$; 'heavy cuts': $F_s = 0.708 - 0.162 \cdot LAI$, $R^2_{adj} = 0.949$; *Relative tiller mortality*: ▽ Wendy, heavy cuts. The line through the data for relative tiller mortality indicates the total relative tiller mortality of vegetative tillers according to the model. Each point is a mean of four plots.

Site filling and tiller mortality related to leaf area index

Site filling and leaf area index were negatively correlated but the relationship depended on the time after cutting (Fig. 7.5). In the first week after cutting, site filling was much lower than later after cutting. Site filling was lower with 'two heavy cuts' than with 'light cuts' (determined for Wendy only). This may be explained from the higher percentage of reproductive tillers - which produce no daughter tillers - with heavy cuts. With heavy cuts, tiller mortality increased dramatically with leaf area indices above 5 (Fig. 7.5).

Leaf area expansion rate per tiller related to temperature

In the 'light cuts' treatment, the net leaf area expansion rate per tiller (A' , $\text{cm}^2 \cdot \text{d}^{-1}$ per tiller) was positively correlated with soil temperature at 10 cm below ground (T_s , °C), in spring 1990. A significant difference in the intercept of the relationship between temperature and A' was found between the two cultivars:

$$A' = -0.048 (\pm 0.037) + 0.018 (\pm 0.003) T_s + 0.070 (\pm 0.019) C$$

with $C = 1$ for Condesa and $C = 0$ for Wendy; $R^2_{adj} = 0.787$, $SD = 0.036$, $n = 14$. This relationship holds for the 'light cuts' treatment. No significant effect of seeding rate on leaf area expansion rate per tiller was found in spring 1990. With 'two heavy cuts', the net leaf

area expansion rate per tiller was 20 % lower on average than with 'light cuts', due to a higher rate of tiller mortality with heavy cuts.

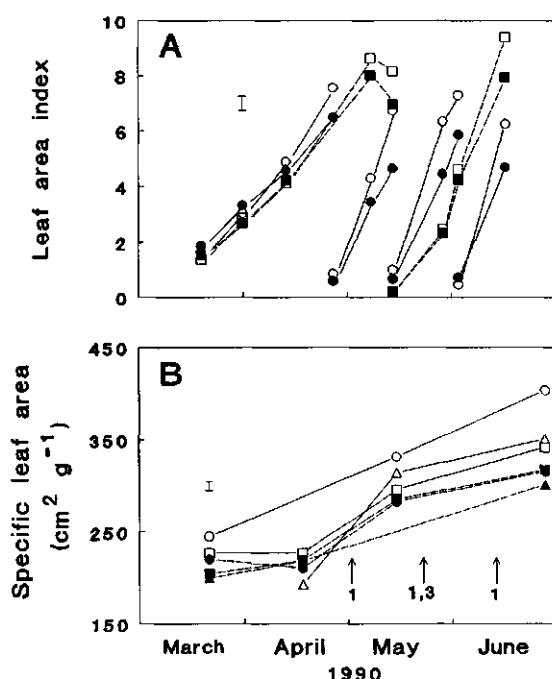


Fig. 7.6. Effect of seeding rate and cutting regime on leaf area expansion and specific leaf area (second harvest year of Exp. 1). A. Leaf area index, B. Specific leaf area. Wendy 40 seeds dm^{-2} : ○ 'light cuts'; □ 'two heavy cuts' Wendy 0.625 seeds dm^{-2} : ● 'light cuts'; ■ 'two heavy cuts' Condesa ('light cuts'): ▲ 0.625 seeds dm^{-2} ; △ 40 seeds dm^{-2} . Error bars indicate LSDs ($P=0.05$) for comparing pairs of means on the same date.

LAI increased fastest at the highest seeding rate, because of the still higher tiller density at the highest seeding rate in spring 1990 and because the leaf area expansion rates per tiller were similar for the seeding rates. This was most pronounced in the first and second regrowth of the 'light cuts' treatment (Fig. 7.6A). The differences in LAI between seeding rates in LAI were not reflected in the amount of herbage harvested, because they were only significant at LAI values higher than 3 (almost complete light interception). After the first harvest in the 'light cuts' treatment, LAI in the 'two heavy cuts' treatment still increased until a maximum of about 8 was reached on 13 May. After the first harvest in the 'two heavy cuts' treatment and the second one in the 'light cuts' treatment, residual LAI in the 'light cuts' treatment was higher than in the 'two heavy cuts' treatment. During the regrowth period, LAI increased much faster with 'light cuts' than with 'two heavy cuts'. This was due to both a higher tiller density (Fig. 7.3A) and a higher net leaf area expansion rate per tiller with 'light cuts' than with 'two heavy cuts' (average net leaf area expansion rate per tiller for Wendy in spring 1990: 0.185 for 'light cuts' and 0.150 $\text{cm}^2 \text{d}^{-1}$ per tiller for 'two heavy cuts'). Increase of LAI of Condesa was not significantly different from that of Wendy - despite its 35 % lower tiller density compared to Wendy - because the mean net leaf area expansion rate per tiller of Condesa was 38 % higher than that of Wendy (0.255 and 0.185 $\text{cm}^2 \text{d}^{-1}$ per tiller for Condesa and Wendy, resp.).

Specific leaf area

The specific leaf area (SLA) increased from March to June 1990 in all treatments (Fig. 7.6B). SLA was lower for Condesa than for Wendy. For both cultivars, SLA was higher at the highest seeding rate. The increase in spring can be explained by the increasing temperatures in spring as SLA increases with increasing temperature (Silsbury, 1971). The effect of density on SLA has not been previously described. The higher SLA at the highest seeding rate may be explained by lower light levels near the developing leaf at soil level, since with decreasing light level SLA increases (Silsbury, 1971).

Table 7.7. Exp. 1. Radiation use efficiency (g DM MJ^{-1}) for herbage accumulation above the cutting height in spring 1990. Averages for the seeding rates are given, since no significant differences between seeding rates were found.

Cultivar	Wendy	Wendy	Condesa
Cutting treatment	'Two heavy cuts'	'light cuts'	'light cuts'
1 March - 1 May	1.3 ^a	0.7	0.7
2 May - 22 May		1.8	1.9
23 May - 12 June	2.1 ^a	1.4	1.5
13 June - 26 June		1.9	2.0

^a for 1 March-22 May and 23 May-26 June, respectively.

Above ground dry matter distribution and radiation use efficiency

For Wendy, the fraction of total above ground herbage accumulation above the cutting height (i.e., the 'harvestable fraction'), was 76 % with 'two heavy cuts' and 56 % with 'light cuts', in spring 1990 (calculated from data on herbage accumulation in the subplots). Seeding rate did not affect the harvestable fraction (results not shown). The harvestable fraction of Condesa with 'light cuts' was 62 %, which was significantly higher than that of Wendy. Radiation use efficiency (RUE) for herbage accumulation above the cutting height increased during spring 1990 (Table 7.7). RUE of Condesa was slightly higher than that of Wendy, because of the higher harvestable fraction. During the second growth period of the heavy cuts treatment, RUE was higher for the heavy cuts, because with heavy cuts more reproductive stem production occurred and no new tillers were formed. No differences between seeding rates in RUE were found (results not shown). The seasonal variation in RUE is caused by seasonal variation in radiation conditions, the CO_2 -assimilation light-response curve, LAI and carbon allocation (Parsons and Robson, 1981). Because of this variation, herbage accumulation may not be simply calculated as the product of RUE and the amount of radiation intercepted by the crop.

7.4.2. Simulation results

Seasonal pattern of tiller density and relationship between tiller density and herbage yield

Simulated tiller density for 'light cuts' exhibited a similar seasonal pattern as was found in Exp. 1 (compare Fig. 7.2 and Fig. 7.7A). Simulation of heavy cuts (cuts at every second light cut) showed lower tiller densities as a result of lower tillering rates and higher tiller mortality rates than with light cuts. The percentage of tillers that were decapitated at harvest dates was higher with heavy cuts, but in absolute tiller numbers the extent of decapitation during the reproductive period of the sward was the same for light and heavy cuts, since the density of vernalised tillers was the same.

Recovery from a low tiller density: effect of leaf appearance rate and leaf size

To simulate the recovery from a low tiller density in summer (caused by for example decapitation with a heavy cut), the model has been run with an initial tiller density on 7 June 1989 of 20 tillers dm^{-2} . To investigate the effect of L_A and leaf area expansion rate per tiller on the recovery of tiller density and on the increase of leaf area index, two values of L_A were used (0.14 d^{-1} and a 20 % lower value, 0.11 d^{-1}) and two values for the scaling factor for the leaf area expansion rate per tiller F (1 and 1.2). The high L_A and low F is the situation for Wendy. The low L_A and high F is comparable to the situation for Condesa. To study the delay caused by the low tiller density, cuts were taken in the model whenever the dry weight of herbage exceeded 1700 kg ha^{-1} . Because of this harvesting scheme, harvest dates were not the same for the four situations. It took about two months, before tiller density stabilised. This rate of recovery corresponds well with experimental results of Davies (1988). Davies (1988) showed that swards starting at about 50 and 130 tillers dm^{-2} reached similar tiller densities after about seven weeks. In the simulations, at the high L_A , tiller density increased fastest, at least initially, since then LAI was still low and therefore F_s was high and tiller bud production limited the tillering rate (Fig. 7.7B,C). With increasing LAI, F_s decreased and therefore the increase of tiller density slowed down or became even negative just before harvesting. After cutting, tillering rates increased again. The highest tiller density in October simulated with a high L_A and low F and the lowest tiller density was simulated with a low L_A and high F . Tiller density was similar for the situations where a high L_A was combined with a high leaf area expansion rate per tiller (high F) and where a low L_A was combined with a low value of F . Differences in leaf area index between the four situations were related to the differences in tiller density and leaf area expansion rate per tiller (Fig. 7.7C). In simulations with an initial tiller density of 100 dm^{-2} on 7 June 1989 it took 15 days until a herbage mass above the cutting height of more than 1700 kg ha^{-1} was reached (results not shown). When starting at 20 tillers dm^{-2} , 10 to 13 days more were needed to reach the same herbage mass. At the high density, the simulated dry weight of herbage harvested during the rest of the year was 9700 kg ha^{-1} . Compared to this yield, the yield reduction was highest at the low L_A and low F (1830 kg ha^{-1}) and lowest at the high L_A and high F (1610 kg ha^{-1}). The yield reduction with high L_A and low F was 1630 kg ha^{-1} and at the low L_A and high F was 1730 kg ha^{-1} . On the basis of these simulations, cultivars with a high leaf appearance rate should show a faster recovery from low tiller density and smaller yield losses during recovery.

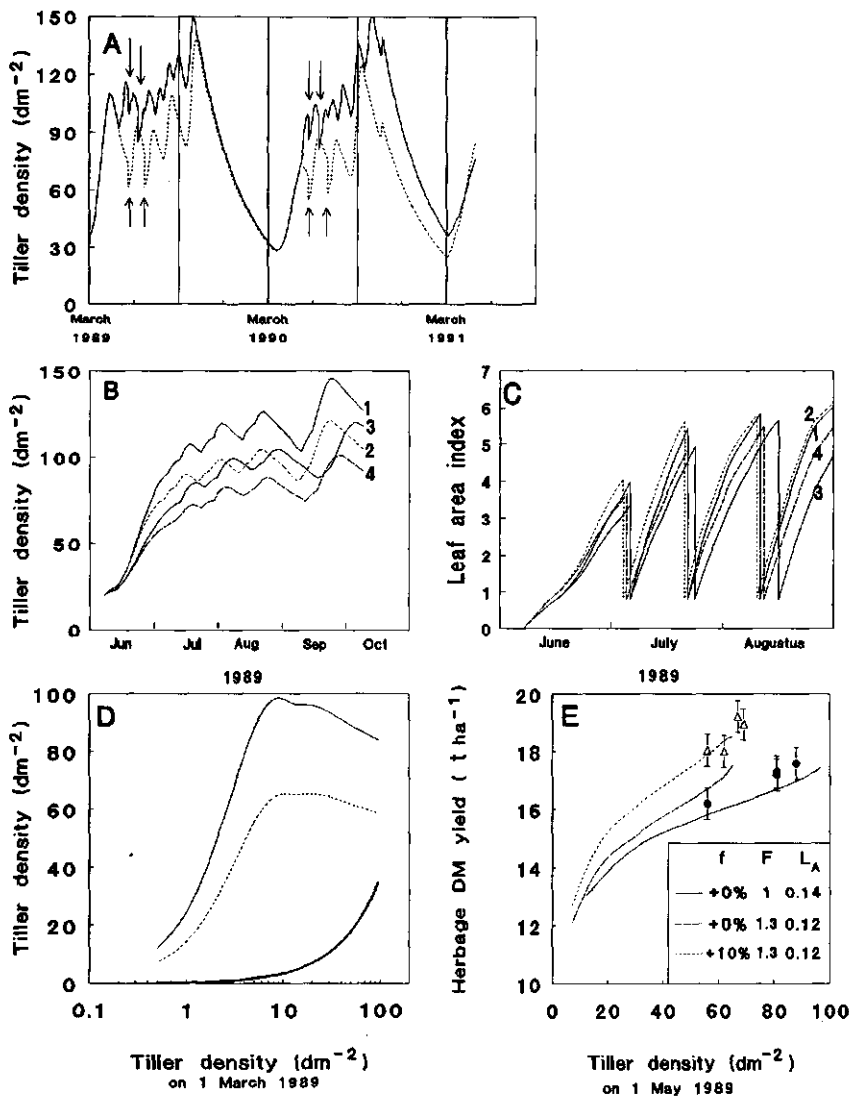


Fig. 7.7. Simulation results. A. Simulated seasonal pattern of tiller density in 1989 and 1990. $L_A(\text{max}) = 0.14 \text{ d}^{-1}$. Solid line: 'light cuts'; dashed line: cuts at every second light cut. Arrows indicate dates on which decapitation occurs. B. Recovery of tiller density from a low tiller density: effect of L_A and leaf size. Lines 1-4 indicate simulations for different parameter values:
 1: $L_A(\text{max}) = 0.14 \text{ d}^{-1}$, $F = 1$; 2: $L_A(\text{max}) = 0.14 \text{ d}^{-1}$, $F = 1.2$.
 3: $L_A(\text{max}) = 0.11 \text{ d}^{-1}$, $F = 1$; 4: $L_A(\text{max}) = 0.11 \text{ d}^{-1}$, $F = 1.2$.
 C. Leaf area index during recovery from low tiller density, parameters as in B.
 D. Relationship between tiller density on 1 March 1989 and tiller density in May 1989;
 - Total tiller density on 1 May 1989: Thin solid line: $L_A(\text{max}) = 0.14 \text{ d}^{-1}$, $F = 1$ (Wendy).
 Dashed line: $L_A(\text{max}) = 0.12 \text{ d}^{-1}$, $F = 1.3$ (Condesa).
 - Thick solid line: density of reproductive tillers on 19 May 1989.
 E. Effect of tiller density on 1 May 1989 on simulated and measured total annual herbage yield with light cuts. Measured yields: ● Wendy, ▲ Condesa. Bars indicate 95 % confidence intervals. F = leaf size factor; f = factor for conversion of assimilates into harvestable dry matter.

Especially at lower leaf appearance rates a lower leaf area expansion rate is expected to result in extra yield reductions during recovery from low tiller densities.

Simulated effect of tiller density on percentage of reproductive tillers

Starting with the same initial tiller density on 1 March 1989, simulated tiller density with parameters for Wendy resulted in about 30 % higher tiller densities than with parameters for Condesa (Fig. 7.7D). For initial tiller densities on 1 March higher than 20 dm^{-2} a negative correlation between simulated density on 1 March and 1 May was found. This was due to a sharp initial increase of the tiller density, after which self-thinning occurred. With increasing initial tiller density, also the density and percentage of reproductive tillers increased in the simulations. However, this is not realistic, since in Exp. 1, the percentage of reproductive tillers was lower with higher tiller density in spring.

Relationship between tiller density and herbage yield

For Wendy, the simulated relationship between tiller density on 1 May and annual herbage yield was not significantly different from the measured relationship (Fig. 7.7E). The relationship did not reach an asymptotic level, because tiller density did not exceed $100 \text{ tillers dm}^{-2}$ for Wendy and $66 \text{ tillers dm}^{-2}$ for Condesa. Simulated herbage accumulation for Condesa was too low when the standard efficiency factor f (Appendix 3) was used. The measured 'harvestable fraction' was higher for Condesa than for Wendy (see Results). Therefore, the model was also run with a 20 % higher value of f for Condesa to show the sensitivity of the model to variation in f . With this higher value of f , simulated yields came close to measured yields of Condesa. Maximum simulated and measured yield of Condesa were reached at a lower tiller density than for Wendy.

7.5 DISCUSSION

Relationship between plant or tiller density and yield

Kreuz (1969) showed with cut swards sown with perennial ryegrass that above 2 to 3 plants dm^{-2} no further response of herbage yield to increasing plant density was found. In that study, annual yields were not higher than $10,000 \text{ kg ha}^{-1}$, since the nitrogen application rate was not very high ($120\text{-}200 \text{ kg ha}^{-1} \text{ yr}^{-1}$). Also the relationship between plant density and herbage yield of perennial ryegrass found by Simons, Davies, and Troughton (1973) showed that above 2 plants dm^{-2} no further increase of herbage yield was found. In the present study, it was shown that - at the nitrogen inputs used - plant density of perennial ryegrass swards may even be as low as 1 plant dm^{-2} without loss of yield compared to swards with higher densities (Fig. 7.1B). In the second harvest year in Exp. 2, even with seeding rates as low as $0.15 \text{ seeds dm}^{-2}$, yields were not lower than with a seeding rate of 10 seeds dm^{-2} . This shows that perennial ryegrass has a large potential for compensating low initial densities. Simulation results also showed that a fast recovery of the tiller density is possible from very low tiller densities. Simulations with a range of tiller densities on 1 March 1989, resulted in a similar relationship between tiller density on 1 May 1989 and annual

herbage yield in 1989 as found in Exp. 1 (compare Fig. 7.1A and Fig. 7.7E).

Compensation for low initial densities through tillering can only be complete, when plants are homogeneously distributed in the sward. In the presented field studies, a homogeneous distribution was created by broadcastly sowing and was further enhanced by the pseudostolon formation. With clumped distributions of plants, e.g. due to locally high mortality rates on spots with urine scorching, yield losses are inevitable even when the mean plant or tiller density is high (Neuteboom *et al.*, 1992). The relationship between tiller density and herbage yield will therefore depend on the extent to which clustering of plants or tillers occurs.

Effect of temperature on leaf appearance rate

At the same soil temperature, different leaf appearance rates were found before and after heading. Higher leaf appearance rates before and after heading have often been reported (Thomas and Norris, 1977, 1979; Davies and Thomas, 1983; Vine, 1983). A different response of leaf appearance rate to temperature is thought to be related to differences between vernalised and unvernalsed tillers (Parsons and Robson, 1980). Leaf appearance rate of non-elongated tillers of Wendy in May 1989 at 13 °C was 0.105 d⁻¹, which was close to the value of 0.109 d⁻¹ for the cultivar S23 reported by Davies and Simons (1979), at that temperature. Also in autumn, the leaf appearance rate at 13 °C of Wendy was close to that reported for S23 by Davies and Simons (1979): 0.06 for Wendy and 0.059 d⁻¹ for S23. However, the relationship with temperature found in Exp. 1 had a higher slope than the relationship of Davies and Simons (1979). Because simulations were compared to results from Exp. 1, the measured relationship is used in the model. In the model, the leaf appearance rate during winter may have been slightly underestimated, since leaf appearance rate was zero when the soil temperature was below 10 °C in the simulations with the measured relation between temperature and leaf appearance rate. Leaf appearance rates of 0.01-0.02 d⁻¹ have been found in winter with soil temperatures below 10 °C (Vine, 1983).

Effect of temperature on leaf area expansion rate

The increase of LAI was modelled as a function of the tiller density and a temperature dependent leaf area expansion rate per tiller. Also in Exp. 1, the net leaf area expansion rate per tiller depended on soil temperature. On average, the net leaf area expansion rate per tiller in spring 1990 was about 30 % lower than the gross expansion rates based on Keatinge *et al.* (1979) for that period. The difference is accounted for by leaf death, which amounted to approximately 30 % of gross herbage accumulation in the simulations.

Aerial tillering and pseudostolon formation

At the very low seeding rates (< 3 seeds dm⁻²), tillering on rooted nodes of elongated tillers contributed largely to increased tiller density and increased homogeneity of the distribution of tillers. This phenomenon is well known for perennial ryegrass and is called pseudostolon formation (e.g. Korte and Harris, 1987; Matthew *et al.*, 1989). In Exp. 2, distances of up to

30 cm between "mother" and "daughter" plants were found. The importance of pseudostolon formation in practical situations may be limited, since the new ramets are easily pulled out by cattle. However, examination of plants in grazed swards show that pseudostolons of 2 to 4 centimeters frequently occur (Korte and Harris, 1987 and own observations). Therefore, pseudostolon formation should be included in models for the simulation of herbage accumulation of heterogeneous grass swards.

In the model, aerial tillering and pseudostolon formation was neglected. Aerial tillers disappeared almost completely before mid summer (Fig. 7.3D) and therefore contributed little to the vegetative spread or persistence of perennial ryegrass in the sward. Korte, Watkin and Harris (1987) reached a similar conclusion for sheep-grazed swards.

Relationship between leaf area index and site filling

In Exp. 1, different relationships describing the effect of leaf area index on site filling were found for the first week after cutting and later on. A similar relationship between site filling and leaf area index was presented by Simon and Lemaire (1987), but they did not make a distinction between the period just after defoliation and later periods. In Chapter 6, also different relationships between leaf area index and site filling were found for the first week after defoliation and later on, in a phytotron study. The lower values of site filling during the first week after defoliation can be explained by a low availability of substrate to developing tiller buds, since all substrate is used by existing tillers (see also Chapter 3). The negative correlation between leaf area index and site filling for the second and later weeks after defoliation was caused by the negative effect of the red/far-red ratio on site filling (Casal *et al.* 1990). The red/far-red ratio of light reaching developing tillers decreases with increasing leaf area index, because the extinction coefficient for red is higher than for far-red light.

Tiller density after winter and percentage reproductive tillers

In Exp. 1, the density and percentage of reproductive tillers was greater at lower seeding rates (and tiller densities) both in 1989 and 1990 (Table 7.5 and Fig. 7.3B). In the model calculations, however, the opposite was found: more reproductive tillers when the tiller density after winter was high (Fig. 7.7D). The assumption may be invalid that all tillers present on 1 March will become reproductive that year. Some tiller mortality among vernalised tillers may occur at high tiller densities (self-thinning). Further, with increasing density the availability of substrate per tiller decreases and this may suppress stem elongation. The model showed realistically the effect of dispersion of the start of stem elongation: decapitation of tillers does not occur in only one cut. In practice, dispersion of decapitation will even be greater, since the postponement and lower rate of internode elongation caused by defoliation in early May (Behaeghe, 1979) was not taken into account. Adequate modelling of the delay of flowering after cutting is however still impossible, since not enough quantitative information is available.

What causes the difference in tiller density between diploid and tetraploid cultivars

The tillering model showed that both the lower L_A and the larger leaf expansion rate per tiller contribute to the generally lower tiller density of tetraploid compared to diploid cultivars. At very low densities, leaf size does not influence the tillering rate, because then tiller bud production still limits the tillering rate (site filling is still maximal). With increasing tiller densities, the leaf area index increases fast and therefore site filling declines. When the relative tiller mortality equals the relative tiller appearance rate, the increase of tiller density stops. With a leaf appearance rate of 0.14 d^{-1} this occurs at an LAI of 4.3 (see eqns. 2a and 2c). With a higher leaf area expansion rate per leaf, this situation is reached at lower tiller densities. This negative feedback of leaf area index on site filling, resulted in a similar rate of increase of leaf area index for large or small-sized tillers after some time (Fig. 7.7C).

Practical implications

From the present study it may be concluded that the (re)seeding of a perennial ryegrass sward can be regarded as succesful when a minimum density of 1 plant dm^{-2} has been achieved with a homogeneous distribution of plants. The homogeneity of the distribution of plants or tillers may be checked by counting plants or tillers in several randomly chosen quadrats and by fitting a negative binomial distribution. In Exp. 2, the tiller density distribution in 0.25 dm^{-2} soil cores was determined in September of the first harvest year (Table 7.8).

Table 7.8. Exp. 2. Tiller density distribution in September 1990. k is the clustering coefficient of the negative binomial distribution. Heterogeneity increases with decreasing values of k .

	Seeding rate (seeds dm^{-2})							
	0.15	0.27	0.5	0.91	1.7	3.3	5.5	10
Mean tiller density (dm^{-2})	27	26	29	53	32	29	42	49
k	0.3	0.3	0.4	0.7	0.8	0.8	2.4	2.0

The clustering coefficient (k) of the tiller density distribution increased with seeding rate. The relationship between mean tiller density and seeding rate was less clear, because at the lower seeding rates many small tillers were formed on rooted nodes of elongated tillers. High values of k indicate a more homogeneous distribution of tillers. Therefore, heterogeneity of the tiller density distribution increased with decreasing seeding rate. A high heterogeneity of the tiller density results in a less efficient light interception by the crop, because of horizontal heterogeneity of the leaf area index. Thus, in practical situations apart from yield losses from low tiller densities, yield losses will also occur when sward deterioration results in a heterogeneous tiller distribution (e.g. with urine scorching). In practice, the determination of the tiller density distribution is often too time consuming. An alternative method for the description of the density and homogeneity of a sward has been suggested by Van Loo

(1991) and Neuteboom *et al.* (1992). The method is based on the measurement of minimal point-to-plant distances. In these studies, it was found that in swards with a homogeneous distribution of 1 plant dm^{-2} (minimum plant density for maximum herbage accumulation), with plants with a diameter of 2 cm, hardly any distances occur of more than 8 cm between randomly chosen points and rooted tillers of nearest plants. Occurrence of distances of more than 8 cm therefore indicates that the plant density is less than 1 dm^{-2} and/or the tiller density distribution is not homogeneous.

In Exp. 1, with heavy cuts, just after the heavy cut tiller density was only 50 % of the density one week before the cut, because of self-thinning at high leaf area index and decapitation of reproductive tillers. This had a negative influence on the yield of a first light cut after the heavy cut. However, tiller density recovered so fast after a heavy cut, that further light cuts after a heavy cut did not yield significantly lower than swards than had always been cut at about 2000 kg ha^{-1} . This means that heavy cuts alone can not be the cause of sward deterioration, as long as plants and tillers remain homogeneously distributed. With additional adverse conditions after a heavy cut, like drought or low nitrogen availability, tillering and leaf area expansion rate per tiller will be reduced and tillers may even die. Then, the lag phase may increase considerably (see also Fig. 7.7C). Recovery of a perennial ryegrass sward is possible from tiller densities as low as 20 tillers dm^{-2} within a few months with light cuts. However, no large open patches should occur and the distribution of tillers should be homogeneous. The positive effect of light cuts on tillering is caused by an increase of site filling as a result of the frequent reduction of the leaf area index.

Both the tillering model and the experimental results showed that - with a homogeneous distribution of plants and tillers - a sward that was reseeded in late summer can establish a high tiller density during the first harvest year also when the tiller density in spring is very low (Fig. 7.7). When tiller densities of diploid cultivars are below 80 dm^{-2} (or below 60 dm^{-2} for tetraploids) on 1 May after a (re)seeding in late summer the year before, annual yield will be more than 1000 kg DM ha^{-1} lower than the yield obtained with maximum tiller densities on 1 May. In the simulations, the maximum for Wendy was 100 tillers dm^{-2} and 66 tillers dm^{-2} for Condesa. According to the model, even with tiller densities on 1 May that are less than 50 % of the maximum tiller density (for example as a result of extreme winter death), annual herbage harvested above 5 cm (with 'light cuts') will still not be more than 2500 kg ha^{-1} lower than with the maximum tiller density on 1 May, because tillering is so very fast at low tiller density. This means that a low tiller density alone should not be the only reason for reseeding of perennial ryegrass swards. Reseeding should only be considered, when the tiller density distribution is very clumped (with values of the clustering coefficient k lower than about 0.7 for the tiller number in 0.25 dm^{-2} or absence frequencies in rings with a radius of 8 cm of more than 10 %).

7.5.1 APPENDIX 1

Equations

Leaf area index

$$\frac{dL}{dt} = A - D_L \quad (1a)$$

$$A = \begin{cases} (a_1 + b_1 T_{soil})(T_v + 1.5 T_g) F, & \text{if } 1 \text{ March} < t < 11 \text{ June} \\ (a_2 + b_2 T_{soil})(T_v + 1.5 T_g) F, & \text{otherwise} \end{cases} \quad (1b)$$

$$D_L = \frac{m}{T_v + T_g} L + \frac{L_A L}{3} \quad (1c)$$

Tiller density

$$\frac{dT_v}{dt} = T_v \cdot F_s \cdot L_A - m \quad (2a)$$

$$\frac{dT_g}{dt} = \begin{cases} 0, & \text{if } t < d_1 \wedge t > d_2 \\ \frac{T_g(\max)}{(d_2 - d_1)}, & \text{otherwise} \end{cases} \quad (2b)$$

$$F_s = \begin{cases} 0.335 - 0.067L, & \text{for } 1^{\text{st}} \text{ week after defoliation} \\ \min(F_s(\max), 0.867 - 0.183L), & \text{otherwise} \end{cases} \quad (2c)$$

$$L_A = \max(0, \min(L_A(\max) \frac{T-c}{d-c}, L_A(\max))) \quad (2d)$$

for dates after 1 March and before 10 June, $c = 0^\circ\text{C}$ and $d = 16^\circ\text{C}$;

after 10 June and before 1 March, $c = 10^\circ\text{C}$ and $d = 18^\circ\text{C}$

$$m = (\mu_m + \mu_L) T_v \quad (2e)$$

$$\mu_L = \max(0, \frac{(0.1 - \mu_m)(L - 6)}{10 - 6}) \quad (2f)$$

$$T_g(\max) = T_v(\text{at } t = 1 \text{ March}) \quad (2g)$$

Herbage production

$$\frac{dW_h}{dt} = f(aP_{crop} - M) - D \quad (3a)$$

$$D_h = \frac{m_v}{T_v + T_g} W_h + \frac{L_A W_h}{3} \quad (3b)$$

$$P_m = P_m^* E_T \quad (3c)$$

$$E_T = \max(0, \min(1, \frac{T_a}{10}, 1 - \frac{T_a - 25}{10})) \quad (3d)$$

$$P_{crop} = f(P_m, \varepsilon, I_o, t, L) \quad (3e)$$

P_{crop} is calculated using a Gaussian integration as described by Spitters *et al.* (1989).

$$P_{leaf} = P_m (1 - \exp(1 - \exp(\frac{\varepsilon I_{abs}}{P_m}))) \quad (3f)$$

$$M = \frac{50}{1500} W_h \cdot 2^{\frac{T-15}{10}} \quad (3g)$$

Harvesting

At harvest dates, L , W_h and T_g are given new initial values:

$$L(\text{aftercutting}) = \begin{cases} = 0.8, & \text{if } W_h \text{ at harvest} < 3000 \text{ kg ha}^{-1} \\ = \max(0, \frac{(6000 - W_h)}{3750}), & \text{otherwise} \end{cases} \quad (4a)$$

$$W_h(\text{aftercutting}) = 0 \quad (4b)$$

$$T_g(\text{aftercutting}) = 0 \quad (4c)$$

7.5.2 APPENDIX 2

List of state variables

L	leaf area index	dimensionless
T_v	density of vegetative tillers	dm^{-2}
T_g	density of reproductive tillers	dm^{-2}
W_h	herbage dry weight above the cutting height	kg ha^{-1}

List of parameters

α	conversion factor	$30/44 \text{ g CH}_2\text{O (g CO}_2\text{)}^{-1}$
ϵ	initial light use efficiency	$14 \text{ g CO}_2 \text{ MJ}^{-1} \text{ (PAR)}$
μ_m	minimum relative mortality rate of vegetative tillers	0.01 d^{-1}
μ_L	relative mortality rate due to high L	d^{-1}
a_1	coefficient in eqn. 1b	$-0.0668 \text{ cm}^2 \text{ d}^{-1}$
a_2	coefficient in eqn. 1b	$0.007 \text{ cm}^2 \text{ d}^{-1}$
a_1	coefficient in eqn. 1b	$0.0282 \text{ cm}^2 \text{ d}^{-1} \text{ }^\circ\text{C}^{-1}$
a_2	coefficient in eqn. 1b	$0.0102 \text{ cm}^2 \text{ d}^{-1} \text{ }^\circ\text{C}^{-1}$
c	base temperature for leaf appearance	$0 \text{ }^\circ\text{C}$ or $10 \text{ }^\circ\text{C}$
d	temperature above which the leaf appearance rate is maximal	16 or $18 \text{ }^\circ\text{C}$
d_1	start date of internode elongation	8 May
d_2	end date of internode elongation	10 June
E_T	factor between 0 and 1 for temperature effect on P_m	
f	factor for conversion of assimilated carbohydrates into harvestable dry matter	$0.3\text{-}0.35 \text{ kg DM (kg CH}_2\text{O)}^{-1}$
F	leaf size factor (1 for Wendy, 1.3 for Condesa)	
$F_s(\text{max})$	maximum site filling	0.693
$L_A(\text{max})$	maximum leaf appearance rate	d^{-1}
P_m^*	rate of light saturated leaf assimilation at $20 \text{ }^\circ\text{C}$	$\text{kg CO}_2 \text{ ha}^{-1} \text{ h}^{-1}$

List of other variables

A	gross rate of increase of leaf area index	d^{-1}
D_L	rate of decrease of leaf area index due to leaf and tiller death	d^{-1}
D_h	rate of decrease of W_h due to leaf and tiller death	$\text{kg ha}^{-1} \text{ d}^{-1}$
F_s	site filling	-

I_{abs}	absorbed radiation (400-700 nm)	$W\ m^{-2}$
I_o	daily radiation (400-700 nm)	$MJ\ m^{-2}\ d^{-1}$
L_A	leaf appearance rate per tiller	d^{-1}
M	daily above ground maintenance respiration rate	$kg\ CH_2O\ ha^{-1}\ d^{-1}$
m	mortality rate of vegetative tillers	$dm^{-2}\ d^{-1}$
P_{crop}	daily gross crop assimilation rate	$kg\ CO_2\ ha^{-1}\ d^{-1}$
P_{leaf}	leaf assimilation rate	$kg\ CO_2\ ha^{-1}\ h^{-1}$
P_m	light saturated rate of leaf assimilation at the prevailing temperature	$kg\ CO_2\ ha^{-1}\ h^{-1}$
t	time	d
T_{soil}	soil temperature at 10 cm depth	$^{\circ}C$
T_a	average daytime temperature	$^{\circ}C$
$T_g(max)$	density of vernalised tillers	dm^{-2}

7.5.3 APPENDIX 3

Seasonal variation in efficiency of harvestable herbage production from assimilate production (f , $kg\ DM\ kg\ CH_2O^{-1}$, based on Lantinga, 1988) and light saturated leaf CO_2 -assimilation rate at $20\ ^{\circ}C$, (P_m^* in $kg\ CO_2\ ha^{-1}\ h^{-1}$, based on Parsons and Robson, 1981 and Lantinga, 1988).

Parameter	Mid-monthly values											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
f	0.3	0.3	0.3	0.35	0.35	0.33	0.3	0.3	0.3	0.3	0.3	0.3
P_m^*	10	15	22.5	45	45	43	40	36	32	27	15	10

CHAPTER 8

GENERAL DISCUSSION

In this chapter, the results from the different studies will be evaluated and some general conclusions are drawn relating to the aims and questions stated in the General Introduction. Effects of various factors on the most important plant parameters related to tillering, leaf area expansion and dry matter accumulation have been summarised in Table 8.1.

8.1 Tillering parameters

8.1.2 Leaf appearance rate

The relative tiller appearance rate was analysed as the product of two parameters: i) the leaf appearance rate per tiller (which determines the tiller bud production rate per tiller), and ii) site filling, a measure for the readiness of tiller buds to develop into tillers. Leaf appearance rates were lowest for tetraploid cultivars, intermediate for diploid cultivars used in production grasslands and highest for the small-leaved diploid cultivar Barry (a 'lawn-type'). Differences in leaf appearance rate between cultivars were generally very constant, because treatment effects were similar for the cultivars used (Chapters 2, 4, 5 and 7). Cutting height negatively affected leaf appearance rate, whereas cutting frequency did not have an effect (Chapter 2). Also, in the field, hardly any effect of infrequent (heavy) cuts on leaf appearance rate was found. Low water potential negatively affected leaf appearance rate only temporarily (Chapter 4). At low nitrogen concentrations ($< 3\%$), leaf appearance rate was about 10 % lower than at nitrogen concentrations of 4 to 5 % (Chapters 5 and 6). Plant density hardly affected leaf appearance rate in the field where nitrogen supply was non-limiting (Chapter 7). When nitrogen was limiting, leaf appearance rate declined with increasing plant or tiller density, because the nitrogen supply per tiller decreased with increasing density (Chapter 6).

There was a positive correlation between temperature and leaf appearance rate and this has been reported frequently for perennial ryegrass (e.g. Davies and Simons, 1979; Davies and Thomas, 1983; Vine, 1983). In spring, higher leaf appearance rates were found than in late summer and autumn at the same temperatures. This effect of the time of the year was also found by Norris and Thomas (1977, 1979). The causes are not fully understood. An effect of hibernation could be involved in spring (Behaeghe, 1979). Vine (1983) states that the shift in temperature response occurs shortly before the onset of double-ridge formation (in January). However, Davies and Thomas (1983) also found a 'spring' response of leaf appearance rate to temperature with vegetative plants.

Table 8.1. Effects of environment, management, leaf area index and genotype on studied plant characters. + + strong positive effect, + positive effect, 0 no effect, - negative effect

Character	Cutting		Lower water potential	Higher N-supply	Higher temperature	Higher LAI	Genetic variation *
	Higher frequency	Lower height					min-max
Leaf appearance rate	0	-	-	+	++	0	0.147-0.175 d ⁻¹
Site filling	-	-	0	++	0	-	0.63-0.64
Tiller mortality	0	0	+ ^a	-	0	+	0.009-0.012 d ⁻¹
Leaf area expansion rate per tiller	-	-	-	++	++	-	31-38 mm ² d ⁻¹
Sheath length	-	-	-	+	+	+	3.3-7.2 cm
Leaf blade length	-	-	-	+	++	-	10-26 cm
Leaf weight ratio	+	+	0	++	0	-	0.71-0.79
Specific leaf area	+	+	-	+	++	-	190-230 cm ² g ⁻¹
Net assimilation rate	0	0	- > 0 ^d	+ ^b	maximum between 10-25 °C ^c	-	16.0-16.5 g m ² d ⁻¹
% Water soluble carbohydrate	-	-	+	-	-	+	19.6-26.0 %

^a based on literature data (see Chapter 4) ^b until a nitrogen concentration in the crop of 3 % (Lantinga, 1985) ^c based on literature data (see Chapter 3) ^d adaptation in newly-formed leaves ^e range within the cultivars or populations studied

Another possibility should also be explored: interactional effects of the way temperature, daylength and daily radiation change. In spring, temperature, daylength and daily radiation increase, while in late summer, they all decrease.

Experimental studies with spaced plants in which light intensity was manipulated, have shown that at very low light levels leaf appearance rates were reduced (Mitchell, 1953). The lower leaf appearance rates at higher plant or tiller density have been explained by this effect of light intensity reaching the apex (Grant *et al.*, 1981). We also found a higher leaf appearance at lower plant densities (Chapter 6). However, in the field either a negative correlation or no correlation at all was found between leaf appearance rate and seeding rate. In the studies of Chapters 5 and 6, no differences in leaf appearance rate between the first week and later weeks after defoliation were found, although large differences in light intensity at soil level (where the apex resides) occurred. In the model of Chapter 7 it was not necessary to include an effect of radiation on the leaf appearance rate for an adequate simulation of the seasonal changes in tiller density. Therefore, it is concluded that the effect of radiation level on the leaf appearance rate is not very important in practical situations.

8.1.2 Site filling

Cutting reduced site filling during the first week after defoliation, but the effect was more pronounced in swards than with spaced plants (Chapters 2, 5, 6 and 7). Low water potential did not significantly affect site filling (Chapter 4). However, site filling was correlated positively with nitrogen concentration in the plant tissue and negatively with leaf area index and with the percentage of incoming radiation intercepted by the sward (Chapter 5, 6 and 7). Casal *et al.* (1990) have shown that the effect of leaf area index on site filling is due to an effect of the red/far-red ratio on site filling. The extinction coefficient of red light is higher than that of far-red light and therefore the red/far-red ratio decreases as the leaf area index increases. In the first week after defoliation site filling was generally much lower than later (Chapter 6 and 7). This cannot be explained by an effect of the red/far-red ratio, since leaf area index during the first week after defoliation is low. The most plausible explanation is that during the first week after defoliation the substrate level in the stubble limits the outgrowth of tiller buds into tillers. This hypothesis was supported by the observed low level of water soluble carbohydrates one week after defoliation and low values of site filling (Chapter 6).

The hypothesis was further tested using a model for tillering, leaf area expansion and growth of spaced perennial ryegrass plants (Chapter 3). In that model, tillering is a function of tiller bud production and the minimum amount of substrate necessary for the outgrowth of a tiller bud into a tiller (W_{min}), which was determined from the size of tillers at the one-leaf stage. Differences between cultivars in tillering rate were partly explained from differences in W_{min} , since small-leaved cultivars need less substrate for the production of the first leaf than large-leaved cultivars. Experimental results on tillering from Chapter 2 agreed well with simulation results. With the model of Chapter 3, it proved possible to explain the differences in tillering rates of spaced plants at different cutting treatments found in Chapter 2, from differences in

leaf appearance rate and assimilate availability to tiller buds.

8.2 Tiller and leaf size depend on the leaf age of a tiller

It was shown that successive leaves on a tiller increase in size during undisturbed or nearly undisturbed growth of plants. Further, it was shown that the relationship between leaf length and the position of the leaf on a tiller (= leaf number counted from the tiller base upwards) was similar for the main tiller and the third tiller under constant environmental conditions (Chapter 2). Therefore, younger tillers will always have smaller leaves. Since the leaf extension duration is more or less constant under constant environmental conditions, the leaf extension rate of expanding leaves of younger tillers is lower than those of older tillers. With extensive tillering, this means that the mean leaf area expansion rate per tiller will no longer increase after some time, because the higher leaf expansion rates on older tillers will be counterbalanced by the lower leaf expansion rates of an increasing proportion of younger tillers and also because of leaf mortality on older tillers. As a consequence, the relative increase of the mean tiller weight of young plants decreases with time (Chapter 4). Leaf lengths with three-weekly cuts at 7.5 cm were not greater than with three-weekly cuts at 5 cm. Therefore, leaf lengths with three-weekly cuts at 7.5 cm are considered to be the maximum attainable. The relationship between leaf length and leaf number on a tiller determined for this cutting treatment was used in the model of Chapter 3 for the simulation of the potential leaf area expansion rates of different tiller age classes.

8.3 Modelling the seasonal variation in tiller density

It proved possible to simulate the seasonal variation in tiller density on the basis of the relation between temperature and leaf appearance rate and the relation between leaf area index and site filling (Chapter 7). Increase of leaf area index was modelled as the product of a temperature-dependent leaf area expansion rate per tiller. Both leaf appearance rate and leaf area expansion rate per tiller were related to temperature using different relationships before and after heading. Parsons and Robson (1980) related the increase in temperature response in spring of potential leaf extension rates to the first stages of floral induction. In the sward model of Chapter 7, the effect of substrate level on site filling was modelled implicitly by using two measured relationships between leaf area index and site filling, one for the first week after defoliation and a second one from the second week after defoliation onwards. Both relationships show a decrease of site filling with increasing leaf area index. For the first week after defoliation a negative effect of a low assimilate availability on site filling is usually found and therefore much lower values of site filling were taken in the model during the first week after defoliation.

With this model, differences in seasonal variation of tiller density between cultivars differing in leaf size and/or leaf appearance rate and between cutting treatments can be explained. tetraploid cultivar Condesa had a lower tiller density than the diploid cultivar Wendy. This differences was shown to be caused by both the lower leaf appearance rate and the bigger leaves of Condesa. With infrequent cuts a lower tiller density is found than with light cuts.

This difference can be explained by a lower site filling and an increased tiller mortality rate at high leaf area index with infrequent cuts.

8.4 The relationship between tiller number and (re)growth

Tiller number or tiller density influences the (re)growth rate through the effect of tiller number on the leaf area expansion rate after defoliation. Differences in growth per plant between cutting treatments were shown to result from a lower tiller number and a lower leaf area expansion rate per tiller with more frequent cuts at lower cutting heights. In Chapter 4, the relative growth rate was shown to be highly correlated with the relative tiller appearance rate. There, it was further shown that the relative growth rate could only be higher than the relative tiller appearance rate for a short time, since the mean tiller size cannot increase indefinitely. Without an increase of the mean tiller size, the maximum relative growth rate of perennial ryegrass would be only about 0.1 d^{-1} (maximum site filling = 0.693; leaf appearance rate = 0.14 d^{-1}).

It has been shown that differences in regrowth rates of perennial ryegrass can be explained by differences in tiller density and in leaf area expansion rates per tiller using a simple growth model with a constant radiation use efficiency (Chapter 5 and 6). In Chapter 5 and 6, leaf area expansion rates were supposed to be the same for all tillers. In Chapter 5, for the last growth period at the high nitrogen supply this was not correct, because tillers of a wide range of age classes were present. For this growth period, simulated yields were higher than measured yields. In the model of Chapter 3, ten tiller age classes were introduced, because of younger tillers have a lower leaf area expansion rate. This resulted in adequate simulation of measured yields.

In the model of Chapter 7 (the sward model), literature data on the effect of temperature on the mean leaf area expansion rate per tiller were used. The sward model, contains only two tiller classes: vegetative and reproductive tillers. Although the sward model does not have the same level of detail in the description of different tiller age classes as the spaced plant model of Chapter 3, tiller density changes, increase of leaf area and herbage accumulation were adequately simulated. Therefore, the use of a mean leaf area expansion rate for all tillers seems justified, although its final justification depends on a stable tiller age distribution.

Both experimentally and theoretically, it was shown in Chapter 7 that tiller density in early May could hardly exceed 70 dm^{-2} for tetraploid cultivars (like Condesa) and 90 dm^{-2} for diploid cultivars (like Wendy). The theoretical relationship shows almost a linear response of herbage harvested above 5 cm to tiller density in early May above 20 tillers dm^{-2} for Condesa (a tetraploid cultivar) and above 40 tillers dm^{-2} for Wendy (a diploid cultivar). Below these densities herbage yield declines very fast with decreasing tiller density. The experimental results confirmed the simulation results for the higher tiller densities. These results were obtained for cuts at yields between 1700-2000 kg ha^{-1} ('light cuts').

8.5 Sink or source-limited growth and specific leaf area

During the development of the models for the relationship between tiller number or density and (re)growth of perennial ryegrass, one of the problems was whether to regard leaf area expansion and growth as source- or sink-limited. In classical growth analysis and in many simulation models leaf area expansion is source-limited and the crop is modelled as if it were one big plant without consideration of tiller dynamics. When leaf area expansion is source limited the number of tillers is not important, since the leaf area expansion rate can then be completely explained from the leaf growth rate by multiplying leaf growth by the SLA. However, regrowth of grass after defoliation is often sink limited and then the leaf area expansion rate is independent of the leaf growth rate and SLA depends on the substrate supply to expanding leaves. With the model in Chapter 3, the higher SLA with frequent cuts at low cutting height could be explained by assuming that leaf area expansion rate was to some extent independent of the leaf growth rate. Further evidence for the independence of leaf area expansion and leaf growth was presented in Chapter 4. As a consequence of strongly reduced leaf extension rates at the low water potential, water soluble carbohydrates accumulated in the leaves. Therefore, a negative correlation between SLA and the water soluble carbohydrate concentration was found. SLA was shown to increase during the first week after defoliation and to decrease later on (Chapter 6). Such a pattern with time was also simulated in Chapter 3. Also, the increasing SLA with increasing plant density in both Chapter 6 and 7 indicate that the reduced mean net assimilation rate at higher plant densities did not negatively affect the leaf area expansion rate per tiller.

However, leaf area expansion is not completely independent of leaf growth. Values of SLA above $600 \text{ cm}^2 \text{ g}^{-1}$ are rare (e.g. Mitchell, 1953; Silsbury, 1971). The maximum value found in the present studies was $550 \text{ cm}^2 \text{ g}^{-1}$. The maximum of $600 \text{ cm}^2 \text{ g}^{-1}$ was introduced in the detailed model of Chapter 3. The actual leaf area expansion rate equals the potential expansion rate as long as substrate supply to leaves can maintain SLA values below the maximum SLA, otherwise actual leaf area expansion rates are lower. In the simulations described in Chapter 3, substrate supply only limited leaf area expansion during the first few days after defoliation. After the first few days after defoliation, assimilation by the new leaf area could already provide enough substrate to sustain the potential leaf area expansion rate. These results hold for the situation with spaced plants. Under sward conditions, the importance of reserves seems to be limited too. In Chapter 6, all regrowth of leaves during the first week after defoliation could be explained from new CO_2 -assimilation without reserves contributing to leaf growth. This confirms observations by Davies (1965) and Alberda (1966) who stated that regrowth rates were only reduced when the concentration of water soluble carbohydrates (WSC) was below about 6 %. Since WSC-concentration is usually much higher than 6 % (typically between 15 and 20 %), WSC-concentration will rarely be limiting regrowth. In the present studies, WSC-concentration was only lower than 6 % one week after defoliation at the higher nitrogen supply levels (Chapter 6).

8.6 The number of unemerged tiller buds and site filling

In the model of Neuteboom and Lantinga (1989) for potential tillering rates of perennial ryegrass, three tillering parameters are included: leaf appearance rate, site filling and the number of inhibited or delayed tiller buds (in short: unemerged tiller buds). In Chapter 4, it was shown that the last two parameters are mathematically strictly related. Thus, it is possible to rewrite the equations of Neuteboom and Lantinga (1989) using only two parameters (leaf appearance rate and site filling).

8.7 Methodology of experiments for studying effects of nitrogen supply

When studying the effect of nitrogen supply to plants or crops to optimise crop husbandry, the timing of nitrogen supply should be similar to that in practice. For Dutch grassland management this means that nitrogen should be supplied immediately after each cut. To gain insight in the effect of nitrogen supply on physiological parameters or morphological characteristics of the plant species, with the ultimate aim of ranking genotypes for these characteristics, genotypes should be compared at similar nitrogen concentrations.

The study presented in Chapter 5 aimed at ranking cultivars with respect to growth and tillering parameters and to study the response of physiological and tillering parameters of perennial ryegrass to nitrogen supply in general. Therefore, a "steady state" nitrogen addition system was chosen. Usually, a "steady state" nitrogen concentration in the plant is accomplished by using an exponentially increasing rate of nitrogen addition (e.g. Ingestadt, 1980; Van der Werff, 1993) in experiments with spaced plants. In this way, constant relative growth rates and "steady state" nitrogen concentration in the plant tissue are realised. However, our interest was to examine growth and tillering parameters in crop situations. Therefore, exponentially increasing nitrogen addition rates could not be used. Instead, nitrogen was added proportionally to the expected growth rate calculated with a model for leaf area expansion, light interception and growth. With this system, it proved possible to create different levels of "steady state" nitrogen concentration in the plant tissue, while the leaf area index increased and the relative growth rate decreased. When adding a fixed amount of nitrogen after each harvest, plant nitrogen concentrations of the fast growing cultivars decline faster than those of slower growing cultivars. This makes the comparison of growth parameters invalid. The differences in nitrogen concentrations found in Chapter 6 at the same nitrogen application rate at different plant densities again stress the importance of using a "steady state" nitrogen addition system when comparing growth parameters of cultivars, since cultivars may easily have different initial tiller or plant densities.

8.8 Implications for plant breeding for higher tillering and regrowth rates

Considerable variation between cultivars was found for leaf appearance rate (in Table 8.1 values for the extreme cultivars of in the cutting experiment with spaced plants are mentioned), but hardly any genetic variation for site filling of spaced plants was found. Selection for fast tillering can therefore best be achieved by selecting for high leaf appearance rate. However, site filling was negatively correlated with leaf appearance rate, because the

populations with a high leaf appearance rate showed a faster tillering rate initially (Chapter 5). At the higher tiller densities, leaf area index increased faster. Therefore, due to the negative effect of leaf area index on site filling, average site filling was lower for populations with a high leaf appearance rate. The prospects of selection for higher site filling are limited. Site filling of spaced plants is close to its maximum value of 0.693 (Table 8.1). Possibly, cultivar differences in the effect of leaf area index on site filling exist, but this was investigated. Selection for higher tillering capacity will lead to increased regrowth rates, when it is not accompanied by a lower leaf area expansion rate per tiller. Selection for lower tiller mortality rates would result in higher tiller densities. In Chapter 5, however, no variation between cultivars was found in relative tiller mortality rates during a growth period with an extreme nitrogen shortage. In the field, Condesa had a slightly lower relative tiller mortality (of vegetative tillers) than Wendy, indicating that some genetic variation in tiller mortality exists (Table 8.1).

In Chapter 5, genetic differences in nitrogen use efficiency have been found. The highest yielding population also had the highest nitrogen use efficiency, the highest "shoot weight ratio" at high nitrogen supply and the highest tiller density. This population was selected from the cultivar Splendor for higher persistency (Den Nijs and Winkelhorst, 1989). The cultivar itself had the lowest yield, lowest nitrogen use efficiency and lowest tiller density (within the group of diploid cultivars).

Large genetic variation was found for leaf morphological characteristics like leaf area expansion rate per tiller, sheath length and leaf blade length (Table 8.1). These characteristics have an important effect on the fraction of herbage accumulation occurring above the cutting height. Leaf blade lengths and sheath lengths are highly positively correlated. Therefore, a trade-off exists between long leaf blades and short sheath lengths concerning the harvestable fraction and the amount of leaf area remaining in the stubble after defoliation. With long leaves the harvestable fraction is high, but since sheaths are also long then, hardly any leaf area will remain in the stubble. This reduces the regrowth rate after defoliation. The model of Chapter 3 can be used to determine the optimal combination of leaf blade and sheath length that maximises harvestable herbage accumulation.

8.9 Implications for grassland management

Recovery potential

This study showed that perennial ryegrass plants have a large capacity for compensating a low plant and tiller density through a high potential tillering rate (the maximum relative rate is about 0.1 d^{-1}) and through a potential for increasing the leaf size at low densities. Further, perennial ryegrass has a capacity of colonizing open patches by rooting on nodes of elongated tillers (ramets). Under cutting, distances between "mother" and "daughter" plant may sometimes be larger than 30 cm at very low plant densities. However, grazing animals will pull out many of the new "ramets". Still, such new ramets of perennial ryegrass have been frequently observed at distances up to 3 cm from the "mother" plant (Korte and Harris,

1987; own observations). Because of the high potential tillering rate, a reseeding of perennial ryegrass swards may even be considered as succesful when at least 1 plant per dm^2 occurs.

It was shown that with a homogeneous distribution of tillers, a density of at least 80 tillers per dm^2 for diploid cultivars and 60 dm^2 for tetraploid cultivars in early May is sufficient for maximum herbage growth during the first harvest year after a late summer (re)seeding (Chapter 7). These critical tiller densities will be higher during drought or limiting nitrogen availability, since then the leaf area expansion rates per tiller are lower (Table 8.1). The lower leaf area increase not only reduces the increase of the growth rate, but also the fraction of herbage accumulation occurring above the cutting height ('fraction harvestable'), because leaf blade lengths are reduced. As previously suggested by Lantinga (1985) for drought periods, reducing the defoliation height will increase the 'fraction harvestable'. No adverse effect on residual leaf area will be found with lower defoliation heights during drought or nitrogen shortage, since also sheath lengths are shorter then.

Effects of heterogeneity

Herbage yield may be substantially reduced when the distribution of the tillers is heterogeneous, even when the tiller density would be sufficient for maximum herbage growth with a homogeneous distribution of tillers. In Chapter 7, it was shown that at low seeding rate not only the mean tiller density was low, but also the clustering coefficient of negative binomial distribution fitted to the tiller density distribution was low (this indicates a clumped distribution of tillers). Annual herbage yield was better correlated with the clustering coefficient and the plant density than with the mean tiller density at the end of the first harvest year. The poorer correlation of yield with the mean tiller density was due to the formation of small tillers on the rooted nodes of elongated tillers at the lower densities. The heterogeneity of the sward may also be assessed by determining the absence frequency (of rooted tiller bases of perennial ryegrass) in rings of different sizes (Neuteboom *et al.*, 1991 and Van Loo, 1991b). Van Loo (1991b) showed in Experiment 2 of Chapter 7 that the frequency of gaps with a radius of at least 8 cm is highly negatively correlated with annual herbage yield (Fig. 8.1). Concluding, a low tiller density should not be the only reason for reseeding of perennial ryegrass swards. Reseeding of perennial ryegrass swards should only be considered when the tiller density distribution has become very clumped (resulting in low values of the clustering coefficient, see Table 7.8) and frequencies of open patches are high.

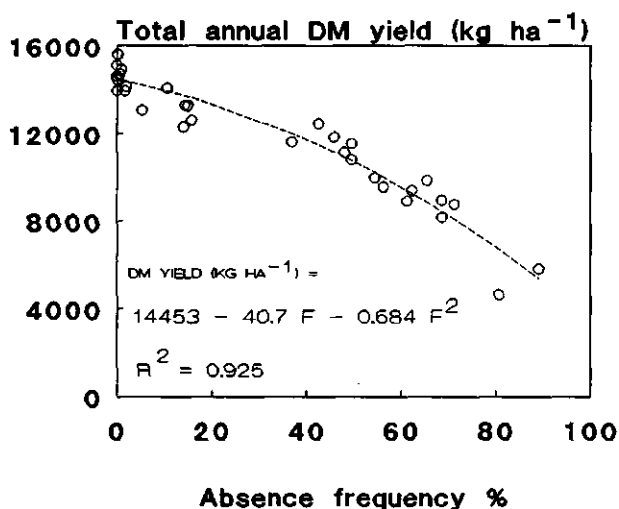


Fig. 8.1. The relationship between total dry weight of harvested herbage in 1990 and absence frequency in rings with radius of 8 cm. Data from Exp. 2 in Chapter 7 (Van Loo, 1991b). Points represent values per plot.

Intuitively, one may argue that increasing the nitrogen application rate could alleviate some of the negative effects on productivity of a low tiller density or a high frequency of open patches. The relative tiller appearance rate increases with increasing nitrogen supply as both the leaf appearance rate and site filling are positively influenced by increasing nitrogen supply. Also, the leaf area expansion rate, SLA, LWR and the harvestable fraction increase with increasing nitrogen supply (Table 8.1). However, with current nitrogen application rates in the Netherlands, nitrogen concentrations in the crop are generally above 3 % - especially with grazing - and therefore rarely limiting for any of these growth parameters. Moreover, with decreasing tiller density and increasing frequency of open patches, the nitrogen uptake in harvested herbage declines and the nitrogen concentration in harvested herbage increases. This increase of the nitrogen concentration further increases the risks of sward damage by trampling and urine scorching. Recovery of open swards can better be promoted by frequent defoliation (continuous grazing or light cuts). Although in spaced plants frequent defoliation results in lower tillering rates, in swards tillering is promoted by a frequent reduction of the leaf area index, because site filling is high at low leaf area index. Also a reduction of the nitrogen application rate can promote tillering in swards, because of the reduced increase of LAI with low nitrogen supply (e.g. Prins, 1983). Therefore, nitrogen application rates should be reduced on swards with a low tiller density or a high frequency of open patches, to avoid unnecessary losses of nitrogen to the environment.

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SUMMARY

Annually, about 10 % (130,000 ha) of the Dutch grasslands is reseeded. The main reason for reseeding is sward deterioration. The current intensive management of perennial ryegrass dominated grasslands - with high nitrogen inputs and high stocking rates - may easily lead to sward deterioration, for example through urine scorching, treading, tiller pulling or by decapitation of reproductive tillers with late cuts. Sward deterioration is manifested by decreasing tiller densities, a more clumped distribution of tillers and an increased frequency of open patches (absence of perennial ryegrass). This leads to a reduced productivity and a reduced nitrogen recovery. Recovery of swards with a reduced tiller density or with open spaces can only occur through tillering of perennial ryegrass, since natural reseeding is rare, because with current grassland management reproductive development is suppressed. The aim of this thesis was to integrate existing knowledge on tillering, leaf expansion rates and dry matter accumulation of perennial ryegrass for a better understanding of the relations between tiller density, dry matter accumulation and the recovery potential of perennial ryegrass swards.

Experiments were carried out to determine tillering rates, leaf area expansion rates per tiller and growth rates under a wide range of environmental conditions, cutting regimes and sward densities. The product of leaf appearance rate and site filling determines the relative tiller appearance rate. The leaf appearance rate determines the rate of tiller bud formation; site filling is a measure of the readiness of tiller buds to develop into tillers. On the basis of these data, several simulation models were developed and validated to relate tiller density to dry matter accumulation of grass swards or plants. Three types of models were used. In increasing order of level of detail and complexity of the modelling of tillering and leaf area expansion:

- i) Simple models based on light interception and a constant radiation use efficiency; this type of model is only valid under conditions with relatively low light intensities. In the model initial leaf area increase depends on the tiller density and the leaf area expansion rate per tiller, but tiller density changes were not modelled (Chapters 5 and 6)
- ii) A sward model (Chapter 7): leaf area increase depends on tiller density and leaf area expansion rate per tiller; in this model a separate module for tiller density changes was included. The module for herbage accumulation above the cutting height was based on a model for crop CO₂-assimilation.
- iii) A detailed morphological model for spaced plants in which leaf area increase depends on the tiller number per plant and on the substrate availability and in which the tillering rate depends on both tiller bud production and substrate availability. (Chapter 3).

In Chapter 2, a study was presented on tillering, leaf expansion and regrowth of three cultivars of perennial ryegrass, at two cutting frequencies and three cutting heights. Tillering rates decreased with increasing cutting frequency and decreasing cutting height, i.e. with

increasing defoliation severity. These effects were generally the same for the three cultivars. The effect of cutting frequency was due to an effect on site filling. Lower cutting heights reduced both site filling and leaf appearance rate. With three-weekly cuts at 7.5 cm (the treatment with the lowest defoliation severity), leaf area per tiller increased with increasing leaf number on a tiller. With increasing defoliation severity, the increase of leaf size with leaf number was less or even negative. Cultivar differences in growth and harvestable biomass were related to differences in sheath and leaf lengths and in growth parameters. With all cultivars, the specific leaf area (SLA) and fraction of biomass allocated to above a stubble height of 2.5 cm increased with increasing defoliation severity. The capacity to increase SLA and the fraction of biomass allocated to leaves with increasing defoliation severity, both help to compensate for the smaller leaf area remaining after cutting. The lower SLA and fraction of biomass allocated to above 2.5 cm may be explained by an 'overflow' of assimilates towards the stubble when assimilation exceeds the demand of leaves.

In Chapter 3, a model for the regrowth after defoliation of perennial ryegrass is presented in which sink-source relations are explicitly simulated. The model was used to evaluate differences between cutting treatments (weekly *versus* three-weekly cuts at 2.5, 5 and 7.5 cm) in tillering and regrowth found in a glasshouse experiment with spaced plants of perennial ryegrass. In the model, the tillering rate is a function of bud formation and development, and of substrate availability to buds. From information on the level of tillers and plant organs (e.g. leaf appearance rate, leaf area expansion rates, tiller bud production, minimum SLA, leaf gross CO₂-assimilation rate), the simulation model could explain phenomena observed on the plant level such as the increase in fraction of dry matter allocated to shoot above 2.5 cm, the increase in SLA and the reduced tillering that occurred especially with the high frequency/low cutting height treatment in the experiment. The model predicts that SLA will increase just after defoliation until a maximum is reached and will decline thereafter. This temporal variation in SLA was largest with the highest cutting frequency and with the lowest cutting height. Further, the model predicts that just after cutting a large fraction of leaf growth comes from the substrate pool in the stubble. However, this fraction declined very fast in a few days after cutting as current assimilation of new leaves provided an increasing proportion of the required substrate for leaf growth.

Tillering and growth parameters of perennial ryegrass cultivars Wendy (diploid) and Condesa (tetraploid) were determined in a hydroponics system at low (-1.3 MPa) and normal (0 MPa) water potential (Chapter 4). At -1.3 MPa, leaf extension rate was reduced by 36 %. Final plant tiller number was 20 % lower, at -1.3 MPa, due to a 12 % reduction of the leaf appearance rate at -1.3 MPa in the first weeks after the start of the treatments. Site filling, the relative increase of tiller number per leaf appearance interval, was high (0.61) - but still lower than theoretically possible - and was only slightly affected by water potential. Site filling was shown to be mathematically related to the number of inhibited plus unemerged tiller buds. Dry matter production was 64 % lower at -1.3 MPa. Relative growth rate (RGR) was 17 % lower at -1.3 MPa, on average, but was more reduced just after the treatments started. Also,

NAR was more reduced by low water potential just after the start of the treatments. SLA was 13 % lower at -1.3 MPa for Wendy, but not significantly reduced for Condesa. Contrary to expectations based on the theory of the functional balance between root and shoot, leaf weight ratio was slightly higher at -1.3 MPa. From comparison of results of this study with literature data, it is concluded that effects of drought in the field on tillering cannot be attributed to low water potential only.

In Chapter 5, population differences in tillering, leaf extension and assimilate distribution between root and shoot among three diploid and three tetraploid populations of perennial ryegrass were evaluated. Three subsequent conditions were used: 1) two steady-state plant nitrogen concentrations (0.027 and 0.042 g N.g DM⁻¹), 2) changing nitrogen availability to zero, 3) changing nitrogen availability to a supra-optimal level. Populations were grown in monocultures and mixtures to investigate the correlation between monoculture and mixture performance. The highest yielding population yielded 40 % more cut herbage than the lowest yielding population. Its nitrogen uptake was 29 % higher and its organic nitrogen concentration was between 5 and 8.5 % lower. Population differences in herbage yields in mixtures were larger, but population order was the same as in monocultures. It was therefore concluded that selection of populations for regrowth characteristics can best be done in mixtures. Stopping nitrogen application reduced tiller density and assimilate allocation to shoots. Both were restored to more than the original values after changing to a supra-optimal nitrogen availability. Analysis of tillering in terms of leaf appearance and site filling showed a negative genetic correlation between the two factors. A simulation model showed the relative importance of tiller density, leaf expansion and assimilate partitioning for regrowth.

Tillering, leaf area expansion, light interception and (re)growth of perennial ryegrass were studied at various plant densities and levels of nitrogen supply (Chapter 6). Substantial compensation for low plant densities occurred by increased tillering rates and increased leaf area expansion and growth per tiller. At low nitrogen supply, the leaf area expansion rate per tiller decreased more with increasing plant density than at high nitrogen supply. Therefore, at low nitrogen supply, the increase in plant and tiller density did not increase the crop leaf area expansion rate and the growth rate as much as at high nitrogen supply. A negative correlation was found between site filling and the fraction of light intercepted by the swards. At low nitrogen supply, site filling decreased faster as the fraction of light intercepted by the swards increased than at high nitrogen supply, because the nitrogen concentration in the plant declined faster. The observed low site filling after defoliation was presumably caused by a low level of reserves in the stubble. Differences in leaf growth between nitrogen and plant density treatments could be explained from differences in increase of leaf area index (product of tiller density and leaf area expansion rate per tiller) and from light absorption by the crop. Leaf area expansion was not strictly related to leaf growth, since SLA increased after defoliation and decreased again later.

Tillering, leaf appearance, leaf area expansion and growth of perennial ryegrass were studied both theoretically using a simulation model and experimentally in two field experiments with a wide range of seeding rates (Chapter 7). The effect of leaf appearance rate and leaf size was determined on tillering and maximum tiller density of a diploid (Wendy) and a tetraploid cultivar (Condesa) differing in these leaf characteristics. Three cutting regimes were used to study the effect of light and heavy cuts on tiller density and regrowth after cutting. Seasonal variation in tiller density was caused by variation in leaf appearance rate, in site filling and in tiller mortality. Site filling decreased as leaf area index increased, but just after cutting site filling was also low. Leaf appearance rate was positively correlated with soil temperature. At the same temperature, however, leaf appearance rate in spring was higher than in autumn. With the simulation model, the seasonal variation in tiller density and in growth rates could be explained using relationships between temperature and leaf appearance and leaf area expansion rate per tiller, between site filling and leaf area index and a seasonal pattern for the maximum leaf assimilation rate. The model showed that the lower leaf appearance rate and the higher leaf area expansion rate per tiller of Condesa contributed to its lower tiller density. Measured dry weight of herbage harvested with cuts at approximately 2000 kg ha⁻¹ ('light cuts') was positively correlated with tiller density. The effect was similar to that predicted by the model. With 'light cuts', the amount of herbage harvested in the first harvest year was only substantially reduced at densities less than 1 plant dm⁻² or less than 80 tillers dm⁻² for Wendy and less than 60 tillers dm⁻² for Condesa. Because of the large potential tillering rates, it was concluded that a low tiller density should not be the only reason for reseeding perennial ryegrass swards. Reseeding should only be considered when the tiller density is very clumped and the frequency with which open patches occur is high.

In Chapter 8 (General Discussion), effects of various factors on tillering, leaf area expansion rate per tiller and on growth parameters have been summarised. Further, some implications from the results for breeding for higher recovery potential and higher regrowth rates after defoliation were discussed. Selection for a higher recovery potential and regrowth rate after defoliation can best be performed in mixtures of genotypes. Concerning grassland management, it was concluded that nitrogen application rates should be reduced on swards with a reduced productivity due to a low tiller density or a high frequency of open patches, to avoid unnecessary losses of nitrogen to the environment. Recovery of open swards can be promoted by frequent defoliation, because frequent reduction of the leaf area index stimulates tillering through high values of site filling at low leaf area index.

SAMENVATTING

Ongeveer 10 % van het Nederlandse produktiegrasland wordt jaarlijks opnieuw ingezaaid (130.000 ha per jaar). De belangrijkste reden voor herinzaai of doorzaai is verslechtering van de zodekwaliteit. Bij het huidige gebruik van door Engels raaigras (*Lolium perenne* L.) gedomineerd grasland met een hoge stikstofbemesting en hoge veedichtheid bestaat een verhoogd risico voor zodebeschadiging, bijvoorbeeld door urineverbranding, betreding, het uittrekken van spruiten en/of door "onthoofding" van generatieve spruiten ten gevolge van te laat maaien. Bij verslechtering van de zodekwaliteit treedt een verlaging van de spruitdichtheid op en vaak ook een meer geclusterde verdeling van spruiten en een hogere frequentie van open plekken (waarin geen Engels raaigras voorkomt). Dit leidt tot een verlaagde produktiviteit en een verlaagde stikstofbenutting. Graszones met een lage spruitdichtheid of met open plekken kunnen zich alleen herstellen door middel van uitstoeling (zijspruitvorming) van Engels raaigras, omdat bij het huidige graslandgebruik de generatieve ontwikkeling wordt onderdrukt waardoor geen zaad wordt gevormd. Het doel van dit proefschrift was om de bestaande kennis op het gebied van uitstoeling, bladoppervlaktetoename per spruit en groei (=droge-stoftoename) van Engels raaigras te integreren om tot een beter begrip te komen van het herstellingsvermogen van Engels raaigras en van de relatie tussen spruitdichtheid en droge-stofproductie.

In veld- en kasexperimenten werd de uitstoelingssnelheid, de bladoppervlaktetoename per spruit en de groeisnelheid bepaald onder uiteenlopende milieu-omstandigheden, ontbladeringsregimes en zodedichtheden. De relatieve spruitverschijningssnelheid wordt bepaald door het produkt van twee factoren: de bladverschijningssnelheid en de knopbenutting ('site filling'). De bladverschijningssnelheid bepaalt de spruitknopaanlegssnelheid (in elk bladoksel wordt een spruitknop gevormd); 'site filling' is een maat voor de snelheid waarmee spruitknoppen zich ontwikkelen en uitgroeien tot een zichtbare spruit. Op basis van gegevens uit deze proeven werden enkele modellen ontwikkeld en getoetst waarmee de relatie tussen spruitaantal of spruitdichtheid en droge-stofproductie van Engels raaigras planten of zones gesimuleerd kan worden. Er werden drie typen modellen gebruikt. In volgorde van toenemend detail en toenemende complexiteit van de module voor uitstoeling en bladoppervlaktetoename:

- i) Eenvoudige modellen gebaseerd op lichtonderschepping en een constante lichtbenuttingsefficiëntie ('radiation use efficiency'); dit type model is alleen bruikbaar bij vrij lage lichtintensiteiten. In dit type model hangt de toename van de bebladeringsindex ('leaf area index', LAI) af van de spruitdichtheid en de bladoppervlaktetoename per spruit, maar worden de spruitdichtheidsveranderingen niet gesimuleerd. (Hoofdstuk 5 en 6).
- ii) een zode-model (Hoofdstuk 7): toename van de LAI hangt af van de spruitdichtheid en de bladoppervlaktetoename per spruit; in dit model is een aparte module voor spruitdichtheidsveranderingen opgenomen. De module voor accumulatie van droge stof in de oogstbare laag was een bestaand model voor CO_2 -assimilatie van Engels

raaigras.

- iii) een gedetailleerd morphogenetisch model voor alleenstaande planten waarin de bladoppervlaktetoename per plant afhankelijk is van zowel het spruitaantal per plant als de beschikbaarheid van substraat. De spruitverschijningssnelheid is afhankelijk van de spruitknopaanlegssnelheid en van de substraatbeschikbaarheid (Hoofdstuk 3).

In Hoofdstuk 2, werden de resultaten gepresenteerd van een onderzoek naar de uitstoeling, bladoppervlaktetoename en hergroei van drie cultivars van Engels raaigras, bij twee knipfrequenties en drie kniphogtes. De uitstoelingssnelheid was lager naarmate de knipfrequentie hoger en de kniphogte lager was (dus naarmate de mate van ontbladering groter was). Over het algemeen reageerden de cultivars op dezelfde manier. Het effect van knipfrequentie op de uitstoelingssnelheid was het gevolg van een lagere site filling bij vaker knippen. Bij de lagere kniphogte was zowel de bladverschijningssnelheid als site filling lager. Bij driewekelijks ontbladeren op 7,5 cm, nam de grootte van volwassen, opeenvolgende bladeren aan een spruit toe. Bij toenemende mate van ontbladering (vaker en korter knippen) was deze toename van bladgrootte met bladnummer minder groot en met name voor het grootbladige type was er bij wekelijks knippen op 2,5 cm zelfs sprake van een afname van de bladgrootte van opeenvolgende bladeren. Rasverschillen in totale groei en in biomassa boven de kniphogte werden gerelateerd aan verschillen in bladschede en bladschijflengte en aan verschillen in groeiparameters. Bij alle rassen namen het specifieke bladoppervlak ('specific leaf area', SLA) en de fractie van de biomassa boven een stoppelhoogte van 2,5 cm toe bij vaker en korter knippen. Zowel de toename van de SLA als van de fractie boven 2,5 cm, helpen om enigszins te compenseren voor het kleinere bladoppervlak in de stoppel bij wekelijks ontbladeren op 2,5 cm. In hoeverre, de lagere SLA en lagere fractie boven 2,5 cm spruithogte bij minder frequent en hoger ontbladeren kan worden verklaard met behulp van een 'overstroom'-model voor de verdeling van assimilaten (meer assimilaten naar de stoppel beneden 2,5 cm wanneer de assimilatie door bladeren hoger is dan de behoefte aan substraat voor bladgroei) is onderzocht in Hoofdstuk 3.

In Hoofdstuk 3 werd een model gepresenteerd voor de hergroei van Engels raaigras na ontbladeren. In dit model is de groei van de verschillende organen (wortel, bladschede, bladschijf) een functie van zowel het substraataanbod (CO_2 -assimilatie en reserves in de stoppel/bladschedes, 'source size') als de behoefte van bladeren aan substraat ('sink size'). Het model is gebruikt om de verschillen in uitstoeling en hergroeisnelheid tussen ontbladeringsregimes gevonden in Hoofdstuk 2 te verklaren. In het model is spruitverschijningssnelheid een functie van de spruitknopaanlegssnelheid (afhankelijk van de bladverschijningssnelheid), de maximale knopbenutting en van de substraatbeschikbaarheid voor spruitknoppen. Met behulp van het simulatiemodel, kon uit eigenschappen van organen (bladverschijningssnelheid, bladstrekkingsnelheid, knopaanleg, minimale SLA, CO_2 -assimilatiesnelheid, e.d.) de toename van de fractie van de groei boven 2,5 cm stoppelhoogte, de toename van de SLA en de lagere spruitverschijningssnelheid worden verklaard, die werden gevonden bij de hoogste ontbladeringsfrequentie en laagste

ontbladeringshoogte in Hoofdstuk 2. Het model voorspelt dat de SLA sterk stijgt direct na ontbladeren tot een maximum is bereikt en daarna weer afneemt. Deze veranderingen van SLA na ontbladeren waren het grootst in simulaties voor frequent en laag ontbladeren. Met behulp van het model kon ook het aandeel van reserves in de stoppel aan de hergroei van blad worden berekend. Direct na ontbladeren is dit aandeel hoog, vooral als weinig bladmateriaal achterblijft na ontbladeren. Het aandeel van reserves in de stoppel aan de hergroei van blad daalt gedurende de eerste dagen na ontbladeren zeer snel, omdat de 'huidige' CO_2 -assimilatie door nieuw bladoppervlak een steeds groter deel van het voor bladgroei benodigde substraat kan leveren.

In Hoofdstuk 4 zijn de resultaten beschreven van een onderzoek naar de effecten van droogte op uitstoeling en groei van twee rassen van Engels raaigras (Wendy, een diploid, en Condesa, een tetraploid ras). Het effect van droogte werd nagebootst door toevoeging van polyethyleenglycol (PEG) aan de voedingsoplossing waardoor de waterpotentiaal van de oplossing verlaagd werd tot -1,3 MPa; de controle zonder PEG had een normale waterpotentiaal (0 MPa). Op deze manier wordt alleen de waterpotentiaal van het wortelmilieu en daarmee in de plant veranderd en niet allerlei andere factoren zoals bijvoorbeeld temperatuur, luchtvochtigheid en mineralenbeschikbaarheid. Bij de lage waterpotentiaal, was de bladstrekkingsnelheid 36 % lager dan bij de normale waterpotentiaal. Aan het eind van het experiment was het spruitaantal per plant 20 % lager bij -1,3 MPa dan bij 0 MPa, ten gevolge van een 12 % lagere bladverschijningsnelheid bij -1,3 MPa gedurende de eerste weken na de start van de behandeling. Site filling, de relatieve toename van het spruitaantal per plant per bladverschijningsinterval, was hoog (0,61 gemiddeld), maar lager dan de maximum waarde van 0,693. Site filling was niet significant lager bij -1,3 MPa dan bij 0 MPa. Site filling en het aantal nog niet uitgelopen spruitknoppen per spruit bleken mathematisch strikt aan elkaar gerelateerd. De absolute droge stof groei was 64 % lager bij -1,3 MPa dan bij 0 MPa. Gemiddeld was de relative groeisnelheid (RGR) 17 % lager bij -1,3 MPa dan bij 0 MPa. RGR was echter sterker verlaagd bij -1,3 MPa gedurende de eerste twee weken na de start van de behandelingen. Ook het verschil in netto assimilatiesnelheid ('net assimilation rate', NAR) tussen -1,3 MPa en 0 MPa was het grootst direct na de start van de behandelingen (NAR lager bij -1,3 MPa). Bij Wendy, was de SLA 13 % lager bij -1,3 MPa dan bij 0 MPa, maar bij Condesa was de SLA niet beïnvloed door de waterpotentiaal van de voedingsoplossing. In tegenstelling tot de verwachtingen van de theorie over de functionele balans tussen spruit- en wortelgroei, was de bladgroei als fractie van de totale groei ('leaf weight ratio', LWR) iets hoger bij -1,3 MPa dan bij 0 MPa. Uit literatuurgegevens blijkt dat in veldproeven waarin het effect van droogte op de spruitverschijningsnelheid is onderzocht, vaak een negatief effect van droogte op de site filling werd gevonden. Dit werd in deze studie niet gevonden en dit laat zien dat effecten van droogte op uitstoeling in het veld niet alleen toegeschreven kunnen worden aan een lage waterpotentiaal.

In Hoofdstuk 5 werd een onderzoek gepresenteerd naar verschillen tussen populaties van

Engels raaigras in uitstoeling, bladverschijningsnelheid, bladstrekkingsnelheid en verdeling van droge stof over wortel en spruit, bij laag en hoog stikstofaanbod. Er werden drie stikstoftoedieningsbehandelingen gebruikt: i) twee 'steady state' stikstofaanbodniveaus waarbij constante stikstofconcentraties in de plant werden bereikt van 0,027 and 0,042 g N.g DM⁻¹, ii) géén stikstoftoediening, iii) supra-optimaal stikstofaanbod. De populaties werden zowel in meng- als in monoculture opgekweekt om de correlatie tussen de prestatie in meng- en monocultuur te onderzoeken. De droge stof opbrengst van de meest produktieve populatie was 40 % hoger dan van de minst produktieve. De stikstofopbrengst van de meest produktieve populatie was 29 % hoger en de stikstofconcentratie van organisch gebonden stikstof was tussen 5 en 8,5 % lager dan van de minst produktieve populatie. Verschillen in hergroeisnelheid en opbrengst tussen populaties was groter in mengsels van populaties dan in monocultures, maar de rangorde was gelijk. Selectie voor hergroei eigenschappen kan daarom het best gedaan worden in mengsels.

Na het stoppen van de stikstoftoediening trad er spruitsterfte op waardoor de spruitdichtheid daalde. Ook de spruit-wortel verhouding werd lager. Zowel spruitdichtheid als de spruit-wortelverhouding herstelden zich tot waarden boven die tijdens de 'steady state'-stikstoftoediening, nadat het stikstofaanbod was verhoogd tot een niveau hoger dan nodig voor maximale groei (supra-optimaal stikstofaanbod). Analyse van de relatieve spruitverschijningsnelheid als product van bladverschijningsnelheid en site filling liet zien dat een negatieve genetische correlatie bestond tussen de twee factoren. Met behulp van een simulatiemodel werd het relatieve belang van spruitdichtheid, bladoppervlaktetoename per spruit en verdeling van droge stof onderzocht. Het verschil in droge stof opbrengst tussen de minst en meest produktieve populatie bleek voor 38 % verklaard te worden door de 11 % hogere 'LWR' van de meest produktieve populatie en voor de overige 62 % door de 63 % hogere spruitdichtheid en de 11 % hogere bladstrekkingsnelheid van de meest produktieve populatie (ten opzichte van de minst produktieve populatie).

In Hoofdstuk 6, werd een studie beschreven naar de interactie tussen plantdichtheid en stikstofaanbod wat betreft uitstoeling, bladoppervlaktetoename, lichtonderschepping en (her)groei van Engels raaigras. De lage plantdichtheid werd sterk gecompenseerd door extra uitstoeling en een hogere bladoppervlaktetoename en groei per spruit. Een hogere spruitdichtheid resulteerde in een snellere toename van de LAI na ontbladeren, vooral als ook het stikstofaanbod hoog was.

Er werd een negatieve correlatie gevonden tussen site filling en de fractie door het gewas onderschept licht. Site filling nam sneller af bij een toenemende lichtonderschepping bij een laag stikstofaanbod dan bij een hoog stikstofaanbod. Direct na ontbladeren was site filling laag, ondanks een lage fractie lichtonderschepping, als gevolg van een lage beschikbaarheid van substraat voor spruitknoppen gedurende de eerste week na ontbladeren. Verschillen in bladgroei bij de verschillende niveaus van stikstofaanbod en plantdichtheid konden worden verklaard uit verschillen in bladoppervlaktetoename (product van spruitdichtheid en

bladoppervlaktetoeename per spruit) en lichtonderschepping door het gewas. De bladoppervlaktetoeename was voor een deel onafhankelijk van de bladgroeisnelheid, aangezien de SLA van nieuwe bladeren sterk toenam na ontbladering en daarna weer daalde. Dit verloop komt overeen met het gesimuleerde verloop van SLA in Hoofdstuk 3.

In hoofdstuk 7 zijn twee veldexperimenten beschreven waarin de uitstoeling, bladverschijsning, bladoppervlaktetoeename en groei van Engels raaigras werden bestudeerd bij zeer uiteenlopende zaaidichtheden. Daarnaast werd een model ontwikkeld waarmee de relatie tussen spruitdichtheid en droge stof opbrengst gesimuleerd kan worden. In de veldstudies en met behulp van het model werd het effect van bladverschijsningssnelheid en bladoppervlaktetoeename per spruit op uitstoeling en de maximum spruitdichtheid van twee in deze eigenschappen verschillende rassen bestudeerd (Wendy en Condesa). Er werden drie oogstregimes toegepast om het effect van lichte en zware snedes op spruitdichtheid en hergroei na maaien te onderzoeken. De seizoensvariatie in spruitdichtheid werd veroorzaakt door variatie in de bladverschijsningssnelheid, site filling en spruitsterfte. Site filling nam af met toenemende LAI. Ook in het veld was site filling direct na maaien lager. De bladverschijsningssnelheid was positief gecorreleerd met bodemtemperatuur. Echter, bij eenzelfde temperatuur was de bladverschijsningssnelheid in het voorjaar hoger dan in het najaar.

Met het simulatiemodel werd getoond dat het seizoensverloop in spruitdichtheid en groeisnelheid kan worden verklaard op basis van uitstoelingssnelheid, spruitmortaliteit, bladoppervlaktevorming en een model voor de droge-stofproductie van Engels raaigras. Hierbij werden relaties gebruikt tussen bladverschijsningssnelheid, bladoppervlaktetoeename per spruit en temperatuur. Site filling werd als functie van LAI berekend. Modeluitkomsten lieten zien dat zowel de lagere bladverschijsningssnelheid als de hogere bladoppervlaktetoeename per spruit van Condesa bijdragen aan de lagere spruitdichtheid van Condesa (vergeleken met Wendy). De gemeten geoogste grasproductie was positief gecorreleerd met spruitdichtheid wanneer lichte sneden werden geoogst. Het model liet eenzelfde verband zien als gemeten was in de veldstudies.

Uit de veldexperimenten bleek dat een herinzaai geslaagd kan worden genoemd als de dichtheid van gevestigde planten tenminste 1 plant dm^{-2} bedraagt. Voor diploïde rassen als Wendy kan bij zo'n lage plantdichtheid toch een spruitdichtheid van 80 spruiten dm^{-2} in mei worden bereikt. Tetraploïde rassen als Condesa hebben dan een slechts een spruitdichtheid van 60 spruiten dm^{-2} , maar vanwege de hogere bladstrekkingsnelheid wordt dezelfde LAI bereikt. Condesa heeft bij een lagere spruitdichtheid zelfs een hoger opbrengs dan Wendy.

Vanwege de hoge potentiële uitstoelingssnelheid van Engels raaigras, geeft een lage spruitdichtheid op zichzelf niet voldoende aanleiding voor herinzaai. Alleen wanneer een lage spruitdichtheid gepaard gaat met een sterk geclusterd voorkomen van spruiten en met een hoge frequentie van grote open plekken zou herinzaai moeten plaatsvinden.

Het herstel van een open grasland kan bevorderd worden door frequent ontbladeren (lichte snedes maaien of standweiden toepassen), omdat het telkens verlagen van de LAI uitstoeling bevordert doordat site filling hoog is bij een lage LAI (veel spruitknoppen lopen ook daadwerkelijk uit). Vanwege de lage stikstofbenutting op grasland met een lagere produktiviteit ten gevolge van een te lage spruitdichtheid of een te hoge frequentie van open plekken, zou de stikstofgift verlaagd moeten worden om onnodige stikstofverliezen te voorkomen.

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Robert van Loo

CURRICULUM VITAE

Eibertus Nicolaas van Loo werd geboren op 11 april 1963 te Nieuwolda. Na het behalen van het diploma Gymnasium B aan het Willem Lodewijk Gymnasium te Groningen in 1981, studeerde hij Biologie aan de Rijksuniversiteit Groningen. In september 1987 studeerde hij cum laude af met als hoofdvak Plantenoecologie en bijvakken Plantenfysiologie en Theoretische Populatiegenetica. Van oktober 1987 tot april 1988 werkte hij als onderzoeker aan een gewasgroeimodel voor de water- en stikstofbeperkte groei van sorghum en millet in het project AGRISK van de Rijksuniversiteit Groningen. Vanaf april 1988 tot januari 1992 werkte hij als assistent-in-opleiding bij de Vakgroep Agronomie van de Landbouwniversiteit Wageningen. Bij deze vakgroep werd het onderzoek voor dit proefschrift uitgevoerd in nauwe samenwerking met de Vakgroep Theoretische Productie-ecologie van dezelfde universiteit. Vanaf 1 januari 1992 is hij als onderzoeker werkzaam bij het DLO-Centrum voor Plantenveredelings- en Reproductie-Onderzoek, op het gebied van de fysiologie van gras.

STELLINGEN

1. Verschillen tussen populaties van Engels raaigras in hergroeisnelheid na ontbladeren berusten in belangrijke mate op verschillen in zijspruitvorming en bladstrekkingssnelheid.

Dit proefschrift.

2. Veredeling in Engels raaigras op een hogere bladverschijningsnelheid zal leiden tot genotypen met een hogere spruitverschijningsnelheid en een hogere spruitdichtheid.
3. Selectie van genotypen van Engels raaigras voor hergroeisnelheid moet worden uitgevoerd op basis van de prestatie van genotypen in mengsels.
4. Het specifieke bladoppervlak geeft een indicatie voor de mate waarin bladgroei wordt beperkt door het aanbod van assimilaten of door morfologische beperkingen aan de grootte van het bladapparaat.

Dit proefschrift.

5. De snelheid van hergroei wordt alleen gedurende de eerste drie dagen na volledige ontbladering voor een groot deel bepaald door de hoeveelheid reserves in de stoppel van een graszode.

Dit proefschrift.

6. Op grasland met open plekken moet de stikstofbemesting naar beneden worden aangepast aan de lagere produktiviteit om verdere achteruitgang van de zode en onnodige stikstofverliezen naar het milieu te voorkomen.
7. Verhoging van de stikstofbenutting uit gras door weidend vee is mogelijk via het adagium: "eiwit omlaag en energie omhoog".
8. Het is onverteerbaar dat de eigenschap verteerbaarheid (nog) niet gebruikt wordt voor beoordeling van rassen van Engels raaigras voor de Nederlandse rassenlijst.
9. Een verhoging van de belastingheffing op energie- en grondstoffenverbruik en een verlaging van de belastingheffing op arbeid zal een grote bijdrage leveren aan de werkgelegenheid en aan een vermindering van de uitstoot van voor het milieu schadelijke stoffen.
10. Premiedifferentiatie bij arbeidsongeschiktheidsverzekeringen op basis van iemands individuele gezondheid of gesteldheid ondermijnt de onderlinge solidariteit tussen verzekerden en moet daarom niet worden toegepast. Het is bijvoorbeeld onwenselijk dat sommige particuliere verzekeringsmaatschappijen bij arbeidsongeschiktheidsverzekeringen voor één-ogigen het verlies van hun goede oog uitsluiten of dit risico slechts tegen hogere premies accepteren.
11. Het enige voordeel van het lage aanvangssalaris van AIO's is dat zij daardoor in aanmerking kunnen komen voor een goedkopere huurwoning.

Proefschrift van E.N. van Loo. On the relation between tillering, leaf area expansion and growth of perennial ryegrass (*Lolium perenne* L.).