Quality Legume-Based Forage Systems for Contrasting Environments

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Edited by

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COST

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Introduction

These proceedings contain papers presented at the final meeting of COST Action 852, *Quality Legume Based Forage Systems for Contrasting Environments*, held at HBLFA Raumberg-Gumpenstein, Austria from 31 August to 2 September 2006. These are the third in a series of proceedings resulting from the Action, the other two presenting papers from workshops in Ystad, Sweden 2004 and Grado, Italy 2005.

COST Action 852 has been very successful and has attracted a large group of scientists from over 20 countries all across Europe. What brought us together is our common appreciation of the forage legumes and our belief that they can play an important role in sustainable agriculture in the future. However, this is not an easy task and there are many hurdles on the way before forage legumes will be considered a group of species that no decent farmer can do away with. Our Action has not and will not find answers to all questions that need to be tackled before this will be realised. But we still believe that our work has moved us a little step forward and, certainly, if we manage to make our work known to the wider scientific community we can proudly claim that we have achieved something worth while.

The papers presented in the current proceedings demonstrate the vibrant energy that can be found within the group. The topics are one way or another all related to our common goal of the Action which is to enhance the uptake of quality legume based forage systems for contrasting environments. The first set of papers looks at legume breeding for different regions of Europe, the second set at sward management in legume forage based systems with special emphasis on nitrogen economy, the third set deals with topics related to the COST 852 Agrodiversity experiment, which is currently being carried out at over 40 sites across Europe in Canada and Australia, and the forth focuses on feeding value of forage legumes across Europe. The final set of papers fall under the heading *Adapting forage mixture composition and plant characteristics* to the ruminant requirement and belong to a mini-workshop combining the interests of Working Group 1 on plant breeding and Working Group 3 on animal nutrition.

Special thanks to Dr. Erich M. Pötsch and his co-workers for taking on the task of hosting the final meeting of COST 852. I would also like to thank the vice-chair and all working group leaders for their contribution and to all the participants for submitting such a wealth of interesting material.

Áslaug Helgadóttir

Chairman COST 852

Contents

POSTER PRESENTATIONS

Austrian agriculture - data and facts

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Keywords - Austrian agriculture, productivity, farm structure

This paper presents a brief insight and introduction into structure, productivity and prospects of Austrian agriculture, which indeed is a decreasing sector but nevertheless an important and indispensable part of the economy and society. *Figure 1* illustrates the structure and distribution of the Austrian agricultural area (AA) which amounts to 3.26 million ha. Of the total farmland 57% is grassland, which is most dominant in the central and western provinces of Austria, whereas arable land and vineyards can be found in the northern and eastern parts of Austria.

According to the agricultural structure survey 2003 there were 190,400 agricultural and forestry holdings in the country,, which is a reduction of 12% compared to the previous survey of 1999. This reduction affected primarily part-time farms, whereas full-time farms remained stable.

The average farm size in Austria is about 18.4 ha AA, the average number of dairy cows per farm amounts to 9 heads which is less than in most European countries (BMLFUW 2006) (*Figure 2*). Nearly 70% both of total AA and of all farms are located in the less favoured areas consisting of mountainous, small and other disadvantaged areas.

Around 52% of all Austrian farms and 70% of all dairy farms can be found in the mountainous area, mainly based on traditionally used permanent grassland. The average milk production per cow is 5,432 kg year^{-1} and on about 75% of all dairy farms the average milk produc-

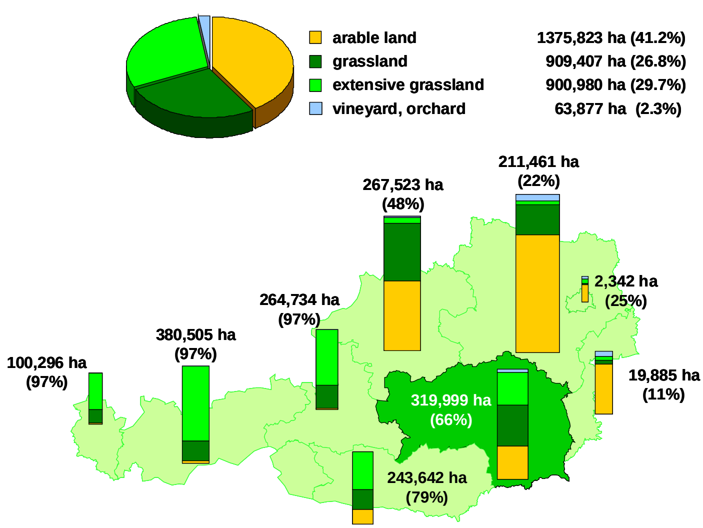

Figure 1: Structure and distribution of the Austrian agricultural area

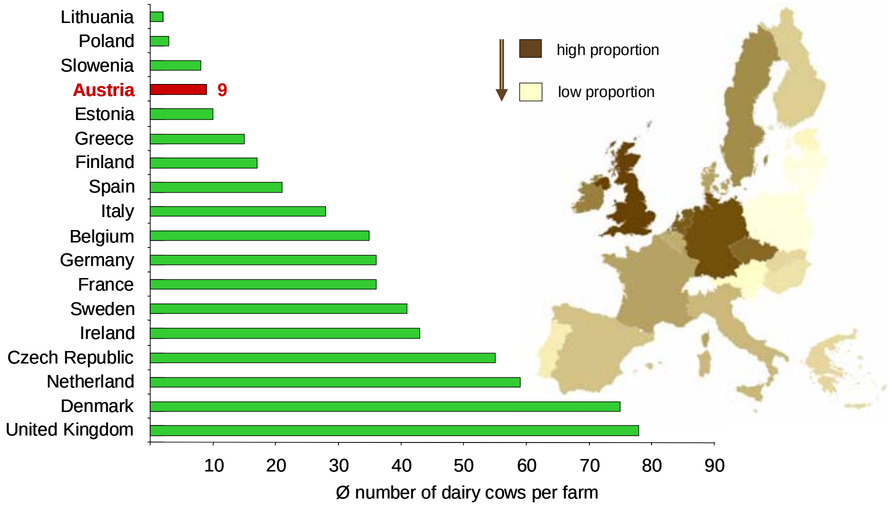

Figure 2: Structure of dairy farm holdings in Europe

tion cow^{-1} year^{-1} is lower than 7,000 kg. Forage from meadows and pastures is the main component of the feed rations on dairy and cattle farms and the input of farm external concentrates has been reduced to a minimum.

About half of this grassland is managed in a very extensive way with a low stocking rate and is cut or grazed once or twice a year. Permanent grassland in the more favourable regions of the mountains can be utilised at least three times a year (silage cut, hay cut, second cut hay or alternatively grazing in the autumn). In some very productive small lowland areas even up to five cuts a year can be harvested.

Table 1 provides some detailed information on the proportion and productivity of different grassland types subdivided in permanent grassland and ley farming areas, which in practice consist of high legume proportion and are therefore strongly related to the topic of COST 852.

Table 1: Structure and productivity of permanent grassland and ley farming areas in Austria (Buchgraber *et al.* 2004)

grassland types	ha	Net yield 1000 t DM year^{-1}	energy yield 1000 GJ NEL year^{-1}	protein yield 1000 t year^{-1}
One cut grassland	55.659	86	724	7,7
Extensive pasture	116.362	114	1.008	11,9
Alpine meadows/pastures	709.479	328	2.954	32,8
More cut meadows	815.945	3.835	35.805	475,4
Cultivated pastures	93.462	347	3.466	49,4
Permanent grassland	**1.790.907**	**4.710**	**43.957**	**577,2**
Grass/clover	58.770	371	3.764	63,1
Temporary grassland	76.501	429	4.276	64,3
Pure clover	8.594	51	513	9,2
Lucerne	12.425	63	592	11,9
Ley farming areas	**156.290**	**914**	**9.145**	**148,5**

Table 2: Organic farming in selected EU-countries (EUROSTAT 2006, FIBL 2006)

Member state	Organic farm area in ha	% of total farm area	Number of organic farms	% of all farms
Austria	**344.916**	**13,5**	**19.826**	**11,3**
Czech Republic	260.120	6,1	836	2,2
Denmark	154.921	5,8	3.166	5,5
Finland	162.024	7,3	4.887	6,0
France	534.037	1,8	11.059	1,7
Germany	767.891	4,5	16.603	4,1
Greece	249.488	3,0	8.269	1,0
Hungary	128.690	2,2	1.583	4,1
Ireland	30.670	0,7	897	0,6
Italy	**954.361**	6,2	**36.639**	1,7
Netherlands	48.155	2,5	1.469	1,4
Poland	82.370	0,5	3.760	0,2
Portugal	206.524	5,0	1.302	0,3
Slowakia	93.943	4,6	117	2,9
Spain	733.182	2,4	16.013	1,4
Sweden	206.579	6,6	3.138	3,9
United Kingdom	690.269	4,4	4.010	1,7
EU -25	**5.529.000**	**3,2**	**137.992**	**1,3**

Contrary to some intensive grassland production areas in Europe, a high natural floristic diversity can be observed on the different grassland types in Austrian, indicating sustainable management (Pötsch *et al.* 2005).

Austria still leads the organic farming in Europe both with respect to the proportion of total AA (13.5%) and the proportion of all farms (11.5%) (*Table 2*).

In 2005 the number of subsidized organic farms rose to 20,104, which means an increase of 2.7% compared to the previous year. The majority of organic farms in Austria are grassland and dairy farms, but a significant increase of organic farming in other land use systems is to be noticed.

In addition to organic farming around 65,000 grassland farms take part in special measures, which require an abolition or reduction of resources leading to yield increase such as mineral nitrogen, easily soluble fertilizer and pesticides. These farms represent a huge potential for the increase of organic farming in the near future.

In 2005 the output from agriculture amounted to 5.4 billion Euros, which is a reduction of -8.1% compared to 2004. This strong decrease was primarily caused by the implementation of the single farm payment schedule following the CAP reform. The number of persons working in agriculture and forestry, calculated on the basis of annual work units, decreased by 1.0% to 187,252 AWU, of which 83% were unpaid (family) workers. Based on the calculations of the aggregate agricultural account the factor income of agriculture per worker dropped nominally by 3.7% in 2005.

The income situation of farm holdings improved on average in 2005, but unlike the average development an analysis by farm types resulted in considerable differences. A strong income improvement was recorded for intensive livestock farms (+17%), feed farms (+11%), mixed farms (+9%) and holdings with a larger proportion of forestry. On mountain farms, income from agriculture and forestry exceeded the level of the previous year by 11%. Due to the favourable ratio between expenses and revenue the income of organic farms was almost 9% above the average for all other farm types. With respect to income, the budget for rural development plays a crucial role in the Austrian agriculture. About 60% of this budget (around 7.1 billion Euros for the period 2000-2006) goes to the Austrian agro-environmental program (ÖPUL), which offers a large number of measures and is very well accepted by farmers. A total of 80% of all farmers take part in this program, that is offered in the whole of Austria and which covers nearly 90% of the total AA.

Nevertheless, it takes strong efforts to maintain grassland and dairy farming in less favoured areas and to keep the landscape open. An open, well structured landscape and maintaining traditional and sustainable farming is an essential assumption especially in regions with a high proportion of tourism. Therefore different strategies have to be considered and adapted to the special conditions and requirements. There will be productive agricultural land use systems, both intensive dairy farming (around 5,000 farms each with 40-50 dairy cows and 300-400 t milk quota) and more traditional dairy farming (around 35,000 farms each with 10 cows and 40-50 t milk quota). Another 30,000 farms will focus on extensive livestock production including sheep and goats, suckler cows, heifer and beef fattening. The already existing system of income combinations has to be enhanced and different types of farm co-operations have to be forced to reduce costs and work load.

In some regions there will also be productive but non-agricultural land management systems with an alternative use of grassland biomass. Grassland could be the basis of a green refinery providing energy, isolation and insulation material, lactic and amino acids, enzymes or even secondary metabolites for special usage. Another strategy to keep the landscape open will be non-productive and non-agricultural land management via cutting or mulching without any use of the biomass.

In future strong efforts have to be made to inform the public about the multifunctional role of agriculture for the whole society. The awareness and sensibility of consumers should be increased to improve sympathy for this endangered economic sector and to raise the acceptance for support. The farmers themselves must improve their efforts for a sustainable management at least by following all relevant laws, guidelines and regulations for production to advance their image.

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Investigating the basis of abiotic stress tolerance in white clover: molecular approaches currently being used in IGER, Aberystwyth

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Keywords: *Trifolium repens*, cold acclimation, drought tolerance, gene expression, introgression

ABSTRACT

Recent advances in molecular biology have facilitated the targeting of specific traits for dissection and will increase the speed and precision of the white clover breeding programme at IGER. This paper provides an overview of the research currently being conducted in white clover on (i) the process of acclimation to low temperatures using analysis of changes in gene expression and identification of candidate genes; (ii) improvement of drought tolerance through marker-assisted introgression and analysis of global changes in gene expression during developing drought using information and resources from the model legume species *Medicago truncatula* as a comparison.

INTRODUCTION

In temperate regions of Europe it is likely that the severity of episodes of abiotic stress will increase in the future as the de-stabilising effects of climate change intensify. As a result, the ability of perennial grassland plants to tolerate low temperatures and reduced water supply will become an increasingly important factor in maintaining productive, sustainable and biodiverse swards. White clover (*Trifolium repens* L.), the most widely-grown perennial legume in cooler climates, is considered to be particularly susceptible to cold and drought stress. In the past, research at IGER has been carried out on cold and drought tolerance in this species at the physiological/biochemical level, and improvements in cold tolerance have been brought about through the traditional plant breeding processes of phenotypic selection and crossing (Rhodes *et al.* 1994). However, plants respond and adapt to environmental stresses not only through physiological and biochemical processes but also through molecular and cellular processes (Yamaguchi-Shinozaki and Shinozaki 2005). Recent advances in molecular biology have facilitated the targeting of specific traits for dissection and this has the potential to increase the speed and precision of plant breeding programmes by providing relevant material for germplasm improvement. This paper gives an overview of research on this topic currently being carried out on white clover at IGER.

APPROACHES AND RESULTS

(i) Cold tolerance

Cold tolerance is a complex trait with several components. We are initially focusing on the important process of cold acclimation (switching to a cold-tolerant physiological state) by analysing plants subjected to low, but not freezing, temperatures. We are taking two approaches to the analysis of cold acclimation: (a) studies of variation in gene expression between plants growing under different temperature treatments; (b) analysis of candidate genes.

(a) Three clonal sets of 25 genotypes from the cultivar Olwen were used. In treatment A ('acclimated'), plants were kept at 2 °C and 8 h daylength for 24 days; in treatment B ('de-acclimated'), plants underwent acclimation as in A, but were then placed in warm conditions (20/10 °C day/night; 12 h daylength) for 14 days; in treatment C ('non-acclimated'), plants were kept in warm conditions (20/10 °C day/night; 12 h daylength) for 38 days. At the end of these treatments RNA was extracted from young plant tissue (i.e. expanding leaves and stolon tips) in each genotype using TRIzol ™ (Invitrogen). Comparisons of gene expression were made using the cDNA-AFLP technique. Initial comparisons were carried out on RNA from each treatment separately, bulked within leaves and stolons. To confirm the pattern of polymorphisms between plants from the different treatments, subsequent analysis with the same primers was carried out on individual genotypes, again comparing the leaf and stolon tissue samples separately. A large number of polymorphic bands were obtained in this analysis. For example, in the case of the bulked leaf samples more than 100 bands from six primer combinations were found. However, the bands scored in the bulked samples represented only about 10% of those present when all genotypes were analysed separately. Thus, polymorphisms identified in bulks represented a suitable means of reducing the overall differences to a more manageable number, which could subsequently be confirmed and sequenced on individual genotypes. Bands that were observed in treatment A only are currently being sequenced and some similarities have been observed with sequences in *Arabidopsis thaliana* (eg. VRN2, a vernalisation control gene). The cDNA-AFLP technique has thus been useful in identifying changes in gene expression across genotypes during cold acclimation. This research is being extended in several directions. For example, leaves from clones of the genotypes of Olwen in the three temperature treatments were analysed for concentrations of the major plant fatty acids, and significant treatment effects were found. In treatments A, B and C levels of the unsaturated fatty acids linoleic acid were 6.99, 6.43 and 5.08 mg/g DW respectively ($P < 0.001$), and linolenic acid were 23.42, 27.00 and 20.55 mg/g DW respectively ($P < 0.001$). These results confirm previous observations that one of the processes occurring during plant acclimation to low temperatures is an increase in the production of unsaturated fatty acids (Lynch and Steponkus 1987, Dalmansdóttir *et al.* 2001, Collins *et al.* 2002). In addition to this research on phenotypic traits, candidate genes identified after further analysis will be confirmed by testing for co-location with quantitative trait loci for cold tolerance in a white clover mapping family (Jones *et al.* 2003) and by the technique of real time-PCR. Further analysis of gene expression is being carried out using microarray technology, employing the model legume species *Medicago truncatula* (*Mt*). This species is taxonomically closely related to other forage legumes and has a small genome that has been successfully mapped and will be fully-sequenced by the end of 2007 (Young *et al.* 2005). It is therefore feasible to exploit this knowledge and apply it to white clover. This is complementary to the cDNA-AFLP approach and will permit bioinformatic analysis of global changes in gene expression during cold acclimation.

(b) Genetic variation in white clover is being exploited to identify functional alleles associated with cold tolerance. A candidate gene approach is being used, currently focusing on regulation of the lipid composition of the plasma membrane. The plasma membrane is the primary site of low temperature injury in plants and maintenance if its functionality during exposure to cold is important (see references in above section). A likely candidate gene involved in this aspect of cold acclimation is TRZIP, which has desaturase properties and may also have a regulatory role. A full length cDNA and genomic sequence has been cloned and molecular markers in the form of single nucleotide polymorphisms (SNPs) have been identified. These have been used to search for allelic variation associated with adaptation to cold in white clover. The germplasm used was a number of white clover populations differing in cold tolerance, produced as part of the COST 814 pan-European project (see Collins *et al.* 2001 for details), plus genotypes from a cold tolerant Swedish cultivar (cv. Undrom) and a Ladino-type accession from Italy. In this material we analysed gene expression patterns during cold acclimation carried out under the same conditions as treatment A in the above section. Levels of expression were measured in leaf laminae and stolon tips separately, and were significantly higher in the former. Both tissue types showed greater expression of TRZIP as acclimation progressed (*Figure 1*), but there were no differences between the populations.

Table 1: Concentration of unsaturated fatty acids (UFA) and freezing tolerance (LT_{50}; estimated temperature causing 50% plant mortality) measured in acclimated plants of 6 white clover populations

Population	UFA (mg/g DW)	LT_{50} (°C)
AberHerald Baseline	29.9	-9.30
AberHerald Belgium	28.7	-7.98
AberHerald Iceland	28.6	-9.09
AberHerald Sweden	30.8	-9.25
Ladino Accession	21.1	-8.77
Undrom	27.8	-12.09

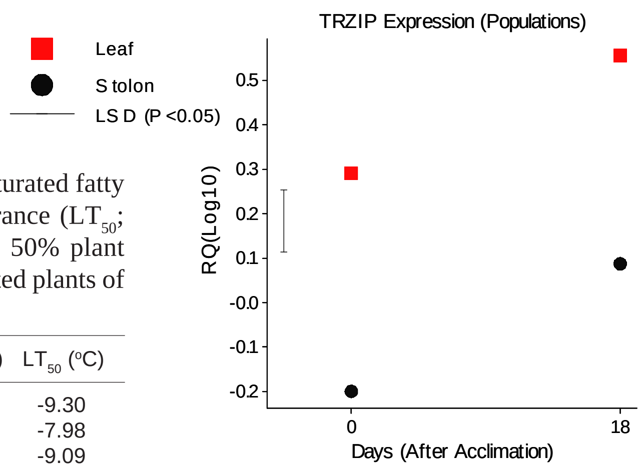

Figure 1: Expression levels of the gene TRZIP during cold acclimation in leaf and stolon tissue averaged over 6 white clover populations

In addition, we carried out an artificial freezing test and analysis of concentrations of fatty acids in leaf tissue of acclimated plants of the same populations in order to relate phenotype to SNP markers. The results are shown in *Table 1*.

There was variation between the populations in both phenotypic traits, but the two parameters were not correlated. It is therefore difficult to associate phenotypic variation with allelic variation in the case of TRZIP. However, the scope of this project will be widened to target other candidate genes which will be identified by a combination of collaboration with researchers elsewhere with similar interests, gene expression analyses such as cDNA-AFLP, data mining and synteny with other crop species.

(ii) Drought tolerance

In forage species agronomic yield, which consists mainly of leaf tissue, is more susceptible to the adverse effects of drought stress than that of cereals or grain legumes.

We are using two approaches to investigate and improve drought tolerance in white clover:

(a) marker assisted introgression

(b) analysis of global changes in gene expression

(a) We are using marker assisted selection to increase the speed and precision of introgression breeding. For improving drought tolerance we are using the closely related species *Trifolium ambiguum* (Caucasian or Kura clover), a rhizomatous species that is stress tolerant and persistent but slow to establish in mixed swards. We have developed backcross hybrids containing rhizomes as well as the stolons of white clover. Third generation backcross plants are considerably more drought tolerant than white clover but show similar levels of N-fixation and agronomic performance (Abberton *et al.* 2001, Marshall *et al.* 2003). Molecular markers for the rhizomatous trait, which takes 18 months to be expressed visibly, are being used in a high-throughput screen system that will speed up the transfer of this trait into agronomically suitable white clover germplasm. Cytological analysis of the *T. repens* x *T. ambiguum* hybrids and backcross material using GISH probes demonstrated strong evidence for recombination and stable introgession between genomes.

(b) In this element of the drought stress programme we (in collaboration with colleagues from Teagasc, Ireland) have compared the changes in gene expression during developing drought in white clover and *Mt* growing in the same conditions in a glasshouse. In this way, a model species approach has been incorporated at the start of the experiment. RNA from both species has been extracted on a time course and has been used to interrogate the *Mt* Affymetrix microarray chip. The following analyses were carried out:

1. Study of the hybridisation of white clover RNA to the Mt chip in comparison to that of *Mt* to the *Mt* chip
2. Differences in expression levels of genes in a droughted Mt plant compared to those in a control (non-droughted) *Mt* plant
3. Differences in expression levels of genes in a droughted white clover plant compared to those in a control white clover plant

The level of cross-species hybridisation (12.4%) was considered encouraging. The results of analyses 2 and 3 on the above list are currently being validated and interpreted.

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Breeding forage legumes for a new era

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ABSTRACT

Agriculture is entering a new era in which there is a greater awareness of and concern over the environmental costs of farming. Grassland agriculture is inherently multifunctional and our challenge for the future is to develop legumes that maintain or improve production whilst enhancing positive functions that increase natural, social and human capital. The research we are undertaking at IGER indicates that forage legumes have the capacity to contribute towards sustainable systems, whilst at the same time, ameliorating some of the effects of climate change by delivering a range of 'non food' functions. Our breeding programmes concentrate on the traditional legumes such as red clover (*Trifolium pratense*) and white clover (*Trifolium repens*), whilst also investigating the potential of birdsfoot trefoil (*Lotus corniculatus*) and the use of a range of novel interspecific hybrids. We conclude that legumes display many attributes that make them essential for the delivery of sustainable agriculture and that we can only exploit these attributes through long term breeding programmes.

Keywords: Legumes, sustainable agriculture, biodiversity, environment, climate change

INTRODUCTION

The intensification of farming following the Second World War led to highly mechanized and specialized farming practices that were heavily dependent on fossil fuels, borrowed capital and chemical fertilizers and pesticides. Although these practices succeeded in increasing output such systems are now associated with declining soil productivity, deteriorating environmental quality, reduced profitability and threats to human and animal health. In the UK the government is promoting a shift in agricultural practice towards stewardship of the environment. This 'Production and Environment' strategy presents a major challenge for UK farmers, and scientific research institutes, such as the Institute of Grassland and Environmental Research (IGER), will play a vital role. IGER can help farming meet these new demands, not only by developing more sustainable legumes, but also through the production of novel and valuable environmental commodities. New attributes will need to be developed if legumes are to contribute to whole systems that reduce environmental impact and enhance ecosystem services. IGER is in a unique position to address these issues as it has access to an exceptional range of genetic resources and can incorporate novel characteristics swiftly into suitable, agronomically acceptable material.

APPROACH

The use and development of sustainable legumes provide opportunities to mitigate the effects of climate change and reduce pollution from pastoral farming compared to conventional grassland agriculture. The research we are undertaking at IGER to develop new varieties of forage legumes suitable for such balanced systems falls into four broad themes.

1. Legume systems with enhanced diversity

The level of function in agro-ecosystems is largely dependent on the degree of plant and animal biodiversity present and this diversity helps provide ecosystem services beyond the production of food, fibre, fuel, and income. We are considering two routes in which the diversity of grassland systems could be improved. In the first we are investigating selection lines within white clover with fixed diverse morphological and physiological traits. These are being used, within a bio-diverse background, to identify specific traits of importance in maintaining competitive ability and promoting plant diversity. In the second, more traditional route, we are endeavouring to improve resource use in grassland through increasing the complexity of sown swards. The use of more complex multi-species grass/legume mixtures (two grasses and two legumes) may overcome instability and increase production through enhanced niche partitioning. To this end we are participating in a pan-European experiment via the EU funded COST 852 programme and this action links 27 research groups in 23 countries and enables hypotheses to be tested over wide environmental gradients.

2. Reducing the environmental footprint

Many bio-indicators of environmental health in farmed areas show declines. Improving P-use efficiency in white clover has the potential to reduce the application of costly phosphoric fertilisers in resource-poor situations. The benefits will be to reduce diffuse pollution of surface and ground water resources, together with the development of highly desirable improvements in sward and microbial diversity. Arbuscular mycorrhizal fungi (AMF) are likely to play a critical role in low-input farming because of their function linking plant and soil processes. Selections for enhanced association with AMF have been made by screening a range of white clover genotypes and these show promise in low fertility bio-diverse environments.

The pasture/ruminant system is inefficient, with only about 25% of the ingested plant protein being incorporated into animal protein. The rest is excreted and represents a potential source of pollution from grazed areas. Improving protein protection in legumes by utilising phenolic compounds could lead to considerably less protein degradation in silo and improved protein utilisation in the grazing animal. *Lotus corniculatus,* through tannins, and red clover, via polyphenol oxidase activity, possess systems that protect protein to a greater extent than other forage legumes such as white clover or lucerne (*Medicago sativa*). In the case of *Lotus* manipulating tannin content could also bring extra benefits by reducing the incidence of animal parasites, preventing bloat and reducing methane emissions from sheep and cattle. Our research centres on understanding the genetic control of these traits and developing divergent populations for use in further studies. The efficiency of N utilisation can also be increased by modifying protein levels in herbage. By utilising self fertile, inbred lines with very poor, or no nodulation we have been able to vary the protein content of white clover plants over a wide range through the differential application of N fertiliser. Despite this variation in protein status prior to cutting, the N content of plant material following ensilement was not significantly different (Winters *et al.* 2004). This suggests that reduced N losses could be achieved by modification of initial protein levels without detriment to the nutritional quality of the final silage.

3. Dealing with the effects of climate change

The increase of greenhouse gasses such as carbon dioxide, nitrous oxide and methane are clear indicators of the effect of human influences on the atmosphere. A lesser known result of photochemical reactions between these gasses and volatile organic compounds is an increased incidence of ground level ozone. These occurrences can cause damage to vegetation, reduce palatability and persistence. White clover is used as an indicator species for ozone damage, since both susceptible and tolerant genotypes are available. Our research indicates that sufficient genetic variation exists between genotypes of advanced white clover germplasm to support the inclusion of this trait as a selection criterion in the development of new varieties. Recent years have seen extreme weather events, including a number of 'floods of the century' and the hottest year on record. Although great variation exists in weather patterns in Northern Europe there is a clear trend towards drier summers and wetter winters. A major element of research aimed at improving tolerance of drought in white clover has been the development of interspecific hybrids with the rhizomatous species *Trifolium ambiguum*. Backcrossing this material with white clover as the recurrent parent has produced plants which are very similar to white clover but with a small amount of rhizome. This material exhibits significantly enhanced drought tolerance in comparison with the white clover parent. At the other extreme legumes are of potential use in the mitigation of flooding through their ability to enhance soil structure. Although the process is not yet fully understood legumes (both white and red clover) are known to improve the diffusivity of soil compared to grasses and this leads to more effective drainage, less water run-off and greater water holding capacity.

4. Sustainable utilisation of the legume

Grassland renovation techniques such as ploughing and reseeding are known to have significant, deleterious effects on the environment (Vellinga *et al.* 2004). A major thrust in our research is to develop management strategies and plant models aimed at sustainable utilisation of the legume, thus reducing the use of invasive management techniques. Enhanced persistence remains a priority and new research in white clover (manipulating population structure), red clover (understanding crown deterioration) and *Lotus* indicate that improvements in this trait are still possible.

CONCLUSION

Legumes display many attributes that make them essential for the delivery of sustainable agriculture and we can only exploit these attributes through long term breeding programmes.

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Breeding forage legumes for better persistence and productivity in legume-based swards

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ABSTRACT

Potentially very valuable legumes unfortunately have drawbacks, as they can be insufficiently resistant to unfavourable abiotic conditions and to diseases. The objectives of this study were to assess the newest local varieties of white clover (cv. Nemuniai) and red clover (cv. Arimaiciai) for persistence and productivity in grass/legume mixtures compared with local varieties and with „wide genetic base“ mixtures of white clover and red clover. Perennial ryegrass (G_1), cocksfoot (G_2), red clover (L_1) and white clover (L_2), with contrasting functional characteristics, were grown on a loamy Endocalcari-Epihypogleyic Cambisol in Dotnuva, Lithuania, (55°24' N 23°50' E) at two sowing densities in varying proportions in mixtures and also in monocultures: plots 1-30 plots contained „narrow genetic base“ legumes (the single Lithuanian varieties) and plots 31-48 contained „wide genetic based“ legumes (a mechanical mixture of different varieties). The productivity and persistence of legumes in swards with local varieties and with „wide genetic base“ mixtures of white clover and red clover were similar. The influence of legumes on total DM yield of swards is not very clear from this single-site experiment.

Keywords: white clover, red clover, yield, mixtures, monocultures

INTRODUCTION

Forage legume sowing in Lithuania was started in the 18th century and since then legumes have been in constant use in crop rotations. Potentially very valuable legumes unfortunately have drawbacks, as they can be insufficiently resistant to unfavourable abiotic conditions and to diseases. Plant breeding is one of the ways in which suitable legume cultivars can be supplied to farmers in order to increase confidence in forage legumes. Legume breeding in Lithuania was started in 1922 and has continued until now. The main legumes used in Lithuania are red and white clovers and lucerne, and the greatest breeding effort is focused on these crops. Various breeding methods have been applied in the development of new varieties: selection (eg. mass negative, mass positive, group and family, individual), intervarietal crossing, experimental polyploidy, and the development of synthetic varieties (Dabkeviciene *et al.* 2001, Sprainaitis *et al.* 2002). Besides conventional methods, embryo polyploidy and intervarietal hybridization have been used in the improvement of red clover herbage and seed yield. The objectives of this study were to assess the newest local varieties of white clover (cv. Nemuniai) and red clover (cv. Arimaiciai) for persistence and productivity in grass/legume mixtures compared with local varieties and with „wide genetic base“ mixtures of white clover and red clover.

MATERIALS AND METHODS

Field studies were conducted on a loamy Endocalcari-Epihypogleyic Cambisol in Dotnuva, Lithuania (55°24' N 23°50' E). Perennial ryegrass (G_1), cocksfoot (G_2), red clover (L_1) and white clover (L_2), with contrasting functional characteristics, were grown at two sowing densities in varying proportions in mixtures and in monocultures in 48 plots: plots 1-30 contained 'narrow genetic base' legumes in monocultures and in mixtures with grasses: G_1 cv. Sodre (high density sowing rate = 18 kg ha^{-1}): G_2 cv. Aukštuole (16 kg ha^{-1}): L_1 cv. Arimaiciai (15 kg ha^{-1}); L_2 cv. Nemuniai (10 kg ha^{-1}). Plots 31-48 were sown with „wide genetic base" legumes composed of a range different varieties in mechanical mixtures. The experiment was established in May 2003 and consisted of monocultures and various mixture combinations ranging from dominance by one (0.7,0.1, 0.1,0,1) or two (0.4,0.4,0.1,0.1) species to centroid mixtures (0.25,0.25,0.25,0.25) at high (100%) and low (60%) sowing densities. In spring 2004 and 2005, and after each harvest, nitrogen was applied to all plots at a rate 40 kg N ha^{-1}. The first harvest was taken on 7 June 2004, with subsequent harvests on 27 July and 21 September. In 2005 two cuts were taken, on 08 June and 10 October.

RESULTS

Red clover and white clover of either narrow or wide genetic base were more productive in monocultures than in mixed swards in 2004 (*Figures 1*and *2*). In the second harvest year DM yields in monocultures and mixed swards were similar. Red clover in the wide genetic base monocultures was slightly more productive than the single variety in 2004 in harvests 2 and 3 (*Figure 1*). The white clover single variety was more productive than the wide genetic base mixture in 2004, but no differences were found in 2005. In mixed swards there was little difference between the yields of the single cultivar red and white clover and the wide genetic base populations in either 2004 or 2005 (*Figure 2*).

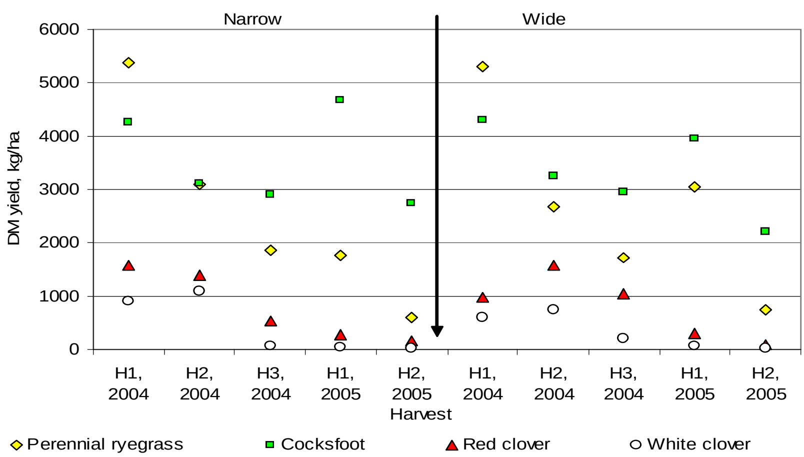

Figure 1: DM yield (kg ha^{-1}) in monocultures with narrow and wide genetic base legumes

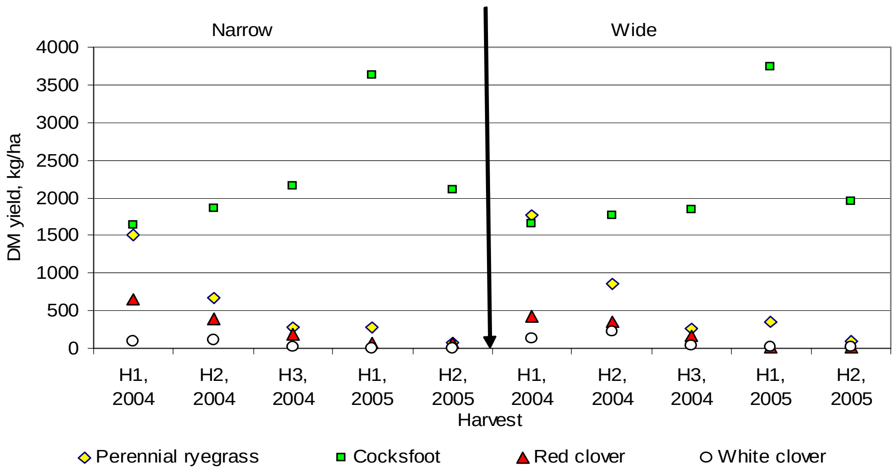

Figure 2: DM yield (kg ha^{-1}) in mixed swards with narrow and wide genetic base legumes

DISCUSSION

The results from our location in 2004 and 2005 suggest that there was no advantage of increased legume genetic heterogeneity in terms of total mixture yield. The influence of legumes on total DM yield of swards is not very clear from this one site experiment, because of unfavourable growing and overwintering conditions. N fertilization probably had a negative effect on the persistence of legumes, especially in second harvest year (2005). In previous experiments we obtained higher DM yield of red clover and white clover in monocultures and in mixtures without nitrogen fertilization in second year of sward use (Kadziuliene and Šarunaite 2005). The slightly better persistence of local legume varieties might be because they were developed using the application of an infection background and selected for their resistance to crown and root rot (Sprainaitis *et al.* 2002).

CONCLUSIONS

The productivity and persistence of legumes in swards with local single varieties and with „wide genetic base" mixtures of white clover and red clover were similar.

It is difficult to draw conclusions about the influence of legumes on total DM sward yield from this one-site experiment.

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Directions and recent achievements in breeding annual forage and grain legumes in Serbia

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ABSTRACT

The Forage Crops Department of the Institute of Field and Vegetable Crops in Novi Sad is the only institution in Serbia involved in breeding annual forage and grain legumes. All breeding programmes are based upon the Annual Forage and Grain Legumes Collection (AFGLC), with more than 1,400 accessions. The main breeding programme is aimed at the development of cultivars with high yields of quality forage and reliable seed yields. The goal of the secondary breeding programme is to develop new cultivars with high yields of grain rich in protein. Other breeding programmes include the development of dual-purpose cultivars of pea and of common vetch with high yields of forage and reduced content of anti-nutritive factors in the grain. Breeding for tolerance to biotic and abiotic stress is interwoven with the main programmes and includes the development of winter cultivars of diverse species, cultivars of pea either tolerant or resistant to pea weevil and cultivars of white lupin tolerant of alkaline and carbonated soil types.

Keywords: breeding, annual forage legumes, grain legumes

INTRODUCTION

Annual forage and grain legumes have a long tradition of cultivation in Serbia. The most important grain legume is soya bean (*Glycine max* (L.) Merr.), while the most widespread pulses are *Phaseolus* beans. Pea (*Pisum sativum* L.) and vetches (*Vicia* spp.) are the most important annual forage legumes and are grown on about 35,000 ha, being utilised as green forage, hay, forage meal, silage, haylage, grain and straw (Mikic *et al.* 2006), as well as being used as cover crops and green manure in organic farming and sustainable agriculture (Cupina *et al.* 2004).

The Forage Crops Department of the Institute of Field and Vegetable Crops in Novi Sad remains the only institution in Serbia involved in breeding annual forage and grain legumes, producing 16 registered cultivars of pea and common (*Vicia sativa* L.), Hungarian (*Vicia panonnica* Crantz) and hairy (*Vicia villosa* Roth) vetches in Serbia and abroad (*Table 1*).

Research on annual forage and grain legumes is being carried out together with the Faculty of Agriculture in Novi Sad and also within projects at a national level, funded by the Ministry of Science and Environmental Protection of Serbia, and within international programmes, such as Grain Legume Technology Transfer Project (GL-TTP), which is an extension of the Grain Legume Integrated Project (GLIP), co-founded by the European Commission FP6 Framework Programme.

Table 1: The cultivars of annual forage and grain legumes developed in the Institute of Field and Vegetable Crops in Novi Sad, Serbia (Mihailovic *et al.* 2005c)

Species and type	Name	Year and Country of Registration
Winter forage pea	NS-Dunav	1977, Serbia
	NS-Pionir	1977, Serbia
Spring dual-purpose pea	NS-Junior	1992, Serbia
	NS-Lim	1992, Serbia
Spring feed pea	Moravac	1994, Serbia
	Jezero	1995, Serbia; 2005, the Ukraine
	Javor	2002, Serbia; 2005, the Ukraine
Winter common vetch	Novosadska 624	1967, Serbia
	NS Sirmium	1979, Serbia
	Kadmos NS	1999, Greece
	Neoplanta	2005, Serbia
Spring common vetch	Novosadska 5590	1967, Serbia
	Beograd	1970, Serbia
	Novi Beograd	1997, Serbia
Winter Hungarian vetch	NS Panonika	1979, Serbia
Winter hairy vetch	NS Violeta	1979, Serbia

ANNUAL FORAGE AND GRAIN LEGUMES COLLECTION

All breeding programmes are based upon the Annual Forage and Grain Legumes Collection (AFGLC), with more than 1,400 accessions of 16 genera and 67 species. The most numerous genera are *Pisum* L., with 568 accessions, *Vicia* L., with 495 accessions, *Lupinus* L. with 164 accessions and *Lathyrus* L., with 84 accessions, while the most numerous species are pea, with 555 accessions, and common vetch, with 287 accessions. Most of the accessions have entered the collection by exchange and donations, while others have been included by selection from hybrid populations, collections of wild populations and landraces and through the purchase of cultivars. The collection is maintained as a field collection at the experimental field of the Institute of Field and Vegetable Crops at Rimski Šancevi, near Novi Sad, on a slightly carbonated chernozem soil. Basic information on the accessions is contained in the AFGLC Passport Database. All activities related to the sustainable utilisation of AFGLC, especially involving breeding and the development of new cultivars, are based upon characterisation and evaluation of its accessions.

BREEDING FOR YIELD

One of the two major breeding programmes in the Forage Crops Department is aimed at the development of new winter and spring cultivars for forage, which would also be suitable for utilisation as green manure. Such cultivars should produce high yields of forage, (i.e. more than 45 t ha^{-1} of green forage and 9 t ha^{-1} of hay in pea and more than 35 t ha^{-1} of green forage and 8 t ha^{-1} of hay in common vetch), with a content of crude protein in the forage dry matter of about 200 g kg^{-1} in pea and about 220 g kg^{-1} in common vetch. Variation in forage yields between years should not exceed 20% and the production of reliable yields of seed is an additional objective. It is desirable that new cultivars for forage should have increased plant height, a moderate number of stems and a high proportion of leaves, either by producing a large number of internodes or by introducing genes for the acacia leaf type (*tl*), in which all tendrils are transformed into leaflets (Mihailovic and Mikic 2004). The most frequently used methods in breeding for yield of forage are individual selection in wild populations and land-races and pedigree and bulk methods in hybrid populations.

The most recent results of this breeding programme are new lines of pea, with L-535 having an increase of yield of both fresh weight and hay of 7%, as compared to the control cultivar NS-Pionir; common vetch, with L-386 and L-387 producing seed yields more than 10% higher than the control cultivar Neoplanta; Hungarian vetch, with G-411, and hairy vetch, with G-410, characterised by more determinate and uniform growth and increased harvest index (*Table 2*). All these cultivars are included in the network of field trials for registration by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia.

Table 2: Average yields of forage of the new cultivars developed in the Institute of Field and Vegetable Crops in Novi Sad, Serbia

Species	Name	Yield of green forage (t ha^{-1})	Yield of hay (t ha^{-1})	Yield of seed (kg ha^{-1})
Pea	L-535	46.2	9.7	1904
Common vetch	L-386	38.1	8.7	1312
	L-387	37.4	8.7	1336
Hairy vetch	G-410	39.7	9.4	984
Hungarian vetch	G-411	29.4	6.5	1141

The goal of the second major breeding programme is the development of new spring and winter cultivars of pea with high yields of grain, meaning more than 4500 kg ha^{-1}, and a content of crude protein in the dry matter of grain of about 250 g kg^{-1}, a thousand seed weight of between 150 g and 200 g and a growing period that ends before the harvest of winter wheat. There are two ways to increase grain yield, and these can be combined with each other. One way is to bring yield components into an optimal relationship, together with determinate growth of the stem to ensure uniform maturity. Another way is to introduce genes for morphological traits such as short internodes (*le*), afila type of leaf (*af*), fasciation of stem (*fa fas*) and strong development of funiculus (*def*), in order to promote yield. In addition to pea, the breeding programme for grain yield also includes faba bean (*Vicia faba* L.). The breeding techniques used to increase grain yield are individual selection and pedigree and bulk methods.

The new lines for fodder of pea L-536, L-537 and L-538 had 5% higher grain yields and a shorter growing period in comparison with the control cultivars Jezero and Javor. The first Serbian lines of faba beans for fodder have shown better preliminary results than the control cultivars from the national list (*Table 3*).

An additional breeding programme, sharing many elements with the previous two, is the development of winter and spring dual-purpose cultivars of pea and common vetch, characterised by high yields of forage, increased grain yield and reduced content of anti-nutritive factors in grain (Mihailovic *et al.* 2005b). The most recent results of this programme include the winter lines of pea L-013, L-015 and L-016, the spring lines of pea P-342, P-823 and P-824 and the spring lines of common vetch L-544, L-719, L-726 and L-749.

Table 3: Average yields of grain of the new cultivars developed in the Institute of Field and Vegetable Crops in Novi Sad, Serbia

Species	Name	Grain Yield (kg ha^{-1})
Pea	L-536	5143
	L-537	5279
	L-538	4908
Faba bean	G-412	4337
	G-413	4443

BREEDING FOR TOLERANCE TO BIOTIC AND ABIOTIC STRESS

Breeding for tolerance to pea weevil (*Bruchus pisorum* L.), the most important pest in pea, is based upon hybridisation between the common pea and the red-yellow pea (*Pisum fulvum* Sm.), with the second one as the pollen donor (Byrne *et al.* 2004). Its goal is the development of either resistant or tolerant lines from hybrid populations, serving for further improvement of the susceptible cultivars with great potential for increasing grain yield.

The development of winter cultivars is closely linked to tolerance of low temperatures, while breeding for drought tolerance is an integral part of the programmes aimed at the development of spring cultivars. Growing most species of lupins (*Lupinus* spp.) on the soil types such as chernozem is not possible without incorporating tolerance of alkaline conditions and high calcium content (Mihailovic *et al.* 2005a).

(RE)INTRODUCTION OF NEGLECTED AND NEW SPECIES

Although once well-known crops such as lentil (*Lens culinaris* Medik.), grass pea (*Lathyrus sativus* L.), bitter vetch (*Vicia ervilia* (L.) Willd.) and faba bean are nearly forgotten in Serbia today. Preliminary results of evaluating lentil, grass pea and bitter vetch for yields of forage and grain show a considerable potential of these species and a sufficient reason for their revival. In the same way, rather encouraging results were obtained in the testing of species such as pigeon pea (*Cajanus cajan* (L.) Millsp.), white (*Lupinus albus* L.) and narrow-leaved (*Lupinus angustifolius* L.) lupins, Narbonne vetch (*Vicia narbonensis* L.) and cowpea (*Vigna unguiculata* (L.) Walp.), which have been previously unknown in Serbia (Mihailovic *et al.* 2005c).

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New annual pasture legumes for Mediterranean conditions

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ABSTRACT

Subclovers and annual medics were commonly used for the improvement of low quality native pastures in southern Europe. Several cultivars of a second generation of annual pasture legumes are now commercially available. The question posed by pasture specialists is whether or not these alternative pasture legumes are really of interest to European farmers. Some of the traits considered important for ley farming systems do not seem so relevant for the establishment of permanent pasture in Mediterranean areas. Preliminary results on the evaluation of these alternative pasture species in Italy are conflicting. Pure swards of several new Australian varieties have shown deficiencies in establishment and re-establishment and lower forage production compared to subterranean clover and burr medic.

Keywords: annual legumes, Mediterranean pastures, pasture legume biodiversity, persistence

INTRODUCTION

Spontaneous annual legumes are an important component of Mediterranean pastures and their survival is strictly linked to their self-reseeding ability. We will refer to annual pasture self-regenerating legumes that show the adaptive advantage of having an annual cycle combined with a „seed escape“ habit. To cope with a high degree of environmental variability, both climatic and pedologic, under the ancient tradition of grazing utilisation, Mediterranean self-reseeding legumes have developed specific strategies to ensure adaptation and reproduction. Complex biological and ecological mechanisms involving seed yield and soil seed bank dynamics allow long-term regeneration of perennial-like stands. These species were introduced to other Mediterranean-type areas, and their naturalisation and diffusion had a remarkable impact on the new environments, particularly in Australia where annual legumes have effectively contributed to sustaining and increasing cereal and animal production. Moreover, the Mediterranean seed market for annual pasture legumes has been heavily reliant in recent decades on the Australian selections from germplasm often originating from the Mediterranean basin.

ANNUAL SELF-RESEEDING LEGUMES AND FARMING SYSTEMS

Annual self-reseeding pasture legumes are the key component of the ley farming and 'phase farming' system supporting wheat/wool industry on millions of hectares in southern Australia. In the last thirty years the annual self-reseeding legumes, mainly subclovers and annual medics, have also been increasingly utilised in Mediterranean Europe for the improvement of low quality native pastures in agro-silvopastoral and agropastoral systems. However, commercial varieties of annual self-reseeding legumes imported from Australia have sometimes proved unsuitable for pasture improvement in southern Europe, mainly due to the different climatic conditions and management systems. These reasons have stimulated different European research institutes to carry out eco-geography studies and selection programmes aimed at the evaluation of local germplasm. In Spain and Italy seven and five varieties of *Trifolium subterraneum* L. sensu latu (subterranean clover) respectively were selected in the 1980s and 90s. New varieties of *Medicago polymorpha* L. (burr medic) were also selected in Italy and France. Unfortunately, despite these new varieties proving to be superior to Australian ones, the lack of European seed multiplication has not allowed their diffusion, except for a few Italian varieties of subclovers multiplied in Australia.

A SECOND GENERATION OF PASTURE LEGUMES

Despite the unequivocal success of southern Australian ley-farming systems, several contemporary factors have challenged their sustainability. This led to a serious re-examination of the pasture legume components required for contemporary ley farming, which resulted in the recognition that alternative legume species with different traits were probably required (Loi *et al.* 2005). Traits sought in the new species are deeper root systems, improved persistence from higher production of hard seed, acid tolerant rhizobial symbioses, tolerance of pests and diseases and ease of harvesting with conventional cereal harvesters. Several cultivars of a second generation of annual pasture legumes are now commercially available, e.g. biserrula (*Biserrula pelecinus* L.), French serradella (*Ornithopus sativus* L.), gland clover (*T. glanduliferum* Boiss) and improved varieties of arrowleaf clover (*T. vesiculosum* Savi.), balansa clover (*T. michelianum* Savi.), Persian clover (*T. resupinatum* L.) and yellow serradella (*Ornithopus compressus* L.), and have been rapidly adopted. Some other species are in an advanced phase of evaluation and are close to being released as commercial cultivars, e.g. *T. formosum* d'Urv. (eastern star clover), *T. spumosum* L. (bladder clover), *T. hirtum* All. (rose clover), *Melilotus albus* Medik (white sweet-clover), *Trigonella balansae* Boiss. and Reuter (*Trigonella* sp.) and *Lotus ornithopodioides* L. (annual birdsfoot trefoil). The availability of a wider range of species for Mediterranean agricultural systems in Australia is of benefit to farmers there, who can thus choose from a wider range of species with different characteristics. The question posed by pasture specialists is whether or not these alternative pasture legumes are really of interest to European farmers. Some of the traits considered important for ley farming systems do not seem so relevant for the establishment of permanent pasture in southern Europe, e.g. insect tolerance, high hardseededness. Preliminary results on the evaluation of these alternative pasture species in the Mediterranean basin are conflicting (Campiglia *et al.* 2005, C. Porqueddu, unpublished data). Pure swards of several new Australian varieties have shown deficiencies in establishment and re-establishment, and lower forage production compared to subterranean clovers that produce higher levels of soft seed and consequently are able to compete against the native flora, usually richer than the Australian ones.

USE OF LEGUMES IN MIXTURES

Traditionally, pasture seed mixtures available in southern Europe consisted of a small number of legume species or 4-5 subclover varieties mainly differing in earliness, and sometimes including low rates of annual grasses such as *Lolium rigidum* Gaudin (annual ryegrass), *Lolium multiflorum* Lam. (Italian ryegrass), *Avena sativa* L. (common oat). Nowadays, it is common to find complex mixtures including up to 20 annual self-regenerating legumes. In some cases eg. pasture improvement in areas with higher rainfall, perennial grasses, such as *Dactylis* *glomerata* L. (cocksfoot), *Phalaris aquatica* L. (bulbous canarygrass), *Festuca arundinacea* Schreber (tall fescue) are utilised in grass-legume mixtures (PERMED 2005). The compatibility of perennial grasses and annual legumes when grown in mixtures has been reviewed by Dear and Roggero (2003), who discussed particularly the effects of perennial grasses on emergence, survival and seed set by annual legumes. It is very important to determine the compatibility of the species to be utilised in the mixtures. According to several authors subclover used in mixture with annual medics and *T. michelianum* tends to dominate from the second year of establishment (Porqueddu *et al.* 2004). Advantages may be achieved by combining seeds of different size (small and large seeds), hardseedness levels and softening patterns with the aim of ensuring long term pasture regeneration with production levels sufficient to reduce inter- and intra-annual fluctuations. Moreover, every species colonises a different ecological niche, so that the inclusion and ideal combination in mixture of new pasture legumes with traditional species could be used to cope better with unpredictable climatic fluctuations and the extremely high heterogeneity of environmental and farming system situations in the Mediterranean area.

CONCLUSIONS

Because farmers in low-input systems cannot afford expensive seed, increased ease of seed harvesting is an essential trait for the development of new pasture legume species. Moreover, the ease of harvesting may play a major role in their adoption because seed production can be achieved locally on the farm scale with conventional cereal harvesters. This may require the evaluation of new native pasture legumes with different traits to those possessed by the currently commercial materials, e.g. legumes with erect habit that set heads on the top of the plants. Simultaneously, the successful development of a European seed industry for Mediterranean pasture species is needed to take advantage of the selection activities carried out by several public research institutions. On the other hand, more efforts in on-farm experimentation and knowledge transfer to farmers about the correct incorporation and management of annual legumes is necessary for their full exploitation.

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Frost resistance and photosynthesis during cold acclimation in Mediterranean annual legumes

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ABSTRACT

This paper summarizes our recent findings about the capacity for cold acclimation and frost resistance in annual legumes native to the Mediterranean region under controlled conditions. Plants from subterranean clover (*Trifolium subterraneum* L. ssp. *brachycalycinum* cv. Clare), balansa clover (*T. michelianum* Savi cv. Giorgia), burr medic (*Medicago polymorpha* L. cv. Anglona) and barrel medic (*M. truncatula* Gaertn. cv. Paraggio) were cold acclimated by growing them at 10/5 °C. Non-acclimated plants developed at 20/15 ºC d/n. In general, the activities of sucrose synthesis pathway were not related to freezing tolerance in a simple way. As a consequence, sucrose did not seem to be the main sugar involved in frost resistance in these species. It might be more related to the accumulation of cryoprotective solutes (soluble sugars and proline) and starch in roots as a carbohydrate reserve for regrowth.

Keywords: cold acclimation, freezing tolerance, photosynthesis, regrowth, sucrose synthesis

INTRODUCTION

The present work summarizes our findings concerning:

1) the ability of annual legumes to maintain or increase its photosynthesis during their development at moderately low temperatures (10 ºC),
2) the biochemical changes that occur during cold-acclimation,
3) the potential agricultural use of frost tolerant legumes by screening for their freezing tolerance under laboratory conditions, and
4) the possible mechanisms for freezing tolerance in the selected analyzed species.

MATERIAL AND METHODS

Plant material and growth conditions

Seedlings from subterranean clover (*Trifolium subterraneum* L. ssp. *brachycalycinum* cv. Clare), balansa clover (*T. michelianum* Savi cv. Giorgia), burr medic (*Medicago polymorpha* L. cv. Anglona) and barrel medic (*M. truncatula* Gaertn. cv. Paraggio) were grown in a greenhouse at 20/15 ºC, 80/90% RH (day/night) and illuminated for 11h/day with a minimum photosynthetic photon flux density (PPFD) of 400 µmol m^{-2} s^{-1}. When five leaves had fully expanded, half of the plants were cold-acclimated by moving them to 10/5 °C day/night temperature regime (cold-acclimated plants, CA). Control plants remained at 20/15 ºC, in the same conditions as the initial growth (non-acclimated plants, NA).

Plant determinations during cold acclimation

Net CO_2 assimilation rate and Chl *a* fluorescence induction kinetics were measured at 10ºC as described by Antolín *et al.* (2005). Stromal and cytosolic fructose-1,6-bisphosphatase (sFBPase) (EC 3.1.3.11) activities were analysed at 25 ºC by measuring NADPH production at 340 nm (Holaday et al. 1992) with the modifications described by Pérez et al. (2001). Sucrose phosphate synthase (SPS) (EC 2.4.1.14) was assayed at 25ºC with saturating substrates by measuring sucrose production at 620 nm (Guy *et al.* 1992). Finally, roots were collected to determine some solutes after cold acclimation (Hekneby *et al.* 2006).

Mechanisms for freezing tolerance

Freezing tolerance was determined by measuring the lethal temperature of 50% of the population (LT50). Frost injury was evaluated after regrowth for four weeks at initial growth

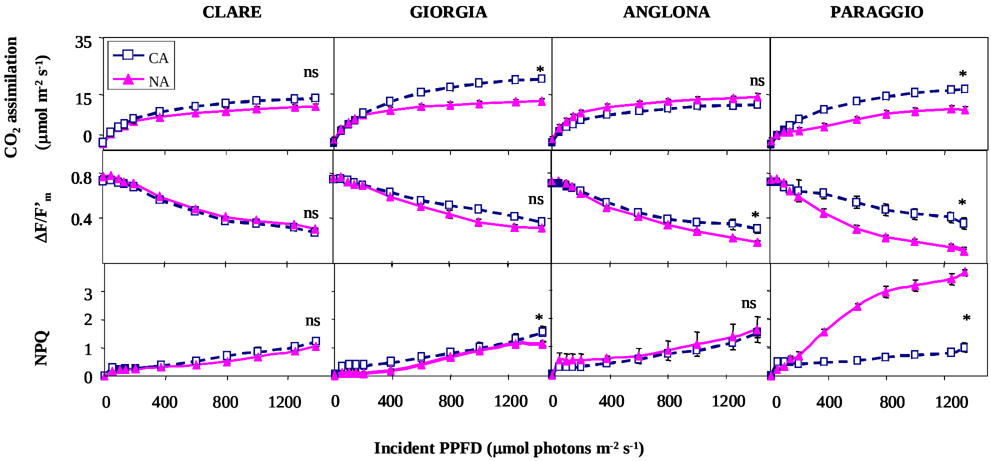

Figure 1: Light response curves of the net CO_2 assimilation rate, photochemical efficiency of PSII (ΔF/F´m) and non-photochemical quenching (NPQ) in leaves of different annual legume species. Measurements were made at ambient CO_2 (330 µmol mol^{-1}) and 10 °C on non-acclimated (NA) and cold-acclimated (CA) leaves (adapted from Antolin *et al.* 2005. *Photosynthetica* 43: 65-74).

Table 1: Maximal photochemical efficiency (F_v/F_m), maximum rate of carboxylation by Rubisco (V_{cmax}) and electron transport capacity (J_{max}) estimated from CO_2-response curves of photosynthesis in different annual legume species. Values are means of 6 determinations. Within each column, means followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test.

Species	Treatments	F_v/F_m	V_{cmax}(µmol m^{-2} s^{-1})	J_{max}(µmol m^{-2} s^{-1})
Clare	NA	0.77 a	24.8 bc	83.5 c
	CA	0.82 a	35.0 bc	119.3 bc
Giorgia	NA	0.82 a	32.4 bc	83.5 c
	CA	0.81 a	95.9 a	328.8 a
Anglona	NA	0.78 a	44.5 b	123.8 b
	CA	0.80 a	29.6 bc	110.8 bc
Paraggio	NA	0.80 a	12.9 c	41.7 d
	CA	0.80 a	23.2 bc	64.3 cd

conditions (20/15ºC, d/n). Finally, a thermal analysis was performed to study the dynamics of ice formation in plant tissues.

RESULTS AND DISCUSSION

Cold acclimation of photosynthesis in Mediterranean annual legumes seemed to be mediated by a multifactorial mechanism. The first response was the enhancement of photosynthesis coupled with high rates of carboxylation by RuBPCO (V_{cmax}) and PPFD-saturated rate of electron transport (J_{max}) (*Figure 1*, *Table 1*) (Hurry *et al.* 1998, Antolín *et al.* 2005). Secondly, there was an increase in NPQ, which reflected a greater extent of thermal dissipation of excitation energy (*Figure 1*). This could be an important mechanism to avoid photoinhibition damage under long-term exposure to low temperatures (Germino and Smith 2000). Thirdly, there was an increase in the activity of a key enzyme for RuBP regeneration, sFBPase, which could lead to increased TCA cycle functioning for starch synthesis (*Figure 2*), and finally, there was a high capacity for sucrose synthesis (Strand *et al.* 2003). In the present study, the enzymatic activities of the sucrose synthesis pathway were not related to freezing tolerance in a simple way because there was a general decrease of SPS in cold-treated plants (*Figure 3*).

Table 2: Lethal temperature for 50% of population (LT_{50}), ice nucleation and freezing temperature in different annual legumes grown at 20 °C (non-acclimated, NA) and at 10 °C (cold-acclimated, CA). Values are means (n = 15). Otherwise as for *Table 1*.

Cultivars	Treatments	Nucleation temperature (ºC)	Freezing temperature (ºC)	LT_{50} (ºC)
Clare	NA	-6.5 bc	-3.3 a	-5.5 a
	CA	-6.7 bc	-4.4 b	-7.8 b
Giorgia	NA	-6.0 ab	-3.4 a	-5.5 a
	CA	-7.6 d	-4.7 b	-8.3 c
Anglona	NA	-7.5 d	-4.5 b	-5.5 a
	CA	-7.5 d	-4.7 b	-8.8 d
Paraggio	NA	-5.4 a	-3.4 a	-5.5 a
	CA	-7.1 cd	-4.6 b	-7.8 b

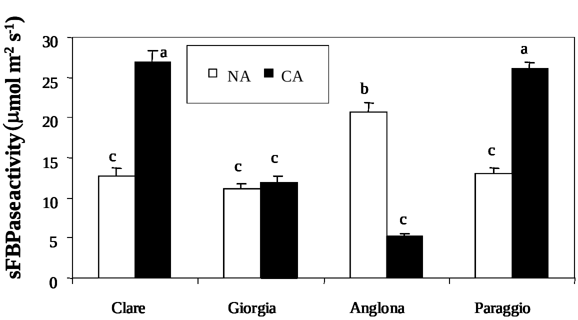

Figure 2: Stromal fructose-1,6-bisphosphatase activity in non acclimated (NA) and cold-acclimated (CA) leaves of different legume species. Values are means (n = 6). Different letter indicates significant differences ($p < 0.05$) according to Tukey´s test (Antolin *et al.* 2005. Photosythetica 43: 65-74).

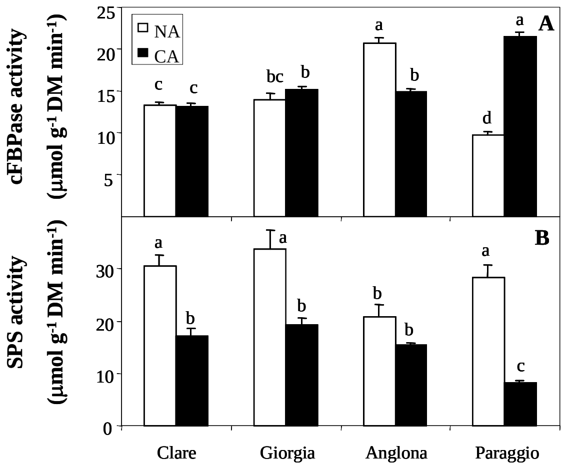

Figure 3: Cytosolic fructose-1,6-bisphosphatase (A) and sucrose phospahte syntase (B) activities on leaves of non-acclimated (NA) and cold-acclimated (CA) plants of different annual legumes. Values are means (n = 5). (Hekneby *et al.* 2006. *Environ. Exp. Bot.* 55: 305-314).

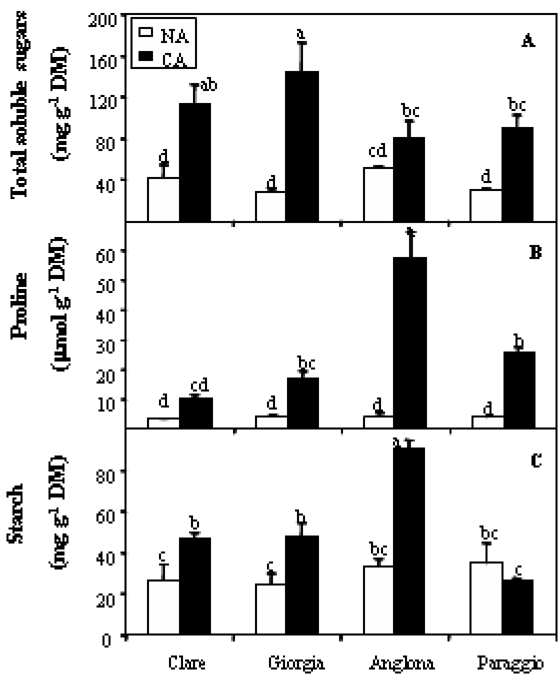

Figure 4: Root solutes of non-acclimated (NA) and cold-acclimated (CA) plants of different annual legumes (Hekneby *et al.* 2006. *Environ. Exp. Bot.* 55: 305-314).

Thus, sucrose seemed not to be the main sugar involved in cold acclimation of these species, although a significant contribution of sucrose in the total soluble sugar pool translocated to roots cannot be excluded.

In roots, cold acclimation induced the accumulation of solutes, such as soluble sugars, proline and starch (*Figure 4*). This process could contribute to the improved frost tolerance (measured as LT50) detected in all cultivars (*Table 2*) (Hekneby *et al.* 2006). Results of thermal analysis suggest different mechanisms during ice formation in CA plants for freezing survival: 1) freezing avoidance by moderate supercooling (in cvs. Giorgia and Paraggio), and 2) cellular capacity to tolerate freezing (in cvs. Clare and Anglona). Both strategies might improve plant survival and regrowth after exposure to sub-zero temperatures (*Figure 5*). It can be concluded that sucrose did not appear to be the main sugar involved in frost resistance in

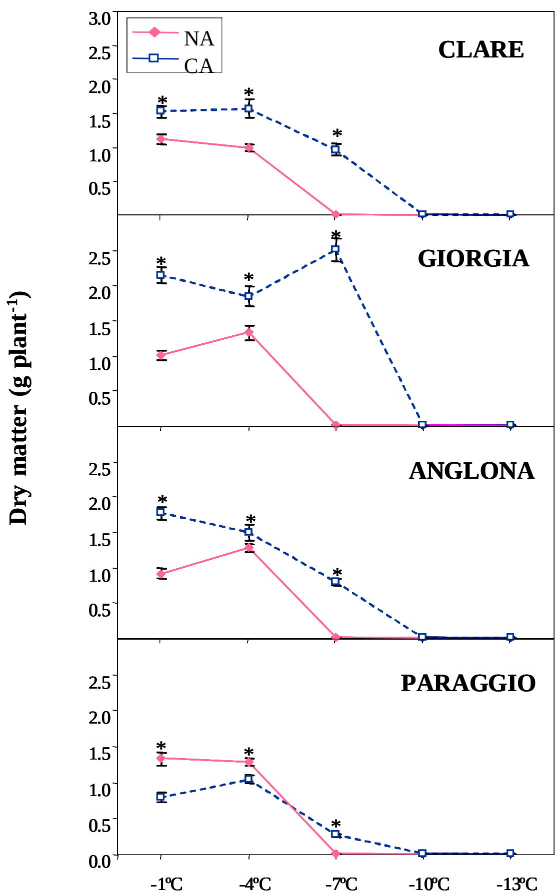

Figure 5: Dry matter production in non-acclimated (NA) and cold-acclimated (CA) plants after regrowth. Values are means (n = 15). The asterisks indicate significant differences (p = ≤ 0.05) between treatments within each species. (Hekneby *et al.* 2006. *Environ. Exp. Bot.* 55: 305-314).

Mediterranean annual legumes. Freezing tolerance and the potential for production of forage mass after freezing in these species seems to be strongly dependent on the accumulation of cryoprotective solutes and starch in roots as a carbohydrate reserve for regrowth.

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Biomass production and unsown species control in rainfed grass-legume mixtures in a Mediterranean environment

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ABSTRACT

The experiment, carried out in North Sardinia (Italy), investigates seasonal and annual patterns of species contribution to yield and unsown species control of different grass-legume mixtures within the common activity of the COST Action 852. Thirty plots were sown in 2002 with pure stands and 4-species mixtures of grass/legume and fast/slow establishing species in different proportions following a simplex design. Data refers to 16 cuts taken between January 2003 to May 2006. Average dry matter yield ranged from 3 to 7 t ha^{-1} in 2003 and 2006 respectively. Biomass distribution along the seasons was markedly improved by perennials: lucerne during summer and cocksfoot mainly in autumn. Within 3,5 years a complete shift in the contribution of sown species occurred, with fast establishing species contributing up to 80% in the first cuts to their complete disappearance and perennial species dominance in the last cuts. Results suggest that the use of mixtures of species of different functional types increases productivity and decreases unsown species growth.

Keywords: grass-legume mixtures, functional groups, simplex design, overyielding

INTRODUCTION

Enhancing sward productivity by exploiting the synergies existing among plant functional groups as an alternative to high artificial inputs is one of the challenges faced by agricultural research. An optimal proportion of grass/legumes and annual self-reseeding/perennials, can favour species' establishment and persistence. Grass-legume mixtures may also have the advantage of stabilizing yield over the growing season, which may be more important than achieving high yields, especially in Mediterranean rainfed conditions. The present paper investigates seasonal and annual patterns of species contribution to yield and unsown species control in grass-legume mixtures with different sown proportion of each species.

MATERIALS AND METHODS

Thirty plots (3 x 3 m) were sown in October 2002 at the Ottava Research Station (Sardinia, Italy), with average annual rainfall of 550 mm, calcareous soil (pH = 7.5) and typically Mediterranean climate. The species used in the experiment represent four functional groups: grass (G), legume (L), fast-establishing (subscript 1) and slow-establishing (subscript 2) species. The sown species were *L. rigidum* cv Nurra (G_1), *Dactylis glomerata* cv Currie (G_2), *M. polymorpha* cv Anglona (L_1) and *M. sativa* cv Mamuntanas (L_2). The plots include pure stands and 4-species combinations, ranging from dominance by one or by two species to total evenness (centroid = equal contribution of all mixture components), following a 'simplex design' (Cornell 1990) duplicated at two sowing densities (high density = 35 kg ha^{-1}, low density = 60% of high density). Herbage samples were taken from 4 fixed quadrats per plot on 16 cuts taken between January 2003 and May 2006. Total yield and the contribution of each component of the mixture to total yield were computed. The effect of mixing functional groups was tested by comparing the yield and resistance to unsown species at the centroid with that at the pure stands. The same responses were also studied through multiple regression in a model-based approach, in order to detect synergisms between the components of the mixture.

RESULTS AND DISCUSSION

1. General trends: average of all treatments

Along the 4 years biomass production was characterized by a strong seasonality, with spring and autumn peaks of production (reaching 4500 kg ha^{-1} in May 2004) and summer slumps of less than 500 kg ha^{-1} (*Figure 1*). Also between-year variations were marked, with a general increase of total biomass from 3000 to 7100 kg ha^{-1} in the first and third year respectively. As expected the contribution of L_1 and G_1 was high in the first year and then decreased progressively (*Figure 2*). This was linked to a marked reduction at re-establishment in seedling density of annuals from autumn 2003 (280 seedlings m^{-2}) to autumn 2005 (30 seedlings m^{-2}) as a result of competition with perennials. Regarding seasonal distribution in summer the highest contribution was given by L_2, in autumn mainly by G_2.

2. Comparing mixtures

In terms of sown species biomass along the years, mixtures outperformed pure stands, apart from L_2 pure stand (*Figure 3*), which was characterized by a high and steadily increasing biomass production. The increase in production over the years was mainly due to the increase of L_2 and G_2 proportions. Since the second year, plots dominated by perennials controlled better unsown species.

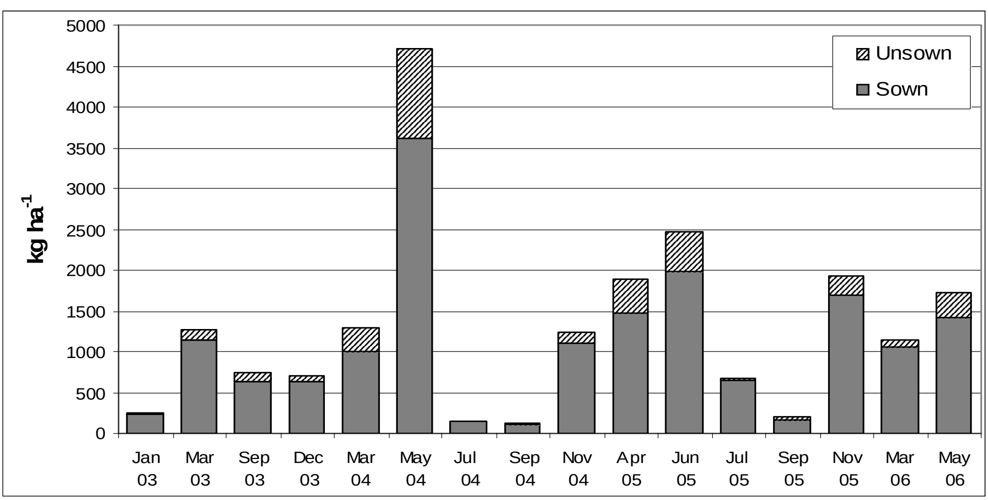

Figure 1: Total dry matter (kg ha^{-1}) of sown and unsown species in each cut during the trial (2003-2006) as an average of all treatments

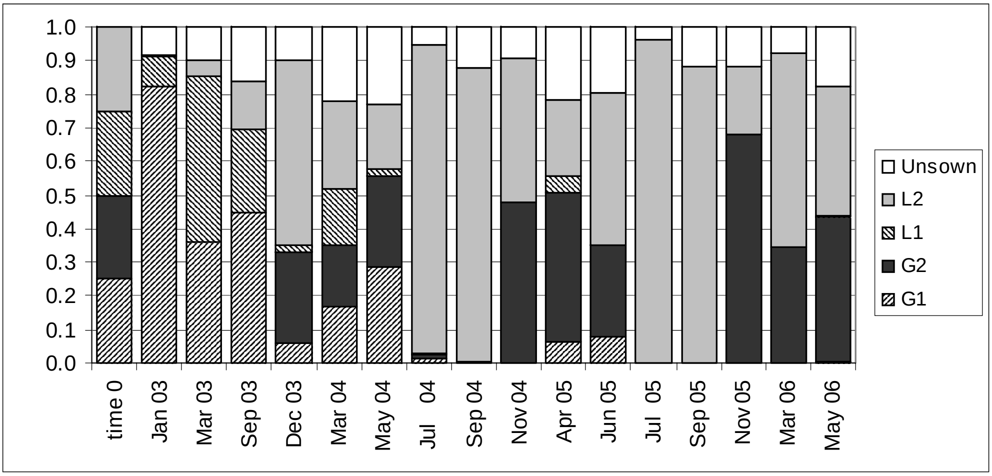

Figure 2: Species contribution to yield (proportion) in each cut during the trial (2003-2006) as an average composition of all treatments

In most seasons increasing evenness, expressed as a measure of the distribution of the relative abundance of species in a community, in the mixture (Kirwan *et al.* 2007) reduces unsown species biomass. Overyielding and transgressive overyielding for annual means of biomass production and unsown species control are reported in *Table 1* for each year.

3. Modelling total yield and unsown species yield

For both response variables (total yield nor unsown species yield), sowing density was never significant. Total yield was affected in 2004 and 2005 by synergistic interaction effects between G_2 and L_1, possibly due to the positive effect of L_1 nitrogen fixation in the first year on the subsequent biomass production of G_2. The asynchrony of growth cycles of G_1 and G_2 in the first year may explain their positive individual interaction effect on unsown species control. In the second and third year, G_2xL_1 and G_2xL_2 interactions reflect the importance of G_2 in unsown species control and the positive grass-legume and inter fast-slow functional group interactions also detected these same years.

The positive effect of evenness on unsown species control in the second and third year was shown by a positive and significant estimate of the parameter evenness in the equations ($P < 0.05$). Best models were selected through F test, showing a maximum fit of $R^2 = 0{,}75$ of

Table 1: Percent change of sown and unsown species of the centroid *vs* a) the average of pure stands (overyielding) and b) the best pure stand (transgressive overyielding)

	a) Overyielding		**b) Transgressive overyielding**	
Year	Sown yield	Weed yield	Sown yield	Weed yield
2003	49	-42	20	-47
2004	159	-92	5	-68
2005	196	-99	8	-59
2006	148	-98	-12	-97

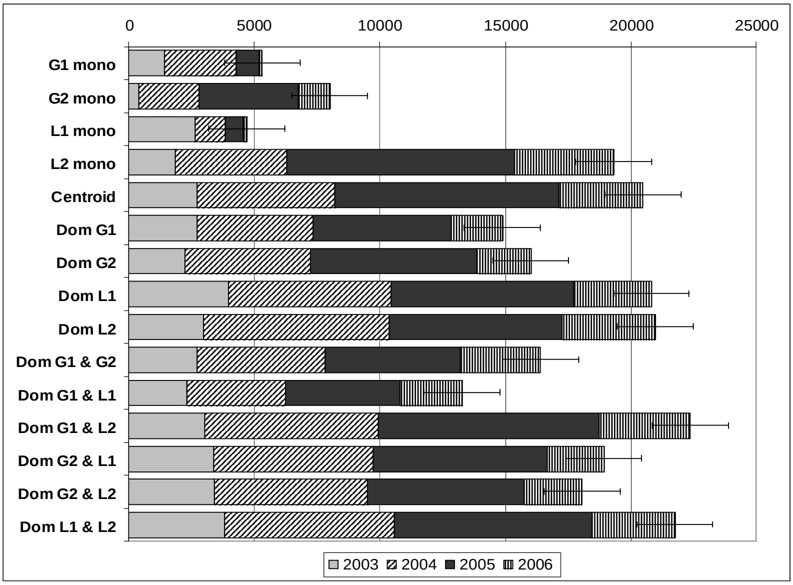

Figure 3: Dry matter yield of sown species (kg ha^{-1}) of each mixture type in each year of trial (2003-2006)

the observed vs predicted values. This suggests the need to further test the models by replacing 'sown proportions' with other 'species proportions' taken at a different point in time.

CONCLUSIONS

In Mediterranean conditions, grass-legume mixtures of species belonging to different functional groups, compared to pure stands proved to: increase productivity and unsown species control, improve seasonal distribution of biomass and soil covering rates. Perennial species in association with annuals play a fundamental role in Mediterranean rainfed low input farming systems. The asynchrony of the growth cycles of Fast/Slow establishing species and Grasses/ Legumes reduces between-year and within-year biomass variations.

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Performance of several forage legumes submitted to different management systems

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ABSTRACT

The objective of this study was to compare the performance of different forage legume species submitted to three different management systems, i.e. grazing, 3 cut and 5 cut system. Depending of the different legume species and management system differences in agronomic performance are expected. White clover, red clover, lucerne and birdsfoot trefoil were established as binary swards with perennial ryegrass. Results of DM yield, N yield and botanical composition of the different legume/grass-swards in 2004 and 2005 are presented. The white clover/grass-swards showed a significant higher performance regarding the DM yield (1059 g DM m^{-2}) under grazing in comparison to all other legume/grass-swards (red clover: 843 g DM m^{-2}, lucerne: 882 g DM m^{-2}, birdsfoot trefoil: 854 g DM m^{-2}). Comparing the DM yield within the two cutting systems, no significant differences were observed, except for birdsfoot trefoil/grass-swards, which had a significant lower DM yield compared to white clover. These results suggest that different legume species can provide high yields in comparison to white clover under cutting systems, while the lower yields of the different legume species in comparison to white clover may limit their utilization in grazing systems.

Keywords: DM yield, forage legumes, grazing, cutting

INTRODUCTION

White clover (*Trifolium repens* L.) is the most important forage legume in legume based swards, because of its persistence under grazing and its high feeding value. However, longer summer droughts and lower DM yield compared to other forage legumes may be disadvantageous. Although several studies investigated possible alternative forage legumes to white clover, there is a lack of studies, which examined simultaneously different forage legumes submitted to different management systems. For this reason the objective of this study was to compare the performance of different forage legume species submitted to three different management systems (grazing, 3 cut system, and 5 cut system). Depending of the different legume species and management system differences in DM yield, N yield and botanical composition are expected.

MATERIAL AND METHODS

White clover, red clover (*Trifolium pratense* L.), lucerne (*Medicago sativa* L., grazing type) and birdsfoot trefoil (*Lotus corniculatus* L.) were established in the late summer of 2003 as well as 2004 as binary swards with perennial ryegrass (*Lolium perenne* L.) as a randomized split-plot design with three replicates. The experiment was carried out at the experimental farm „Lindhof" of the University of Kiel (Germany). The four mixtures will be abbreviated as WCG (white clover/perennial ryegrass), RCG (red clover/perennial ryegrass), LGG (lucerne/perennial ryegrass) and BTG (birdsfoot trefoil/perennial ryegrass). For the 3 cut system the legume/grass-swards were harvested either 4 times (2004) or 3 times (2005), i.e. the first cut in spring was at ear emergence of perennial ryegrass and afterwards at every 50±5 days, as described in the common protocol of COST 852. For the 5 cut system the legume/grass swards were harvested 5 times in 2004 as well as in 2005, i.e. the first cut was at detectable node of the grass and subsequent cuts after 30±3 days. For the grazing system, the legume/grass-swards were rotationally grazed 5 times/year in 2004 just as 2005, using „Limousin" heifers.

To obtain the DM yield, the fresh forage was weighted, dried (58 °C) and corrected for residual water content (drying, 105 °C). A subsample was separated into the fractions legume species, perennial ryegrass and weeds, afterwards dried and weighted to determine the botanical composition. After drying and milling (1 mm) bulk samples were analysed for their N content to calculate the annual N yield.

All data were submitted to analysis of variance and means were compared using LSD, while the white clover/perennial ryegrass swards are used as control (SAS, proc mixed). The probabilities were adjusted using the Bonferroni-Holm test.

RESULTS

For the annual DM yield and annual N yield the interaction species*system was significant. The results of the different legume/grass-swards under the different management systems are shown in *Table 1*.

Table 1: Total annual DM yield (g DM m^{-2}), (SE = 44.4) and N yield (g N m^{-2}), (SE = 1.25) of the different legume/grass swards

	3 cut system		5 cut system		grazing	
species/system	DM yield	N yield	DM yield	N yield	DM yield	N yield
WCG	805.5 ^{b}	18.6 ^{b}	718.2 ^{b}	21.9 ^{b}	1059.2 ^{a}	26.0 ^{a}
RCG	895.5 ^{a}	22.0 ^{a}	678.5 ^{b}	21.1 ^{a}	842.9 ^{*a}	24.8 ^{a}
LGG	877.6 ^{a}	23.8 ^{a}	712.6 ^{b}	25.0 ^{a}	882.1 ^{*a}	26.9 ^{a}
BTG	736.1 ^{a}	17.2 ^{a}	528.4 ^{*b}	14.1 ^{*a}	854.5 ^{*a}	15.7 ^{*a}

* significant different to WCG ($P < 0.05$) within system ^{a,b} significant differences between systems $P < 0.05$)

Under grazing the WCG produced a significant higher DM yield (1059.2 g DM m^{-2}) compared to all other legume/grass-swards (RCG 842.9 g DM m^{-2}, LGG 882.1 g DM m^{-2}, BTG 854.5 g DM m^{-2}). No significant differences between WCG and any other legume/grass-sward could be found for the 3 cut system. Considering the DM yields in the 5 cut system, BTG were the only legume species, which had a significant lower DM yield compared to WCG. Comparing the management systems within legume species, all legume/grass-swards produced a significant higher DM yield under grazing compared to the 5 cut system. No significant differences could be found when the 3 cut system and the grazing system were compared. An exception was WCG, which produced a lower DM yield in the 3 cut system compared to grazing. Regarding the N yield, BTG was the only sward, which had a lower N yield in the 5 cut (14.1 g N m^{-2}) and under grazing (15.7 g N m^{-2}) compared to WCG, with 21.9 g N m^{-2} and 26.0 g N m^{-2} respectively. Comparing the different management systems within the different legume/grass-swards, no significant differences could be found for any legume/grass-sward. An exception is WCG, which showed a higher N yield under grazing (26.0 g N m^{-2}) compared to the two cutting systems (3 cut with 18.6 g N m^{-2}, and 5 cut with 21.9 g N m^{-2}).

For the legume proportion of total annual DM yield, the interaction legume species* system*year was significant. In 2004 all legumes had a higher proportion in the cutting systems compared to WCG. In the grazing system only LGG (48.8%) showed a higher legume proportion compared to WCG (34.4%) (*Table 2*). Considering the cutting systems in 2005, no significant differences could be found in comparison to WCG, except BTG in the 5 cut system (13.0%). BTG had a lower legume proportion also under grazing (8.5%) compared to the WCG (29.0%). Regarding the different management systems within legume species in 2004, the grazing system decreased the legume proportion of all legume species significantly in comparison to the cutting systems, except WCG (*Table 2*).

Table 2: Legume yield (%) as proportion of total annual DM yield (SE = 4.2)

species/system	2004 3 cut	2004 5 cut	2004 grazing	2005 3 cut	2005 5 cut	2005 grazing
WCG	35.9 [a]	39.1 [a]	34.4 [a]	32.4 [b]	53.3 [a]	29.0 [b]
RCG	63.7 [*a]	65.1 [*a]	45.5 [b]	41.1 [b]	61.8 [a]	46.1 [*b]
LGG	64.8 [*a]	73.9 [*a]	47.8 [*b]	44.9 [b]	59.7 [a]	42.1 [*b]
BTG	54.1 [*a]	50.6 [*a]	26.6 [b]	20.5 [a]	13.0 [*a]	8.5 [*a]

[*] significant different to WCG ($P < 0.05$) within system [a,b] significant differences between systems ($P < 0.05$)

DISCUSSION

Differences in N yield between the different swards compared to WCG could be explained by the variable legume proportions. As shown in *Table 2*, BTG reached low legume proportions, especially in 2005, which resulted in significant lower N yields in the 5 cut system and the grazing system compared to the WCG ones. On the other hand a higher DM yield of WCG also resulted in a higher N yield. BTG is mentioned as a forage legume, which is not very easy to establish and is adapted to moderate grazing (Sheaffer *et al.* 1992, Brummer and Moore 2000). The results show a good performance of BTG in the 3 cut system.

CONCLUSION

BTG should be considered very critically as an alternative forage legume for WCG, as it showed a lower performance in the 5 cut and grazing systems compared to WCG. Because of their comparable performances, RCG and LGG can be used as alternatives to WCG especially in cutting systems. WCG showed the highest performance in the grazing system. The results suggest that different legume species can provide high yields in comparison to WCG swards under cutting systems, while the lower yields of the different legume species in comparison to WCG may limit their utilization in grazing systems.

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Spatial variation of yield and red clover content in organic mixed swards

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Keywords: *Trifolium pratense*, clover content, variation, variogram

ABSTRACT

It is known that the variation of yield amount and especially the clover content of a sward is high inside a field. The aim of this study was to determine the variation of the yield level and clover content of mixed swards and at the same time geostatistical methods were introduced for these purposes. In eastern Finland 100 or 105 plots of 15m² were placed inside two two-hectare fields with distances of 2-10 metres of each other. Plots were harvested twice in a growing season in 2004-2005. Quantity and clover content of yields were analysed by dry weight and the spatial variation of these characters was determined with model-based spatial interpolation. The spatial dependence was modelled with variogram by statistical programmes before interpolation. The variation of the yields of mixed sward was very high on both experimental fields. In Juva the range of dry matter yields was 1 600-3 900 kg ha^{-1} cut^{-1} and in Sotkamo 1 600-5 500 kg ha^{-1} cut^{-1}. According to the variogram, the spatial dependence vanished when the distance between field plots reached 50 metres. The clover contents of these swards varied 0-100% (dry matter) in both experimental fields and all cuts.

INTRODUCTION

In organic farming grasslands are typically red clover-timothy-tall fescue (*Trifolium pratense*, *Phleum pratense*, *Festuca arundinacea*) leys in Finland. Cropping of red clover based swards is becoming more common also in conventional farming due to its good characteristics. The clover content of a sward is an important factor in determining the optimal harvesting time of mixed swards (Rinne and Nykänen 2000) as well as in calculations of nitrogen balances and planning of animal feeding. It is known, that the variation of yield amount and especially the clover content of a sward is high inside a field (Gagnon *et al.* 2003). The quantity and speed of variation is not known. The aim of this study was to determine the variation of the yield level and clover content of mixed swards. At the same time geostatistical methods were introduced for these purposes. The results of the study help in choosing sampling places from the field for the yield and clover content determination.

MATERIALS AND METHODS

Two field experiments were conducted in eastern Finland, Juva (60°53''N 27°53''E) and Sotkamo (64°11''N 28°13''E). Juva fields had been under organic farming for over 20 years and Sotkamo fields started to be converted in the establishment year of the experiment in 2003. Swards were established with barley (*Hordeum vulgare*) as a nurse crop and fertilized with cow manure. 100 (Sotkamo) or 105 (Juva) plots of 10 x 1.5 m² were placed inside these two-hectare fields with distances of 2-10 metres of each other. The experimental plots were placed as evenly as possible around the fields to get a clear and the best possible view of the variation of the property under the study. It is essential for the spatial dependence (the distance, where measurements are not dependent on each other anymore) determination to have enough experimental plots on the field within various distances, which in these fields was between 2-10 meters. The field was firstly divided in bigger 30 m x 30 m sized squares and inside these squares, 3-5 smaller plots were placed. Groups of small plots were planned beforehand.

Plots were harvested twice in a growing season in 2004-2005. Yield samples were dried over night in 105°C for dry matter determination and dry yield calculation. Clover content was analysed with Near Infrared Reflectance Spectroscopy (NIRS) from dried and milled samples.

The variation of the measured variables in the fields was studied with model-based spatial interpolation (kriging). With this method it is possible to interpolate the value of the property under the study on whatever point of the field, although the measurement would originally have been made in a certain point. Compared to other interpolation techniques this method produces also a standard error in addition to a value itself. Standard error describes the accuracy of the predicted value. This is useful knowledge considering how densely the value of the variable should be measured to get a clear view of the whole field. Before the interpolation the spatial dependence was modeled with variogrammes. Statistical analyses were made with SAS-software and VARIOGRAM-, NLIN- and KRIGE2D procedures.

RESULTS

The variation of the yields of mixed swards was very high on both experimental fields. In Juva the range of dry matter yields was 1 600-3 900 kg ha^{-1} cut^{-1} and in Sotkamo 1 600-5 500

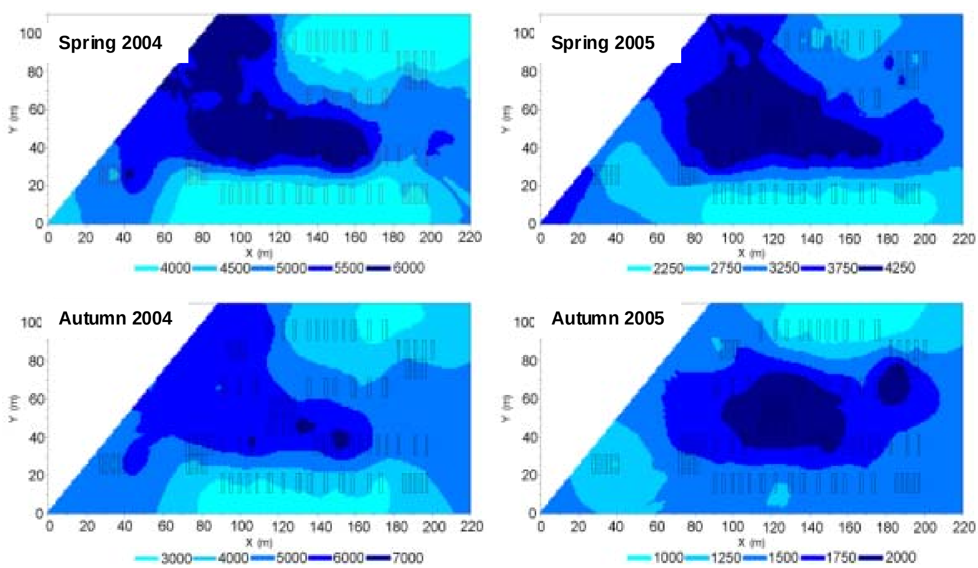

Figure 1: Spatial variation of yields (kg ha^{-1}, dm) of swards in Juva in 2004 and 2005

kg ha^{-1} cut^{-1}. The yield level diminished in Juva in the second production year, which was not observed in Sotkamo. In Juva, the highest yields in 2004 were found in slightly different spot than in 2005 (*Figure 1*). In 2005 an interesting phenomenon in some spots of Sotkamo field was, that the yield of the first cut was compensated of the second cut in a way that in some

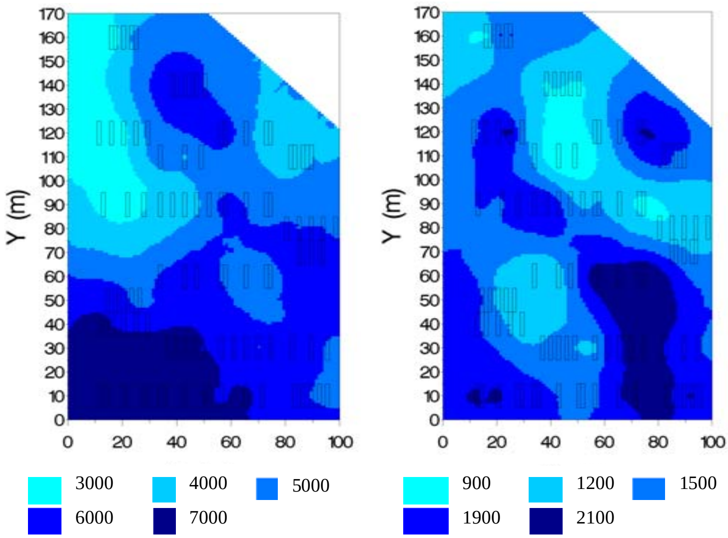

Figure 2: Spatial variation of yields (kg ha^{-1}, dm) of swards in spring and autumn in Sotkamo in 2005

parts of the field second cut yield was higher and in other parts lower than in the first cut (*Figure 2*).

According to the variogram, the spatial dependence vanished when the distance between field plots reached 40-60 metres. However it is good to notice, that the spatial dependence was quite low already, when the distance between plots was 20-30 metres. This means that appropriate distance between samples for yield determination would be 30-50 metres.

The clover contents of these swards varied 0-100% (dry matter) in both experimental fields and all cuts. The mean value in all cuts varied from 45% to 65%. In Juva, the figure of clover content of first cut was different from second cut as the highest clover contents were in one spot of the field in first cut and in two spots in the second cut. The mean value of all plots for both cuts in Juva was quite at the same level, but in Sotkamo, the clover content of second cut was clearly higher (*Figure 3*). The spatial dependence vanished until 30-50 metres, but appropriate distance between samples for clover content determination is about 20 metres.

CONCLUSIONS

The information of the spatial analysis helps to understand the variation of different properties in fields. It is evident to recognize those factors, which are causing variation. According these results, the weather conditions and their co-effects with other factors in field can explain only a small part of the variation. Chemical, physical and microbiological properties of the soils of fields have been determined and in near future they will be studied as possible accounted factor for the spatial variability of sward yields and clover content.

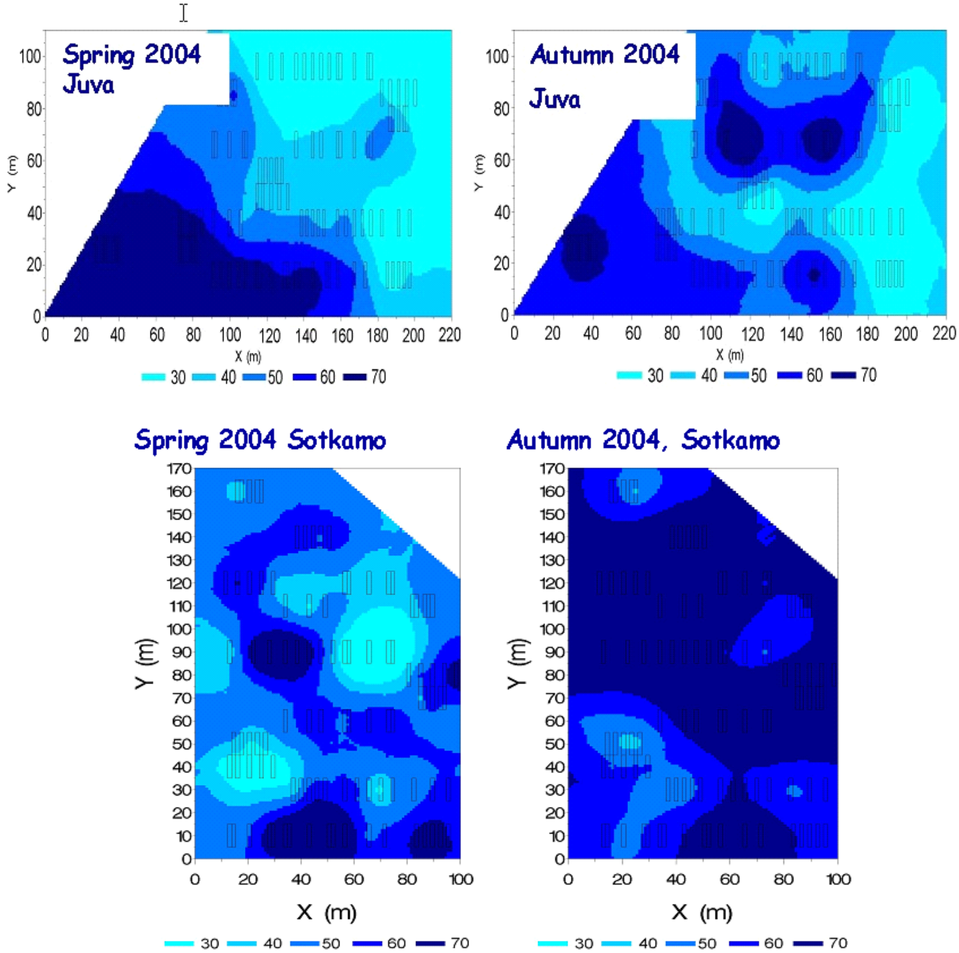

Figure 3: Spatial variation of clover contents (%, dm) of swards in Juva and Sotkamo in 2005.

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Influence of the last grazing time on the white clover/grass swards performance

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ABSTRACT

Two field experiments were carried out on a light loamy gleyic cambisol. With two different grass/clover mixtures the date of the last utilisation in autumn was varied in two weeks intervals between September 1 and November 1. In the sowing year this last utilisation was cutting while it was grazing in the four years of use. In the years of use, mixtures were grazed 4-5 times per season. A higher average dry matter yield was produced by the sward sown with *Festulolium* and white clover (*Trifolium repens*). Perennial ryegras (*Lolium perenne*) and white clover sward was less productive. Its yield was by 0.50 t ha a^{-1} lower. The highest dry matter and digestible protein yield was obtained for both swards when the last grazing in the autumn had been performed in the middle of September or October. When the last grazing had been performed at the end of September or the first days of October, the herbage yield significantly declined. After four years of use a higher percentage of perennial ryegrass thinned out. A higher percentage of *Festulolium* persisted in the sward. In dry matter yield more of white clover (13.9-16.4%) was in the sward with ryegrass.

Keywords: *Trifolium repens*, *Lolium perenne*, *Festulolium*, last grazing time

INTRODUCTION

Winter survival and longevity of grasses depend not only on the weather conditions but also on the state of grasses in the autumn, when their wintering shoots and roots are intensively accumulating reserves of nutrients (Stout 1987, Sardana and Narwal 2000). The long-term productivity of the sward depends on the species composition of grasses, frequency of use, nitrogen fertilisation, and timing of the last cut. The effects of these various factors on the sward can be very diverse in various geographic locations (Van Keuren 1988, Taneja *et al.* 1994). When utilising pastures in regions of a more humid climate, it is very important to finish grazing at the right time so as to allow the sward to accumulate the necessary amount of nutrients and to prevent excessive trampling or poaching of the sward by livestock. Sward trampling results in the reduction of sward productivity and the occurrence of forbs, moreover, it requires extra costs for the elimination of sward surface defects, especially when combined management (grazing and cutting) is applied on the sward. Consequently, the differences in the optimum time of the last cut are relatively great for different sward types and depend on many factors. The objective of the present study was to determine the time of the last cut in the sowing year and years of use of two pasture swards of *Festulolium* and perennial ryegrass each sown with white clover.

MATERIALS AND METHODS

During the period 1998-2004 field experiments were conducted on a gleyic light loamy cambisol. Soil pH varied between 6.4-6.8, humus content was 3.2-3.5%, available

P 104-112 mg kg^{-1} and K 118-130 mg kg^{-1}. Two experiments were set up in 1998 and 2000. Vetch and oats mixture was undersown with two types of grass mixtures. Perennial ryegrass (cv. 'Zvilge') 15 kg ha^{-1} was mixed with white clover (cv. 'Suduviai') 2 kg ha^{-1}. The other mixture was composed of *Festulolium* (*Lolium multiflorum* x *Festuca pratensis*) 'Punia' 18 kg ha^{-1} and white clover 2 kg ha^{-1}. After harvesting of vetch and oats mixture for forage, the swards in the autumn of the sowing year were cut and in the years of use were grazed. The date of the last utilisation in autumn was varied in two weeks intervals between September 1 and November 1. The swards were used for four years. In the spring of each year of use the swards were fertilised with P_{60} K_{60}. The annual rate of nitrogen fertiliser N_{120} was applied at N_{40} in spring and after the second and third grazing. Plot size was 2.5 m × 10.0 m. The treatments were replicated 4 times and were grazed 4-5 times with a herd of dairy cows. Herbage yield was measured by cutting half of the plot before grazing. The botanical composition (grasses, clover, forbs) of the samples was measured after separation and is presented as dry matter weight. The yield data and their analysis were statistically processed using variance analysis.

RESULTS AND DISCUSSION

A delay in grazing from September 1 to October 15 resulted in an increase in herbage dry matter yield for both swards. When grazing was performed on November 1, a significant reduction in the herbage yield occurred. Under Lithuania's conditions grass vegetation stops in the third ten-day period of October due to the weather becoming cold, often accompanied by the first snow. Grass leaves turned brown, the swards lodged and this determined the reduction in the herbage yield of the last grazing.

The highest annual herbage dry matter yield was obtained in the first year of use. The sward composed of *Festulolium* and white clover tended to yield better. The yield of the swards of the first year of use responded more to the timing of the last grazing compared with the swards of later years. The highest herbage dry matter yield for both swards was obtained when grazing was finished on September 15 and October 15. In the swards of the second year of use the effect of the timing of the last grazing on the herbage yield in *Festulolium* sward was found to be insignificant, and in the sward of perennial ryegrass herbage yield significantly declined when grazing had been finished on October 1 and November 1. When the sward had been grazed on October 1, the herbage re-grew a little and was utilising nutrient reserves for re-growth. Utilisation of nutrient reserves for autumn re-growth of sward often tends to decline the productivity of the sward in the following year (Stout 1987). Grasses persist best in the sward and yield best when they are cut relatively early at the beginning of September or late, shortly before the end of the growing season. In the third year of use the sward yield variation relationship remained the same as in the first year of use - the highest yield was obtained having finished grazing on September 15 and October 15. In the fourth year of use completion of grazing from September 15 to November 1 did not result in significant yield differences for both swards. Thus, younger swards respond more to the timing of the last grazing compared with older ones. Averaged data suggest that the highest yields of digestible protein 829-837 kg ha^{-1} and metabolyzable energy 58.6-62.3 GJ ha^{-1} were obtained when grazing had been finished on September 15.

Delay in the last grazing resulted in a marked deterioration of herbage nutritive value. When herbage had been cut on October 1, this gave already a significant reduction in crude protein content and an increase in crude fibre. When grazing had been performed on November 1, crude protein content in herbage declined to 181-185 g kg^{-1} DM. Significant reductions occurred in herbage crude ash, P, K, Ca contents, while the content of crude fat remained similar (*Table 1*). After four years of use a higher percentage of perennial ryegrass thinned out. In the fourth year of use *Festulolium* accounted for 65.8-72.8 % of the dry matter yield, perennial ryegrass accounted for 57.6-61.4%. White clover accounted for 13.9-16.4% of dry matter yield in *Festulolium* sward, and in ryegrass sward white clover accounted for 9.8-14.1%. A higher percentage of forbs was found in ryegrass sward.

Table 1: Dry matter yield, proportion sown grasses and chemical composition of dry matter yield of the last grazing. Average of four years of sward use from two trials 1999-2004.

Date of the last grazing	Yield t DM ha^{-1}	Proportion of sown grass %	Proportion of white clover %	Crude protein g kg^{-1}	Digestible protein kg ha^{-1}	Crude fibre g kg^{-1}	Crude ash g kg^{-1}
			Festulolium + white clover				
1 September	6.05	69.6	12.2	212	759	238	87
15 September	6.54	72.8	10.1	205	837	249	83
1 October	6.06	69.2	14.1	193	766	255	86
15 October	6.50	71.0	10.5	192	768	266	75
1 November	6.10	65.8	9.8	181	757	259	64
			Perennial ryegrass + white clover				
1 September	5.51	61.0	15.9	221	756	238	85
15 September	5.84	61.4	16.4	216	829	236	87
1 October	5.42	58.6	15.7	203	724	249	80
15 October	5.99	57.6	16.4	198	766	254	77
1 November	5.38	58.0	13.9	185	706	265	70
$LSD_{0.05}$	0.27	9.0	5.1	10.3	32.7	16.7	8.1

CONCLUSIONS

In pastures dominated by perennial ryegrass and *Festulolium* with white clover it is not advisable to perform the last grazing at the end of September - beginning of October. For such swards it is better to finish grazing at the end of grass growing season in the middle of October. Younger swards (of the sowing year and first year of use) are more sensitive to the timing of the last grazing compared with older swards.

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N_2O exchanges over different grass-clover mixtures

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Keywords: N_2O fluxes, emission fluxes, gas exchange, grassland

ABSTRACT

During the vegetation period 2005 gas exchange rates of nitrous oxide (N_2O) have been quasi-continuously measured with static chambers at the Swiss workgroup 2 COST 852 site in Ruemlang near Zurich. The measurements were focussed on plots including pure stands of clover and grass as well as standard mixtures with different fertilizer levels (10, 30, 90 kg N ha^{-1} $regrowth^{-1}$) and harvested 5 times per year. Our results allow an insight into short term processes (hourly to seasonal scale). Besides the N-input, also the plant community and their status have a major influence on the emissions. Of the environmental variables, the soil water content has by far the largest influence: humid conditions cause large N_2O emissions.

INTRODUCTION

N_2O is an important greenhouse gas. With an atmospheric lifetime of about 200 years, its contribution to global warming is about 5%. Agriculture contributes to the total annual N_2O emission by about 30% (Houghton *et al.* 1996). It is thus crucial to eliminate unnecessary N_2O emissions to the environment. In addition, gaseous losses contribute to the total N-losses. In order to optimise the N-efficiency, all N-losses should be minimised. In this paper, we investigate the annual gaseous N-losses in the form of N_2O as a function of the applied fertiliser and as a function of the sward composition (pure stands and mixtures).

MATERIALS AND METHODS

In order to estimate the gaseous N_2O losses, static chambers were used on 6 different swards: highly fertilised white clover (CH), lowly fertilised white clover (CL), highly fertilised ryegrass (GH), lowly fertilised ryegrass (GL), moderately fertilised mixture of grass and clover (MM) and highly fertilised grass-clover mixture (MH). A detailed description of the methods used for the determination of the N_2O emission flux can be found in (Flechard *et al.* 2005).

Table 1: Measurement periods and total number of measurement days within the measurement period for the different swards in 2005. In addition, the applied fertiliser is given.

Plot	CH	CL	GH	GL	MH	MM
Measurement	13/4-30/11	13/4-11/5 8/6-28/6	13/4-8/6 28/6-25/7	13/4-11/5 8/6-28/6 26/7-30/11	30/5-0/11	30/5-8/6 28/6-30/11
Measured days/ total days	193/231	33/231	42/231	139/231	180/231	164/231
Applied fertiliser [kg N ha^{-1} yr^{-1}]	450	50	450	50	450	150

Table 1 summarises the measurement periods for the different swards. In addition the number of measurement days within the measurement period and the applied fertiliser is given.

The swards are fertilised and cut 5 times a year. *Figure 1* shows the dates of the cutting and fertilising events.

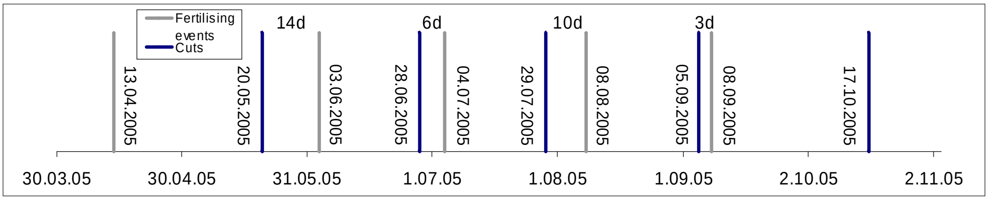

Figure 1: Trigger events (cuts and fertilisation) in 2005

Due to both equipment availability and weather conditions, the time span between the cuts and the consecutive fertilisation varies between 3 and 14 days. The measurements were carried out quasi-continuous (one measurement every two hours). Due to the large amount of technical equipment, measurements could only be carried out on 4 swards at a time.

RESULTS AND DISCUSSION

Emissions after cuts and fertilising events, integrated fluxes

From the measured linear concentration increase or decrease while the chambers are closed, emission or uptake fluxes can be calculated. In order to judge the total gaseous N_2O emissions, the measured fluxes are integrated over time (integrated fluxes). An important result is that the average daily N_2O emissions after the cut are higher than those after the fertilising events for all plots except GL as shown in *Table 2* (averaged integrated fluxes in [g N ha^{-1} d^{-1}]).

Table 2: Integrated fluxes after fertilising events and after cuts. Integrated fluxes are the cumulative fluxes for the measurement period (cuts or fertilising events); averaged integrated fluxes are the daily averages for the measurement period (Av. = averaged, int. = integrated, meas. = measured).

	Fertilising events			Cuts		
2005 (total s)	Int. flux as meas. [g N ha^{-1}]	Av. int. flux [g N ha^{-1} d^{-1}]	Meas. days	Int. flux as meas. [g N ha^{-1}]	Av. int. flux [g N ha^{-1} d^{-1}]	Meas. days
GL	437	**11.0**	40	97.3	3.79	26
GH	617	20.7	30	591	**30.4**	9
CL	564	35.6	17	-	-	-
CH	5628	118	48	13017	**308**	42
MM	529	13.3	40	764	**17.2**	44
MH	1107	28.3	39	1382	**32.3**	43

The measurements showed a distinct dependence of the emitted N_2O on the soil moisture: Strong emissions correlate with high water filled pore space (WFPS) as exemplarily shown in *Figure 2*.

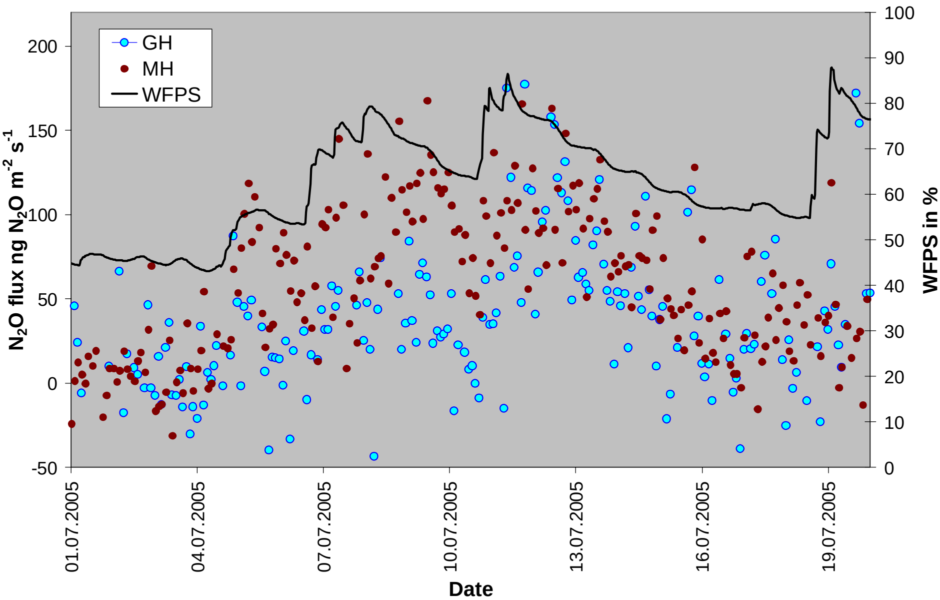

Figure 2: Measured N_2O fluxes and water filled pore space (WFPS) for two plots. The emission fluxes follow the course of the WFPS.

Emission factors

From the measured fluxes, emission factors can be calculated. They are defined as the emitted kg N divided by the total kg N applied. *Table 3* gives the emission factors of the year 2005 for all 6 measured swards.

Table 3: Integrated fluxes and emission factors of the measured swards. The asterisks indicate swards that have not been measured the entire vegetation period; the integrated annual flux can not be directly compared to the other swards.

Plot	Clover fraction [%]	Integrated annual flux [kg N ha^{-1}]	Fertilisation [kg N ha^{-1} yr^{-1}]	Emission factor [%]	Emission factor incl. N-fixation [%]
CH	97	37.10	450	8.24	6.82
CL	95	2.08*	50	10.40	1.73
GH	0	2.68*	450	0.99	0.99
GL	5	1.36	50	2.71	1.94
MH	30	4.14	450	0.92	0.88
MM	45	1.94	150	1.30	0.69

Table 3 shows that CH has as expected the highest annual integrated flux. The higher the fertilising level, the higher is the integrated annual flux. An analysis of the emission factors shows a different result: the emission factor is higher for lower fertilising levels. It also seems that the mixture is favoured in terms of lower N_2O emissions. The pure clover systems show the highest emission factors. When taking into account the additional N-input from fixation, the emission factors are reduced (last column of *Table 3*).

CONCLUSION

The quasi-continuous measurements on 6 different swards showed a clear reaction of the systems both to cutting and fertilising events. The major driving mechanism for N_2O emissions is the WFPS: for WFPS values above 60% there are significant emissions regardless of the vegetation stage. A distinct dependence on the temperature was not found. A higher amount of applied fertiliser leads to higher overall emissions. The emission increase is not linear as the emission factors (emitted N_2O on applied N_2O) show: the emission factors for the higher fertilised swards are lower than for the lower fertilised swards. Fertilised clover monocultures lead to very high emissions. In mixtures, the gaseous N-loss in the form of N_2O is decreased in comparison to monocultures. The coexistence of grass and clover on a single plot reduces the emissions.

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Recovery of fertilizer nitrogen in grass/clover mixtures in the COST 852 experiment

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ABSTRACT

The clover proportion in grass/clover mixtures might influence the recovery of fertilizer N because of its potential effects on N availability and N sink. We investigated the influence of botanical composition and N fertilization level on the recovery of fertilizer N in the plants after a ^{15}N labeled ammonium nitrate application on grass/clover mixtures and pure stands in the COST 852 Agrodiversity experiment. The botanical composition of the mixtures having a clover proportion of up to about 50% did not influence significantly the fertilizer N recovery and the amount of N taken up from the soil. In the clover pure stands however, fertilizer N recovery and N uptake from the soil were much lower. The amount of fertilizer N that was not recovered increased with increasing N fertilization although the percentage of plant N derived from the fertilizer was higher at the high than at the low fertilization level. We conclude that under the conditions of our experiment and at N fertilization levels up to 30 kg ha^{-1} $regrowth^{-1}$, increasing clover proportion up to 50% does not reduce fertilizer N recovery and soil N uptake and therefore does not increase the risk of N leaching during plant growth.

Keywords: nitrogen, fertilizer recovery, clover proportion

INTRODUCTION

The efficient use of nitrogen (N) fertilizer requires knowledge about its recovery in the crop. Clover might reduce fertilizer N recovery in grass/clover mixtures because of the additional N source from symbiotic N_2 fixation (increased N availability). But the larger biomass production of grass/clover mixtures compared to grass pure stands (Kirwan *et al.* 2007) might increase the mineral N uptake (increased N sink) and thereby favour a high fertilizer N recovery. The aim of this study was to assess the influence of the N fertilization level and the botanical composition on the fertilizer N recovery in grass/clover mixtures.

MATERIAL AND METHODS

Pure stands and four species mixtures of *Lolium perenne* L., *Dactylis glomerata* L., *Trifolium pratense* L. and *Trifolium repens* L. were sown in autumn 2002 according to the common protocol of the COST action 852 experiment. In April 2003 all swards received 30 kg N ha^{-1} to complete establishment. Afterwards, the plots were fertilized with either 10 (N1), 30 (N2) or 90 (N3) kg N ha^{-1} to each of the 5 yearly regrowths. The fertilizer application in April 2005 was performed with ^{15}N labeled ammonium nitrate on subplots (1.5 x 1.4 m) that did not receive any previous ^{15}N application. At the subsequent harvests, the ^{15}N:^{14}N-ratio and the total N content were determined in the harvested plant material of each sown species and in pooled samples of unsown species, in order to assess the proportion of N derived from symbiotic fixation (%Nsym) and the proportion of N derived from the fertilizer application

(%Nfert). The amount of harvested N derived from the fertilizer application (Nfert) and from symbiotic fixation (Nsym) was then calculated from the total N content in the plants and their dry matter yield. The amount of harvested N derived from the soil (Nsoil) was calculated from the difference between the total N harvested (Ntot) and the sum of Nfert and Nsym. For N2, these values were also assessed for the first harvest of 2003 following a single ^{15}N application on subplots other than those labeled in 2005.

RESULTS AND DISCUSSION

Plant functional type (grass, clover) and N fertilization level strongly influenced %Nfert (*Figure 1*). In the mixtures, at the first harvest after the 2005 labeled fertilizer application, %Nfert averaged 7, 21 and 40% in grasses, and only 0.7%, 1.4% and 10% in clover, for N1, N2 and N3 respectively. In pure stands, grass and clover tended to have higher %Nfert than in mixtures (*Figure 1*).

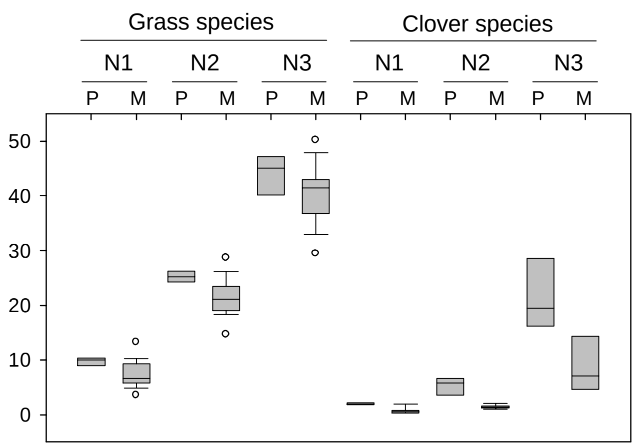

Figure 1: Box plot of the percentage of N derived from fertilizer (%Nfert) found at the 1st harvest of 2005 in grass and clover grown in pure stands (P) or in mixtures (M) at 3 different levels of N fertilization (N1 = 10, N2 = 30, N3 = 90 kg N ha^{-1} $regrowth^{-1}$). The box plots show the median, the 10th, 25th, 75th and 90th percentiles.

No difference in %Nfert was observed between the two grass species. In white clover %Nfert was slightly higher than in red clover. In the second regrowth after ^{15}N labeling, %Nfert from the labeled fertilizer application dropped below 10% in all species and at all N levels (results not shown). The %Nfert values at the first harvest of 2003 were very similar to those found in 2005 for the corresponding N fertilization level (N2), indicating that the differences between grass and clover were consistent over the years.

Nfert was around 19 kg N ha^{-1} throughout the range of clover proportion in the mixtures fertilized at N2 (*Figure 2* and *3*). Therefore the botanical composition of the mixtures having a clover proportion of up to about 50% did not influence significantly the fertilizer N recovery. This probably was because for these mixtures, the increasing amount of symbiotically fixed N with increasing clover proportion did not provoke a decrease in Nsoil but was accompanied by a corresponding increase in total N uptake by the sward. Both Nfert and Nsoil were much lower in the clover pure stands than in the mixtures (*Figure 2* and *3*). At N3, all swards except the clover pure stands shifted toward grass rich stands (*Figure 3*), so that the effect of clover proportion on Nfert cannot be assessed for this level of N fertilization.

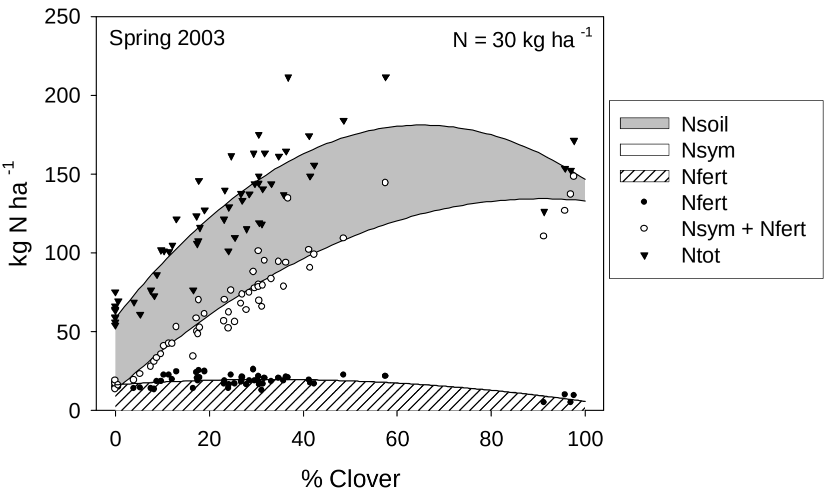

Figure 2: Effect of the yield proportion of clover on the harvested amount of N derived from one fertilizer application (Nfert), from symbiotic fixation (Nsym) and from the soil (Nsoil) for the N2 level in spring 2003. Symbols represent actual values and the shaded surfaces show regression estimates.

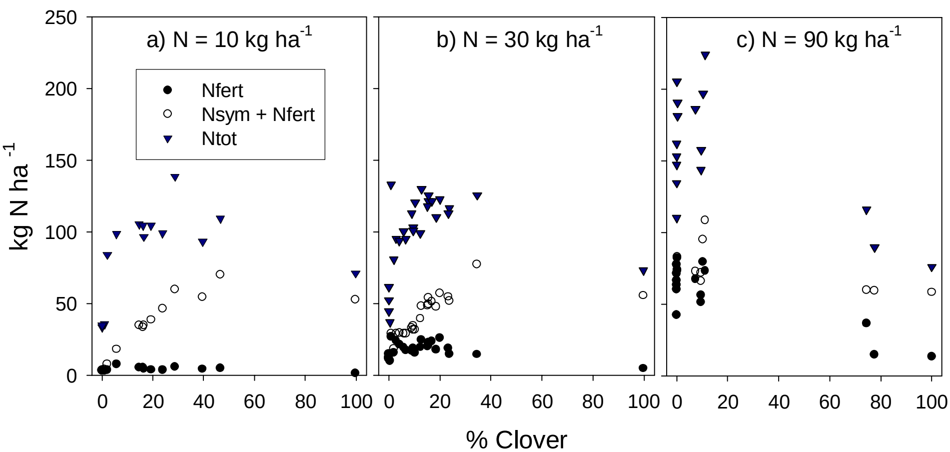

Figure 3: Effect of the yield proportion of clover on the quantity of harvested N derived from symbiotic fixation (Nsym) and derived from one fertilizer application (Nfert), and on the total amount of harvested N (Ntot). The swards were fertilized with either (a) 10, (b) 30 or (c) 90 kg N ha^{-1} per regrowth^{-1} and were harvested in spring 2005.

The percentage of the applied fertilizer N that was recovered in the harvested biomass increased with increasing N fertilization (in average over all mixtures, 43, 58 and 63% of fertilizer N recovered at N1, N2 and N3 respectively), probably because the mineralization/immobilization turnover in the soil was, proportionally to the amount of applied N, higher at the low than at the high N fertilization level (Barraclough *et al.* 1985). Nevertheless 5.6, 12.3 and 32.9 kg of fertilizer N were not recovered at N1, N2 and N3 respectively. The amount of fertilizer N that was not recovered therefore increased by a rate of 0.34 kg N per additional kg of N applied per ha within the experimental fertilization rage.

CONCLUSIONS

- The low %Nfert in clover grown in mixtures and the low Nfert and Nsoil in the clover pure swards indicate that clover is a weak competitor for mineral N sources and relay on symbiotic N2 fixation for the bulk of its N.
- In the conditions of our experiment and at N fertilization levels up to 30 kg ha^{-1} $regrowth^{-1}$, increasing clover proportion up to 50% does not reduce mineral N uptake from the soil and the fertilizer and therefore probably does not increase the risk of N leaching during plant growth.
- At very high clover proportions in the sward, large amounts of available soil and fertilizer N are left in the soil, which creates a risk of N losses to the environment.

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Nitrogen use efficiency of lucerne under conditions of water deficiency stress

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ABSTRACT

A pot trial was carried out at the Institute of Forage Crops, Pleven to determine the nitrogen use efficiency of lucerne after application of mineral fertilizer and manure under the conditions of optimal moistening and water deficiency stress. Ammonium nitrate and well rotted cattle manure were tested at rates of 70 and 210 mg N kg^{-1} soil. Water deficiency stress was imposed for ten days at the stage of active vegetative growth of the plants. It was found that the nitrogen use efficiency for both levels of water supply was the highest for 70 mg N kg^{-1} soil applied as manure. Water deficiency stress decreased the agronomic efficiency of mineral fertilizer and manure application by 30 and 42%, and by 26 and 39%, respectively. Lucerne sensitivity index to water deficiency stress was the lowest at 70 mg N kg^{-1} soil applied as manure. Water deficiency stress decreased the efficiency of soil-plant system, and this decrease was lower when manure was applied.

Keywords: nitrogen use efficiency, mineral fertilizing, manure, water deficiency stress, lucerne

INTRODUCTION

A plant's high-yield ability as related to nitrogen fertilization is assessed as nitrogen agronomic efficiency (Novoa and Loomis 1981, Below 1995). When using mineral fertilizers, 30 to 50% of mineral nitrogen is lost as elementary nitrogen in atmosphere, which greatly decreases their efficacy (Kostov and Lynch 1998). Water deficiency is an important stress factor and lucerne is more tolerant as compared to most of forage crops. Nevertheless its productivity was negatively affected, particularly during the first year of development. Crops treated with nitrogen and subjected to water deficiency stress had better growth and better productivity as compared to those, depending only on N from symbiotic N_2-fixation (Antolin *et al.* 1995). According to Letey (1977) and Vasileva and Kostov (2002) plants treated with manure were more resistant to water deficiency stress as compared to these treated with mineral fertilizer. The aim of this study was to determine the nitrogen use efficiency of lucerne after application of mineral fertilizer and manure under the conditions of optimum moistening and water deficiency stress.

MATERIALS AND METHODS

A pot trial with lucerne variety Victoria and leached chernozem soil type was carried out at the Institute of Forage Crops, Pleven. Pots of 10 L capacity were used. Four plants were grown in each pot. Four replicates per treatment were used. The following treatments were tested:

1. $Control_1$ - unfertilized with N + PK(N_0PK) + 80% Field Capacity (FC)
2. Mineral N_1 + PK (MN_1PK) + 80% FC
3. Organic N_1 + PK (ON_1PK) + 80% FC
4. Mineral N_2 + PK (MN_2PK) + 80% FC
5. Organic N_2 + PK (ON_2PK) + 80% FC
6. $Control_2$ - unfertilized with N + PK (N_0PK) + 40% FC
7. Mineral N_1 + PK (MN_1PK) + 40% FC
8. Organic N_1 + PK (ON_1PK) + 40% FC
9. Mineral N_2 + PK (MN_2PK) + 40% FC
10. Organic N_2 + PK (ON_2PK) + 40% FC

The following rates of fertilizing were applied: N_0 - unfertilized, N_1 - 70 mg N kg^{-1} soil, N_2 - 210 mg N kg^{-1} soil, P - 110 mg P kg^{-1} soil, K - 110 mg K kg^{-1} soil. Ammonium nitrate and well rotted cattle manure were used. Phosphorus was applied as triple super phosphate and potassium as potassium chloride. The plants from treatments 1 to 5 were grown under optimum water supply (80% FC). Water deficiency stress was imposed at the active vegetative stage for the plants from treatments 6 to 10 (irrigation was stopped till the soil moisture dropped to 40% of FC for 10 days). The average data for two cuts are given. Efficacy of the soil-plant system was calculated using the formula as suggested by Bowen and Zapata (1991): Nitrogen use efficiency (an agronomic parameter) - (mg top mass/mg N applied); nutrient uptake efficiency (an ecophysiological parameter) - (mg total N in plant/mg N applied). Lucerne sensitivity index to water deficiency stress was calculated using formula of Dimitova and Mehandjieva (1990). The experimental data was statistically processed using the software products SPSS and Excel for Windows 2000.

RESULTS AND DISCUSSION

Efficacy of the soil-plant system expressed by agronomic and ecophysiological parameters changes under the effect of fertilizing. Nitrogen use efficiency for both levels of water supply was the highest for 70 mg N kg^{-1} soil applied as manure (*Table 1*). The exceeding as compared to the same rate applied as mineral fertilizer was 57% (optimum moistening), and 67% (water deficiency stress). No significant differences between two forms of fertilizers were found for the higher tested rates.

Water deficiency stress decreased the agronomic efficiency of mineral fertilizer and manure application by 30 and 42%, and by 26 and 39%, respectively. An analogous trend was found for the ecophysiological parameter. When applying 70 mg N kg^{-1} soil as manure, the ecophysiological parameter values under water deficiency stress were 60% higher than those at the same nitrogen rate applied as mineral fertilizer. Water deficiency stress decreased the ecophysiological efficacy by 38-39% for mineral fertilizer, and slightly, by 21-33% for manure.

Lucerne sensitivity index to water deficiency stress was in correspondence with the above data. For both studied rates of manure the index of sensitivity to water deficiency stress (*Figure 1*) was lower, as compared to that when using mineral fertilizer (by 15% for 70 mg N kg^{-1} soil, and by 6% for 210 mg N kg^{-1} soil). Lucerne showed the lowest sensitivity to water deficiency stress when using 70 mg N kg^{-1} soil applied as manure. The lower plant sensitivity when using manure was also in conformity with forage productivity (Vasileva and Kostov

Table 1: Efficacy of the soil-plant system under application of mineral fertilizer and manure in forage lucerne

Treatments [a]	Agronomic parameter [b]	Ecophysiological parameter [c]
	Under optimum water supply (80% FC)	
1. Control$_1$ N_0PK	-	-
2. MN_1PK	198	11.9
3. ON_1 PK	310	14.5
4. MN_2 PK	70	3.9
5. ON_2 PK	77	4.3
	Under water deficiency stress (40% FC)	
6. Control$_2$ N_0PK	-	-
7. MN_1 PK	138	7.2
8. ON_1 PK	230	11.5
9. MN_2 PK	41	2.4
10. ON_2 PK	47	2.9
SE (P=0.05)	35	1.6

[a], MN_1, mineral N, 70 mg N kg^{-1} soil; ON_1, organic N, 70 mg N kg^{-1} soil; MN_2, mineral N, 210 mg N kg^{-1} soil, ON_2, organic N, 210 mg N kg^{-1} soil; P, 110 mg P kg^{-1} soil; K, 110 mg K kg^{-1} soil; [b], mg top mass/mg N applied; [c], mg total N in plant/mg N applied

2002). Water deficiency stress decreased the efficacy of soil-plant system, and this decrease was lower when manure was applied.

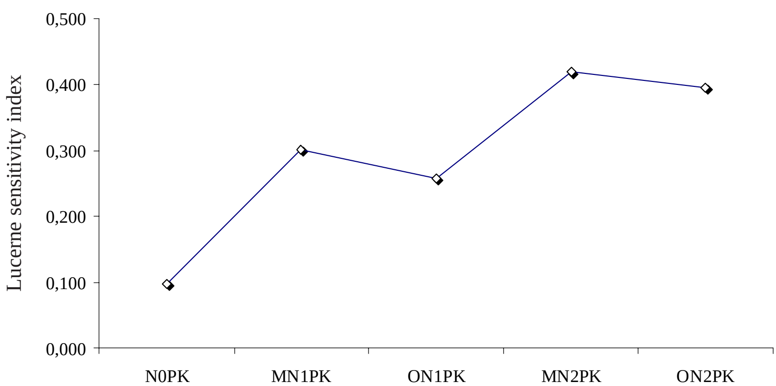

Figure 1: Lucerne sensitivity index to water deficiency stress after mineral fertilizer and manure application

CONCLUSIONS

Nitrogen use efficiency for both levels of water supply was the highest for 70 mg N kg^{-1} soil applied as manure. Water deficiency stress decreased the agronomic efficiency of mineral fertilizer and manure application by 30 and 42%, and by 26 and 39%, respectively. Lucerne sensitivity index to water deficiency stress was the lowest at 70 mg N kg^{-1} soil applied as manure. Water deficiency stress decreased the efficiency of the soil-plant system, and this decrease was lower when manure was applied. However, the results obtained can be only considered for the soil type and experimental conditions used.

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N-content and N-export of grass/legume mixtures in the COST 852 experiment in Belgium

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ABSTRACT

Lolium perenne (Lp), *Phleum pratense* (Pp), *Trifolium pratense* (Tp) and *Trifolium repens* (Tr) were sown in monoculture and in 5 combinations at a recommended and a low -60% of recommended- seeding rate in September 2002. There were two sets of treatments: one fertilised with 150 kg N and the other with 225 kg N ha^{-1} $year^{-1}$ during 2003-2005. The plots were harvested 4 times a year. At each cut DM yield has been measured and a grabbed subsample was separated into Lp, Pp, Tp, Tr and unsown species, dried and weighed. The samples for dry matter determination were analysed by NIRS. Dry matter yield, botanical composition, crude protein content and the N-export were calculated for the cycle 2003-2005. Considering the means of the 5 combinations, seeding rate had a negative effect on the amount of legumes (Tp+Tr) in the mixture, the crude protein content, the absolute dry matter yield and N-export. An increase of the N-fertilisation from 150 to 225 kg N ha^{-1} resulted in a lower amount of legumes (Tp+Tr) in the mixture, a small increase of the crude protein content, a substantial positive effect on absolute dry matter yield and N-export.

Keywords: N-content, N-export, grass/legumes, COST 852

INTRODUCTION

In Flanders as well as in the surrounding countries, farming moves towards less intensive agriculture. This opens new perspectives for legume based forage crops e.g. red and white clover, which are mostly sown together with grasses (*Lolium* species). Farmers are very interested in the yield potential and the feeding value of these grass/clover mixtures. They are not very familiar with the management of these 'new' forage crops. In the Flemish COST 852 experiment the influence of seeding rate and level of N-fertilisation on the behaviour of grass/clover mixtures are studied. In this paper attention will be paid to crude protein content, dry matter production and the export of nitrogen in cutting conditions.

MATERIALS AND METHODS

The basic design with 30 plots included monocultures and mixtures of four species: *Lolium perenne* (Lp), *Phleum pratense* (Pp), *Trifolium pratense* (Tp) and *Trifolium repens* (Tr) in various densities and in a recommended and low (60% of the recommended) seeding rate (*Table 1,2*). This set was fertilised with 150 units of N ha^{-1} $year^{-1}$. An additional set of 18 plots contained a subset of the various densities (*Table 2*) and was fertilised with 225 units of N ha^{-1} $year^{-1}$. So, 4 monocultures and 5 combinations of grasses and legumes, sown in 2

Table 1: Species, cultivars and recommended seeding densities (R) for monocultures in Flanders

Species			Cultivar	Seeding rate
100Lp	Fast developing grass	*Lolium perenne*	Ritz	40 kg ha^{-1}
100Pp	Slow developing grass	*Phleum pratense*	Comer	15 kg ha^{-1}
100Tp	Fast developing legume	*Trifolium pratense*	Lemmon	12 kg ha^{-1}
100Tr	Slow developing legume	*Trifolium repens*	Merida	10 kg ha^{-1}

Table 2: Seeding rates of each species in the multi species plots

		Species (% seeds in the mixture)			
		Lp	Pp	Tp	Tr
1	70Lp	70	10	10	10
2	70Pp	10	70	10	10
3	70Tp	10	10	70	10
4	70Tr	10	10	10	70
5	25-25-25-25	25	25	25	25

seeding rates and fertilised at 2 levels of N could be evaluated. There were no replicates in Merelbeke but the experiment with the same design was set up in several West European countries.

The experiment was sown on 3rd of September 2002 on a sandy loam soil with a pH 6.1, a very low organic matter content (11.1 g kg^{-1}), a very low cation exchange capacity (<10 cmol kg^{-1}, a base saturation of 30.8%, and a low content of C and N (Karyotis *et al.* 2004).

The N- fertilisation was fractioned per cut as follows: 60-50-40 N ha^{-1} for the 150 N level and 75-60-60-30 N ha^{-1} for the 225 N level. The experiment was cut 4 times a year.

At each cut DM yield has been measured and a grabbed subsample was separated into 5 fractions (Lp, Pp, Tp, Tr and unsown species), dried and weighed separately. The samples used for dry matter determination were dried, milled and analysed in the laboratory for the N-content by NIRS. The dry matter yield, the botanical composition of the dry matter, the crude protein content and the N-export were calculated for the cycle 2003-2005.

RESULTS AND DISCUSSION

Considering the 5 grass/legume mixtures over the 3 year period, the lower seeding rate had a slightly negative (-4% of the dry matter) or no effect on the quantity of legumes in the mixture. A decrease with 40% of the recommended seed density (60% R) resulted in a lower crude protein content (-0.3% units), a lower dry matter yield ha^{-1} (-3.5%) and consequently in a lower N export ha^{-1} (-5,8%) (*Table 3*).

An increase of the N fertilisation slightly decreased the proportion of the legumes (-3% of the dry matter) and had almost no effect on the crude protein content (+0.1% units). The dry matter yield increased substantially with 7.6% (= 1.3 ton DM ha^{-1} $year^{-1}$) as well as the N export : +7.8% (= 31 kg N ha^{-1} $year^{-1}$). There was a big variation in the proportion of legumes in the different cuts but on average over the years there is a good balance between legumes and grasses, even in the higher N level. The quantity of unsown species over the 3 year period was negligible in the two N levels (respectively 0.6% and 1.2% for the 150 and 225 N level).

Table 3: Crude protein content, DM yield and N export in relation to N fertilisation and sowing density (COST 852 experiment - Merelbeke 2003-2005)

N level	Seeding density	Tp+Tr % of total dry matter	Crude Protein %	DM yield Ton ha^{-1} 2003-2005	DM yield %	N export kg ha^{-1} 2003-2005	N export %
150N	R[(1)]	45	14.0	53.9	100.0	1213	100.0
	60% R	41	13.7	51.7	95.9	1137	93.7
225N	R	40	14.1	57.6	100.0	1302	100.0
	60% R	40	13.8	55.9	97.0	1232	94.6
150N	average	43	13.9	52.8	100.0	1175	100.0
225N	average	40	14.0	56.8	107.6	1267	107.8

R[(1)]: recommended seeding density

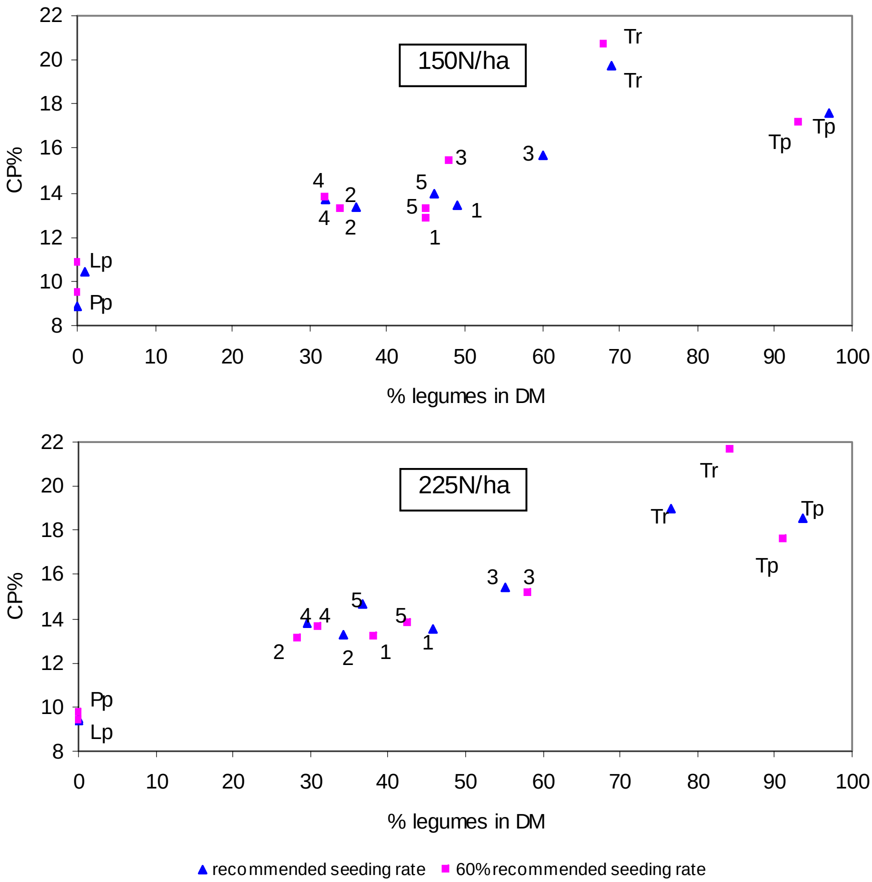

Figure 1: Effect of the seed mixture composition on the proportion of legumes in the sward and on the crude protein content (COST 852 experiment - Merelbeke 2003-2005). Explanations figures: see *Table 2*.

Under cutting conditions, at levels of 150-225 kg N ha^{-1}, it is normal to become very low protein contents in monocultures of grasses (8.9%-10.9%). In these circumstances pure stands of legumes have a favourable protein content (17.1%-21.6%) (*Figure 1*). Mixture 3 with 70% red clover seeds results in the highest legume content in the sward and the highest crude protein content of all the grass/legume mixtures.

CONCLUSION

Considering the means of the 5 combinations, seeding rate had a negative effect on the amount of legumes (Tp+Tr) in the mixture, the crude protein content, the absolute dry matter yield and N-export. An increase of the N-fertilisation from 150 to 225 kg N ha^{-1} resulted in a lower amount of legumes (Tp+Tr) in the mixture, a small increase of the crude protein content, a substantial positive effect on absolute dry matter yield and N-export. The mixture with 70% red clover seeds resulted in the highest legume content in the sward and the highest crude protein content of all the grass/legume mixtures.

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Effects of sward diversity on potentially mineralizable nitrogen: a multisite COST 852 study

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ABSTRACT

The experimental framework provided by the COST 852 Agrodiversity experiment allows the testing of possible plant diversity effects on soil parameters such as soil fertility. Soil fertility was assessed by quantifying the potential mineralizable nitrogen (PMN) in the soil after 3 years of sward development. Diversity effects on soil fertility are being investigated at 24 sites that are distributed in 13 European countries and Canada, and cover a large environmental gradient, including contrasting climate and soil types. We expect the multi-site analysis of PMN within the Agrodiversity experiment to be useful to help understand the influence of plant diversity on soil fertility.

INTRODUCTION

A significant effort has been made since the 1990s to comprehend the role of biodiversity in ecosystem processes, through both experimental and observational studies (Tilman *et al.* 1996, Hector *et al.* 1999). Within the Agrodiversity experiment (COST Action 852), the diversity effect in mixed swards was compared with monocultures. Sward evenness significantly enhanced productivity (Kirwan *et al.* in press) and reduced invasibility (Coll *et al.* this volume). The experimental design (Kirwan *et al.* in press) allowed the separation of evenness effects from identity effects, and therefore the explicit testing of the contribution of each species and functional group to several ecosystem processes, as well as other possible effects as diversity is increased.

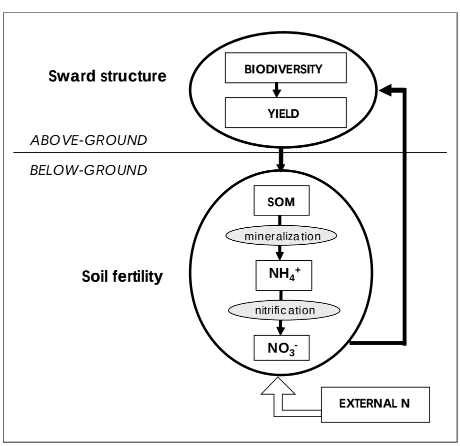

Figure 1: Idealised relationships between soil fertility, and above and belowground processes

Increased diversity has been proposed to result in a higher productivity as a consequence of complementarity in resource use (Loreau 1998, Spehn *et al.* 2000). The increased nutrient uptake can therefore lead to a subsequent reduced N leaching (Drinkwater 1998, Scherer-Lorenzen *et al.* 2003, Spehn *et al.* 2005). In addition, the legume content in the sward may increase soil N, with an effect on non-legume species production (Palmborg 2006). The experimental framework provided by the Agrodiversity experiment allows the testing of possible diversity effects on soil parameters such as soil fertility. Our hypothesis is that soil fertility associated with the organic fraction could be affected by plant evenness (*Figure 1*). This effect could be particularly relevant if there is contribution to soil fertility by legumes. Soil fertility can be assessed by quantifying the potential mineralizable nitrogen (PMN) in the soil. A common effort has been started within the COST 852 Agrodiversity experiment to analyze PMN across multiple sites. We present the extent of this common effort and report preliminary results in one of the sites where the experiment was established, located in Gòsol (Eastern Pyrenees).

METHODS

Soil fertility was assessed by quantifying PMN in the soil after 3 years of sward development. Potential mineralizable nitrogen is considered an indicator of the ability of soils to supply N to plants and soil organisms (Bundy and Meisinger 1994). The mineralization of organic N in standardized conditions can give a comparative indication of fertility in the soil organic fraction among the different evenness treatments. PMN was determined by ammonium production during anaerobic incubation of soil samples (5g of fine earth) for 14 days at 40 ºC (Bundy and Meisinger 1994, *Figure 2*). Anaerobic conditions were achieved by waterlogged incubation (12.5 ml H_2O) and filling the tubes with N_2 to avoid oxygen diffusion. After incubation, NH_4^+ was extracted by KCl and determined colorimetrically. Initial soil NH_4^+ content was assumed to be almost negligible, so it was not analysed.

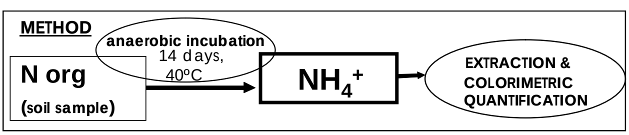

Figure 2: Illustration of the analytical method to determine potential mineralizable nitrogen (PMN)

Monocultures and 4-species mixtures with varying proportions were sown in 30 plots in Gòsol (Eastern Pyrenees) at two densities, one 60 % of the other. The species in the experiment were: *Lolium perenne*, *Dactylis glomerata*, *Onobrychis viciifolia* and *Medicago sativa*. The effect on PMN of sowing density and either evenness (three levels: low, high and medium) or legume content at sowing (five levels, from pure legume to pure grass monocultures) was tested using two-way ANOVA. See Kirwan *et al.* (in press) for details on experimental design.

RESULTS AND DISCUSSION

Diversity effects on soil fertility are being assessed across 24 sites, distributed in 13 European countries and Canada. These sites cover a wide environmental gradient, and include contrasting climate and soil types. Preliminary results from the Gòsol site suggest a positive effect of evenness on soil fertility. Mean estimated PMN was 130.1 $\mu g\ g^{-1}$ in monocultures, and 149.2 $\mu g\ g^{-1}$ in high diversity mixtures and centroid or maximum evenness mixture, or equal proportion of each species (significant differences between both treatments, $P = 0.033$). There was a tendency for PMN to be enhanced with increased legume content ($P = 0.065$). Therefore, plant diversity resulted in enhanced N fertility (PMN) at the Gòsol site. This was probably due to enhanced quantity and quality of organic matter inputs associated with increased plant diversity and productivity. As expected, legumes played an important role on PMN. Preliminary results suggest that the level of PMN in legume monocultures (146.9 $\mu g\ g^{-1}$) was more different from that in grass monocultures (113.2 $\mu g\ g^{-1}$, $P < 0.007$) than it was from that in the maximum evenness treatment (145.8 $\mu g\ g^{-1}$, $P < 0.017$). More sophisticated analyses will assess whether evenness has an effect over and above that of the influence of legumes.

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Estimate of N-fixation in selected plots of the COST 852 common experiment in Iceland

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Keywords: N-fixation, clover, seed mixture, ^{15}N label, field experiment

ABSTRACT

The aim was to estimate the degree of N-fixation in different mixtures of red clover (*Trifolium pratense*), white clover (*Trifolium repens*), timothy (*Phleum pratense*) and smooth meadow grass (*Poa pratensis*) in selected plots of the common experiment of COST 852. Plots were watered with ^{15}N solution of ammonium nitrate over two growing seasons during the first and the second harvest years in 2004 and 2005. Two harvests were taken on each plot. Red clover had survived poorly and in many plots it had disappeared completely. Total N-fixation increased with higher percentage of clover, resulting in three times more fixation (N kg ha^{-1}) in Harvest 2 compared to Harvest 1 in summer 2004 and six times more in 2005. About 90-99% of total N in the clover species was derived from fixation. In Harvest 1 in both years the % N derived from fixation was higher in red clover than in white clover. The N-transfer between clover and grass was not measured in this experiment but the value for atom % ^{15}N was higher in timothy than in smooth meadow grass.

INTRODUCTION

Biological nitrogen (N) fixation is of great importance for agriculture and legume-based grassland mixtures play a central role in providing substantial amounts of N for agricultural ecosystems. There has been increasing interest in more complex mixtures in recent years as an alternative to white clover based grassland. There are various advantages of using multi-species mixtures. Different species can compensate each other, e.g. in the utilization of available resources, and they increase the stability and productivity of the sward (Tilman *et al.* 1996). Different root morphology of the plants may play a role in resource use complementarity as, for example, timothy has a fibrous and shallow root system which doesn't spread laterally, while smooth meadow grass has roots that can penetrate deep into the soil (Grime *et al.* 1988). Recent studies have shown that multi-species pastures can produce higher dry matter yield and fix more N than ryegrass-white clover pastures (Goh and Bruce 2005).

White clover and red clover are the most important legumes of temperate pastures. Numerous values for N fixation of white clover have been published, while measurements for red clover are more restricted. On average, 80% of the total N found in white clover grown in mixed grass swards is derived from the atmosphere and N fixation rates of up to 545 kg N ha^{-1} year^{-1} have been recorded (Carlsson and Huss-Danell 2003). However, considerably lower values have also been presented. Fagberg and Sundqvist (1994) reported up to 99 kg ha^{-1} of fixed N in Northern Sweden (65° N) and the amount of fixed N has been calculated to range from 40 to 169 kg N ha^{-1} in Iceland (Helgadóttir and Kristjánsdóttir 1993).

For red clover, N fixation rates of up to 373 kg N ha^{-1} $year^{-1}$ have been published (Carlsson and Huss-Danell 2003). Nesheim and Oyen (1994) measured lower values in northern Norway (128 kg N ha^{-1} $year^{-1}$), and it has been estimated that red clover can fix from 62 to 88 kg N ha^{-1} $year^{-1}$ in Iceland (Sveinbjörnsson 1997). In general, values of N fixation published for white clover are higher than for red clover (Abberton and Marshall 2005).

The objective of the current study was to estimate the degree of N fixation in different mixtures of red clover, white clover, timothy and smooth meadow grass in Iceland.

MATERIALS AND METHODS

N-fixation was measured in selected plots of the common experiment of COST 852 during the first two harvest years in summer 2004 and 2005, at Korpa Experimental Station in Iceland (64°30'N/21°15'E). A total of 11 plots with different sowing proportions of smooth meadow grass, timothy, red clover and white clover (see *Table 1*) were fertilised with 40 kg N ha^{-1} and watered with 3 kg N ha^{-1} of 10% $^{15}NH_4$+$^{15}NO_3$ fertiliser in early spring and after each cut. In 2005 two additional plots were included to ensure the presence of red clover. The ^{15}N fertiliser was applied to a fixed plot of 1m² from the centre of which 0.25 m² were harvested twice during growing season. The herbage was separated into the four sown species and unsown species. The samples were dried, ground and analysed for ^{15}N:^{14}N ratio and total N content at the Cornell Isotope Laboratory, USA.

Table 1: Nitrogen fixation in selected plots of the common experiment in 2004 (1st harvest year).

	Harvest 1				Harvest 2			
Plots G1:G2:L1:L2[1)]	Total Yield kg DM ha^{-1}	Clover kg DM ha^{-1}	Nitrogen-fixation kg N ha^{-1}	% N-fixation in clover	Total Yield kg DM ha^{-1}	Clover kg DM ha^{-1}	Nitrogen-fixation kg N ha^{-1}	% N-fixation in clover
70:10:10:10	2024	116	3.5	98.3	652	188	-	-
10:70:10:10	1576	484	16.2	96.2	2148	913	34.7	96.8
10:10:70:10	1152	108	3.2	97.7	1084	296	10.6	96.2
10:10:10:70	1272	168	4.0	96.5	836	451	14.3	96.5
30:30:30:30	1128	192	5.1	97.0	1288	548	18.8	94.9
40:40:10:10	1372	172	4.9	97.5	824	372	12.5	98.0
40:10:40:10	1812	84	2.5	97.4	860	208	7.8	96.2
40:10:10:40	1860	308	9.7	97.8	1332	658	27.3	96.6
10:40:40:10	1240	148	3.9	97.7	1220	325	11.3	97.3
10:40:10:40	1156	196	5.2	96.3	976	488	17.5	96.8
10:10:40:40	1064	244	6.7	97.2	1396	794	30.3	97.5
70:10:10:10	2480	144	4.6	98.1	1172	354	13.3	97.4
10:70:10:10	748	8	0.2	98.4	668	12	0.5	98.5
10:10:70:10	744	8	0.2	93.2	380	24	0.8	98.0
10:10:10:70	956	52	1.5	96.6	1260	372	11.3	97.2
30:30:30:30	1516	124	3.6	96.0	1476	327	11.8	96.9
Mean	1381	160	4.7	97.0	1098	160	14.0	97.0
s.d.	474.6	117.3	3.95	1.27	415.2	117.3	10.09	0.89

[1)] Species proportions at sowing. G1: *Phleum pratense*, G2: *Poa pratense*, L1: *Trifolium pratense* og L2: *Trifolium repens*

RESULTS AND DISCUSSION

The total fixation was generally low, ranging from 2-52 kg N ha^{-1} year^{-1}, and it was closely related to the amount of clover in the harvest (2004: r = 0.884, P < 0.001; 2005: r = 0.837, P < 0.001) (*Table 1* and *2*).

The establishment of all species in the experiment was very good. However, during the first winter there was severe winter kill of clover, especially red clover. This explains the low fixation rates in several of the plots. Total N-fixation increased with a higher percentage of clover, resulting in three times more fixation (N kg ha^{-1}) in Harvest 2 than Harvest 1 in 2004 and six times more in 2005. The spring of 2005 was exceptionally cold which could explain the difference between years. About 90-99% of total N in red and white clover originated from fixation. In Harvest 1 in both years the %N derived from fixation was higher in red clover than in white clover (2004: P = 0.021, 2005: P = 0.024) but the same trend was not found in Harvest 2. It has been demonstrated that white clover decreases N-fixation more than red clover when soil N becomes more accessible (Böller and Nösberger 1987). A possible explanation could be that the shallower root system of white clover has a greater ability to compete for soil mineral nitrogen. N-transfer between clover and grass was not measured in this experiment but the value for % ^{15}N excess was higher in timothy than in smooth meadow

Table 2: Nitrogen fixation in selected plots of the common experiments in 2005 (2nd harvest year).

Plots G1:G2:L1:L2[1)]	Harvest 1 Total Yield kg DM ha^{-1}	Harvest 1 Clover kg DM ha^{-1}	Harvest 1 Nitrogen-fixation kg N ha^{-1}	Harvest 1 % N-fixation in clover	Harvest 2 Total Yield kg DM ha^{-1}	Harvest 2 Clover kg DM ha^{-1}	Harvest 2 Nitrogen-fixation kg N ha^{-1}	Harvest 2 % N-fixation in clover
70:10:10:10	2612	100	1.4	97.3	1208	490	5.6	98.0
10:70:10:10	1844	120	1.7	94.6	1876	484	4.7	97.2
10:10:70:10	2040	160	2.4	96.4	1840	604	10.1	96.7
10:10:10:70	1732	48	0.7	94.7	1400	347	5.7	96.1
30:30:30:30	1476	92	1.4	95.8	2452	547	8.9	95.5
40:40:10:10	1900	188	2.7	92.9	1940	853	12.9	96.9
40:10:40:10	2152	140	2.1	97.4	1676	666	10.7	94.9
40:10:10:40	2340	96	1.3	95.8	1784	491	7.5	96.2
10:40:40:10	1788	128	2.1	96.9	2216	766	12.9	95.8
10:40:10:40	1792	44	0.7	94.2	1432	245	3.9	96.9
10:10:40:40	1496	12	0.2	96.3	1352	253	4.4	97.3
0: 0: 0:100	1108	8	0.1	90.5	668	56	1.4	94.6
70:10:10:10	3196	236	3.3	96.0	2180	1012	23.9	96.7
10:70:10:10	1152	32	0.4	97.6	824	56	1.5	96.3
10:10:70:10	1728	80	1.1	97.8	1072	236	5.6	98.4
10:10:10:70	2172	124	1.6	93.3	2096	706	16.6	96.4
30:30:30:30	1480	92	1.3	98.1	1356	510	11.2	98.2
10:10:10:70[2]	3056	76	1.4	99.2	720	160	4.5	99.1
30:30:30:30[2]	1160	32	0.4	96.5	804	220	5.6	98.8
Mean	1907	95	1.4	95.9	1521	458	8.3	96.8
s.d.	587.4	60.2	0.89	2.10	545.6	270.3	5.56	1.25

1) Species proportions at sowing. G1: *Phleum pratense*, G2: *Poa pratense*, L1: *Trifolium pratense* og L2: *Trifolium repens*

2) Higher seeding rate

grass (P < 0.001). This could partly be explained by more rapid growth of timothy compared to smooth meadow grass, resulting in greater accumulation of N. However, it could also indicate that smooth meadow grass is a better receiver of fixed N.

CONCLUSIONS

Despite the low yields of white and red clover in the COST 852 common experiment in Iceland, the %N derived from fixation was very high.

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Diversity and resistance to invasion in a multisite grassland experiment

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ABSTRACT

Biological invasions have a big impact on the earth's ecosystems. Species richness has been postulated as an important variable associated with resistance to invasion. We studied the effects of plant diversity on invasion in experimental grassland swards where species diversity was manipulated to achieve monoculture swards and mixtures with varying evenness. Our results showed that the average proportion of unsown species was less in mixtures than in any monoculture and that this result was generally consistent across 21 sites, indicating a strong relationship between diversity and resistance to invasion in these grasslands.

INTRODUCTION

Species richness has been theoretically and empirically postulated as an important variable associated with emergent ecosystem properties, such as resistance to establishment of invasive species. Some authors have described species-rich communities as less invasible because of an increase of biotic resistance (Knops 1999, Tilman, Levine 2000). This resistance to invasion has been hypothesized to be the possible result of sampling and complementarity effects (Kennedy 2002, Symstad 2000). The COST 852 Agrodiversity experiment allows the study of how species composition, functional group diversity and species diversity affect the invasibility of grasslands. Our aim was to answer the following questions: is invasibility reduced in more species-rich mixtures and are mixture effects on invasion consistent across multiple sites?

METHODS

We measured invasion success by experimentally manipulating levels of resident plant species diversity in an experiment repeated at 21 different sites, of which one was located in Canada and the remaining in Europe. Sites included Nordic, Mediterranean, Atlantic and continental climates. Using a simplex design to create a diversity gradient, plots were sown with one of 11 mixtures consisting of varying proportions of four species plus their monocultures. Mixtures were composed of two grasses and two legumes. Several times in the year following establishment of the experiments vegetation was cut to a height of 5 cm in each plot, and a vegetation subsample was separated into five groups: four sown species and the unsown.

We computed the average proportion of unsown species for each of 11 mixtures and four monocultures at each site and across all the sites.

RESULTS AND DISCUSSION

In all sites the proportion of unsown species was less in the average mixture than in the average monoculture (*Table 1*). Averaged over all sites our data showed consistent negative effects on invasibility when we increased species richness, the average proportions of unsown species for each monoculture was greater than the average for any mixture (P = 0.001, *Table 2*). Many of these sites were included in the analysis of Kirwan et al. 2007 that showed, on average, considerably higher yield in mixtures than monocultures. Perhaps part of the reason for the reduced level of unsown species in mixtures lies in competition from the higher biomass of sown species in these mixtures. Under these conditions, it may be more difficult for unsown species to invade the mixed communities.

Table 1: Means percentage of unsown species (%) in 21 sites

SITE	Mono %	Mixture %	SITE	Mono %	Mixture %
Belgium	8.0	2.1	Poland	32.7	19.3
Iceland	37.5	4.6	Poland	26.5	1.8
Iceland	46.2	5.1	Spain	30.8	8.1
Ireland	30.4	3.9	Sweden	1.4	1.0
Italy	21.7	9.6	Sweden	1.4	0.2
Lithuania	39.7	30.9	Sweden	8.1	2.4
Lithuania	28.3	10.1	Switzerland	9.0	2.8
Lithuania	13.7	0.8	Wales	12.4	1.6
Netherlands	10.9	9.0	Wales	28.5	14.1
Norway	0.9	0.0	Canada	24.4	14.0
Norway	14.6	5.8			

Table 2: Mean proportion of unsown species averaged over 21 sites for 11 mixtures and 4 monocultures

Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mon-Mix	Mon	Mon	Mon	Mon	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix
% Unsown	10.0	16.2	23.7	31.8	6.7	6.3	7.3	5.9	9.3	5.4	8.9	6.3	6.9	6.8	7.4

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Nitrogen mineralisation dynamics in European soils

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ABSTRACT

Mineralisation of nitrogen (N) was studied under aerobic conditions in 24 soils selected from various European districts. The soils studied originate from different parent materials located mainly on alluvial or eolian deposits and they have been developed under different climatic conditions such as continental, or temperate (cold, Mediterranean etc.). The cumulative net mineralized N over the incubation period ranged from 27.22 to 215.38 mg/kg soil, representing a small percentage of the soil nitrogen which greatly varied. The increased values of ammonification at certain samples may be attributed to the specific site conditions which favor this stage of mineralisation process. Close relationship was found between soil organic matter and soil N indicating that N is mainly bound in the soil organic matter. Moreover, cumulative net mineralized N was closely related to potentially mineralized nitrogen (N_0) and to soil organic mater, while relation was also found between pH and C/N. In most cases, the estimated rates of N mineralisation are not negligible and can be taken into consideration as a potential source in the crop-soil system, minimizing the quantity of applied N fertilizers.

Keywords: nitrogen, mineralisation, immobilization, nitrification, ammonification

INTRODUCTION

The process of mineralisation depends mainly on temperature, moisture, aeration, acidity, soil organic matter and soil texture (Mafongoya *et al.* 1998). Several studies to evaluate N mineralisation were based on laboratory or field incubation of disturbed soil samples (Stanford and Smith 1972). Quantifying the contribution of indigenous soil organic N mineralisation to the soil-plant mineral N balance, to allow accurate credits for mineralized N in fertilizer recommendations, requires in situ investigation in specific production systems (Ma, *et al.* 1999). The main objectives of this study were: i) to quantify the amount of mineralisable N in a wide range of soils in Europe ii) to determine the dynamics and the distribution of N among soils in grassland areas developed under various conditions (i.e. climate, topography and parent materials) and iii) to determine the pattern of mineralisation process and to assess the importance of N as a potential source of plant-available form.

MATERIALS AND METHODS

Results regarding soil properties (Karyotis *et al.* 2004) of the experimental sites are used in this study. Net N mineralisation was determined in aerobic incubation experiments by using the method of Stanford and Smith (1972). This process was assessed after 2, 4, 8, 12, 18 and 24 weeks. Leaching was performed by adding 100 ml of 0.01 M $CaCl_2$ followed by 25 ml of N free-nutrient solution. After each incubation, NO_3^--N and NH_4^+-N were determined by a FIAstar 9000 Analyzer. The potentially mineralised nitrogen (N_0) was estimated by the following non-linear equation (Stanford and Smith 1972): $N_t = N_0 (1- e^{-kt})$, where N_t is the cumulative amount of N mineralized at specific time interval (t, weeks), N_0 is the potentially mineralized N, and k is the first-order rate constant.

RESULTS AND DISCUSSION

Previous studies (Karyotis et al. 2004) indicated that basic properties of the examined soils greatly differ. The content in soil organic carbon and nitrogen (N) varied between 7.85 and 49.43 g/kg, and from 0.50 to 3.41 g/kg, respectively. The amount and distribution of soil organic N may be influenced by factors related to topography to soil properties and farming practices that affect biomass production.The C/N ratio in the soils ranged from 9.84 to 32.33 indicating different degree of decomposition of soil organic matter. Statistical analysis showed that organic matter correlates with organic N, indicating that this element is mainly bound in the organic matter fraction of soils.

$$Y_{\text{org. matter}} = 3.56 + 12.72X_N \quad (n = 24, R^2 = 86.3^{***})$$

Pre-incubation soil leachates of inorganic N varied between 4.43 to 95.26 mg/kg (*Figure 1*) and consisted mainly of nitrates and might be the result of conversion of the ammonified organic N into nitrates. Cumulative net N mineralisation in soils was between 27.22 and 215.38 mg/kg as it is illustrated in *Table 1*. Ammonification rates were higher than nitrification in the first 8 weeks of incubation. In general, the rates of nitrification in the incubated soils were lower (average 35.5 mg/kg) than ammonification (average 44.6 mg/kg), the relatively elevated ammonification may be attributed to the specific site conditions which favor the stage of ammonification. Soil acidity restricts mineralisation in soil (Raath and Saayman 1996), but in this investigation the impact of pH cannot be explained due to complexity of the sites.

Differences may be ascribed to specific site conditions which affect the activity of soil microorganisms responsible for ammonification and nitrification. It was reported that the amounts of N mineralized during incubation were not significantly correlated with total N in soils

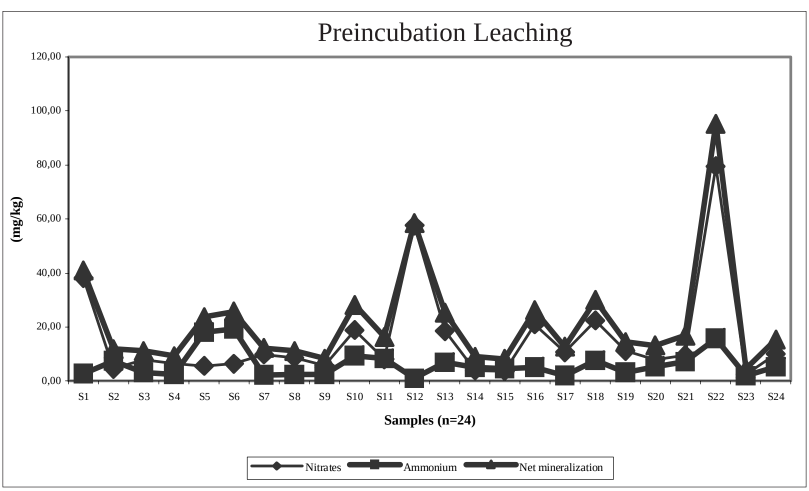

Figure 1: Mineral nitrogen from soil leachates during pre-incubation

(Tabatabai and Bremner 1972) and results of this investigation are in accordance to these findings. However, the following linear regression was found between pH and C/N ratio:

$$Y_{pH} = 5.38 + 0.09X_{C/N}, \quad (n = 24, R2 = 47.8^{***})$$

Other relations:

$$Y_{Ncum} = 40.78 + 0.88X_{ammonif} \quad (n = 24, R^2 = 65.0^{***})$$

$$Y_{Ncum} = -24.72 + 4.07X_{org.\ matter} \quad (n = 24, R^2 = 62.6^{***})$$

The values of N_0 ranged between 59.35 and 429.74 mg/kg and the constant k varied from 0.05838 to 0.08237. The following close relation was found between the cumulative net mineralised N and the values of N_0:

$$Y_{Ncum} = 2.2916 + 0.4776X_{N0} \quad (n = 24, R^2 = 98.6^{***})$$

The decline of mineralisation varies considerably with time amongst the examined soils and ranges between 1.71 and 9.67% of the soil organic nitrogen (*Table 1*). This study was carried out under controlled laboratory conditions. In the field, edaphic-climatic conditions are more variable and the activity of soil microorganisms can be affected by the daily fluctuation of temperature, soil moisture and aeration. Contribution of the mineralized N to plant growth should be appreciated, although biochemical processes should be considered and clarified.

Table 1: Net $N_{min.}$, potentially mineralised nitrogen N_0 and kinetic parameters (t = 24 weeks)

Samples	Country	N_{cum} mg/kg	$\mathbf{N_0}$ mg/kg	**k** 2b/a	O.M. %	N g/kg	C_{org} g/kg	N_{min}x100/N_{soil}	C/N
S1	Denmark	53,67	107,27	0,06761	*1,8*	*1,23*	*14,33*	*4.36*	*11,65*
S2	Belgium	48,39	103,34	0,07199	*1,11*	*0,496*	*8,16*	*9.67*	*16,45*
S3	Sweden	41,89	80,30	0,06816	*2,12*	*1,34*	*16,54*	*3.13*	*12,34*
S4	Sweden	65,02	112,36	0,05873	*2,32*	*1,57*	*19,09*	*4.14*	*12,16*
S5	Iceland	200,68	404,31	0,06418	*3,72*	*2,46*	*36,16*	*3.16*	*14,70*
S6	Iceland	215,38	429,74	0,06633	*4,02*	*2,94*	*40,11*	*2.68*	*13,64*
S7	Greece	41,05	85,15	0,07330	*1,12*	*0,522*	*7,85*	*7.89*	*15,05*
S8	Lithuania	80,41	150,24	0,06353	*2,26*	*1,522*	*18,07*	*5.29*	*11,88*
S9	Lithuania	63,77	118,87	0,06776	*1,98*	*1,118*	*14,80*	*5.69*	*13,24*
S10	Bulgaria	83,25	162,53	0,06513	*1,86*	*1,098*	*13,40*	*7.57*	*12,20*
S11	Bulgaria	63,01	123,02	0,06769	*2,27*	*1,252*	*16,69*	*5.04*	*13,33*
S12	Holland	45,86	101,34	0,07301	*2,24*	*1,502*	*20,44*	*3.06*	*13,61*
S13	Spain	62,16	124,34	0,06954	*2,61*	*2,36*	*59,54*	*2.63*	*25,23*
S14	Switzerland	83,78	186,98	0,07502	*2,4*	*1,76*	*20,32*	*4.76*	*11,55*
S15	Switzerland	65,16	145,20	0,07386	*2,24*	*1,42*	*18,52*	*4.59*	*13,04*
S16	Spain	201,66	423,36	0,06974	*5,8*	*3,41*	*45,51*	*5.91*	*13,35*
S17	Spain	54,37	99,97	0,05838	*2,43*	*1,98*	*21,26*	*2.74*	*10,74*
S18	Spain	59,00	162,69	0,08237	*3,07*	*1,78*	*49,43*	*3.31*	*27,77*
S19	Spain	27,22	59,35	0,07164	*2,27*	*1,59*	*47,15*	*1.71*	*29,65*
S20	Italy	44,30	85,44	0,06742	*1,61*	*1,19*	*29,18*	*3.72*	*24,52*
S21	Italy	49,14	94,60	0,06766	*1,72*	*1,13*	*36,54*	*4.35*	*32,33*
S22	Great Britain	168,85	363,26	0,07007	*4,28*	*3,28*	*32,27*	*5.15*	*9,84*
S23	Finland	57,00	98,96	0,05865	*3,76*	*2,33*	*30,33*	*2.45*	*13,02*
S24	Finland	47,09	86,72	0,06603	*2,84*	*2,06*	*20,96*	*2.28*	*10,17*

CONCLUSIONS

The amount of leached inorganic N during pre-incubation varied considerably among the soils and can be ascribed mainly to the origin of N (different plant residues) and secondary to parent material. Cumulative net N mineralisation and the values of N_0 also varied and the relatively elevated ammonification may be attributed to the specific site conditions which favor this stage of mineralisation. Soil acidity seems not to influence importantly mineralisation.

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Measurements of community shift and convergence in multispecies plant mixtures

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ABSTRACT

Multispecies mixtures, set up in the simplex design and analysed with the RGRD (relative growth rate difference) method, allow us to evaluate the effects of plant species interactions and environmental conditions on changes in species proportions. Using this approach, this paper suggests measurements and graphics to evaluate the shift of communities and convergence/divergence patterns.

To display species proportions in a simplex design an unfolded tetrahedron is presented. The inscribed predicted proportions of species mixtures show simultaneously the shift of four species. Experimental data revealed that the species' identity and environmental conditions are the most important factors in causing shifts in community structure.

A convergence index based on the Euclidean distance (CI_{ED}) is suggested to evaluate convergence/divergence patterns. A simulation study revealed that the CI_{ED} provides valid information on convergence/divergence. The index had a small positive bias and a type I error rate (5.8%) only slightly higher than the expectation under the null hypothesis.

Keywords: relative growth rate difference, convergence index, percent similarity, Euclidean distance

INTRODUCTION

Analyses of single species performances over time are well established (Gibson *et al.* 1999), but methods to evaluate changes in entire communities are less known. Experiments set up in the simplex design and analysed with the relative growth rate difference (RGRD) method (Connolly and Wayne 2005) allow the effects of species identity, species interactions, and abiotic conditions on community composition to be evaluated. Using these methods, this paper suggests graphics to evaluate the shift of communities and convergence/divergence patterns (Leps and Rejmanek 1991). A new convergence index based on the Euclidean distance is introduced.

MATERIALS AND METHODS

Data from an experiment on wetland species dynamics (Suter *et al.* 2006) was used to demonstrate species identity effects and convergence patterns. Mixtures of five species were grown for two years under four environmental conditions produced by combining two water and two nutrient levels. The five species were: *Carex elata*, *Lycopus europaeus*, *Lysimachia vulgaris*, *Carex flava* and *Mentha aquatica*. Data were analysed with the RGRD method (Connolly and Wayne 2005), and community shifts were displayed using the tetrahedron plots developed by Suter *et al.* (2006): a triangle displaying species proportions for experiments in a simplex design is extended with three more triangles; the resulting figure illustrates an unfolded tetrahedron. The inscribed predicted proportions of species mixtures simultaneously show the shift of four species. In this paper, only results for low nutrient conditions are presented.

To measure community shift, a convergence index (CI_{PS}) based on percent similarity has been proposed by Suter *et al.* (2006). A similar convergence index, based on the Euclidean distance (CI_{ED}), is defined as follows:

$$CI_{ED} = \frac{\left\{ \frac{1}{n} \sum_{j<k}^{n} ED_{jk} \right\}_{Start}}{\left\{ \frac{1}{n} \sum_{j<k}^{n} ED_{jk} \right\}_{End}}$$

with n = number of mixture pairs and $ED_{jk} = \sqrt{\sum_i \left(x_{ij} - x_{ik}\right)^2}$, where x_{ij} and x_{ik} are the observed proportion of species *i* in mixture *j* and *k*, respectively. The CI_{ED} is greater than one if convergence occurs while it is smaller than one with divergence.

The bias and the type I error rate of the CI_{ED} were evaluated with a simulation study. The starting conditions were 50 mixtures with four species randomly sampled from a uniform distribution. The final conditions were computed by randomly adding a normally distributed value to each of the starting proportions. Species proportions within a mixture were always controlled to add up to one. The CI_{ED} was calculated for 1000 simulation steps, and bootstrapping was applied to estimate the 95% confidence interval. Under the null hypothesis the index is expected to be significantly different from one in 5% of the cases.

RESULTS

The illustration of mixture shift in the wetland communities demonstrated convergence: The tetrahedron-like figure formed by the fitted lines for the final proportions (*Figure 1:* hatched lines for low water conditions) was clearly smaller than the tetrahedron of the starting mixtures (dotted lines). All the mixtures shifted towards *Carex elata*; that is, independent of the starting proportions, the final proportion of *C. elata* always increased. This behaviour reflects a strong species identity effect. With the high water level the mixtures also shifted towards *L. europaeus* (*Figure 1:* continuous lines), and the degree of convergence was slightly less pronounced.

The simulation revealed that the CI_{ED} had a slightly positive bias (0.0075) resulting in a mean of 1.0075. In total the CI_{ED} was significantly different from one in 5.8 % of the cases. The rate with the index being significantly greater than one (3.8%) was higher than the rate with the index being smaller than one (2%).

DISCUSSION

Convergence implies some form of negative feedback between the dominance of species and their performance and can arise with stronger intra- than interspecific competition (MacAr-

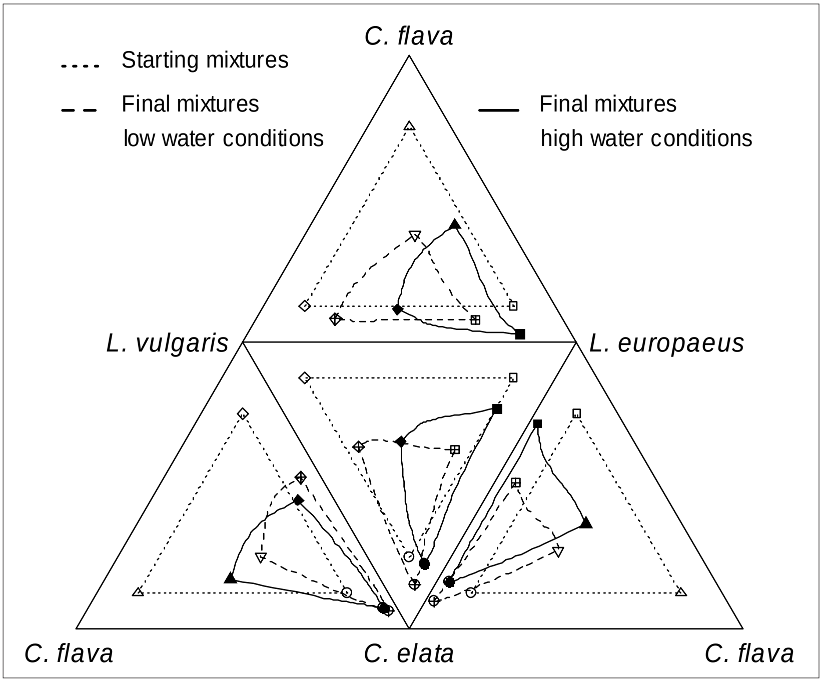

Figure 1: Tetrahedron plot to reveal changes of plant communities over time. The hatched and continuous lines are predicted mixtures, the same symbols indicate the same stands. If no shift occurred, the final mixtures would lie on the dotted line. The species with the least biomass production was omitted (*Mentha aquatica*). From Suter *et al.* (2006).

thur and Levins 1967). In the present case, convergence was partly caused by a negative feedback, but also by the identity of *C. elata* through the large difference in relative growth rates between *C. elata* and the remaining species (Suter *et al.* 2006). The higher water level modified the convergence pattern by changing the species competitive ability. Abiotic conditions influence the species' competitive performance, which in turn affects the community structure (Liancourt *et al.* 2005).

The low bias for the CI_{ED} suggests that this index is a valid measurement for convergence/ divergence patterns. The increased error rate for the CI_{ED} being greater than one can be explained by the positive bias. The overall type I error rate of 5.8% was only slightly higher than the expectation under the null hypothesis. This increased value may have been caused by the present simulation algorithm.

CONCLUSIONS

The tetrahedron plot and the convergence index CI_{ED} are helpful tools in showing plant community changes over time. They help in evaluating to what extent the observed shifts in community composition can be explained by species identity, interactions between species and abiotic conditions.

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Arthropod response to plant evenness in mixed legume-gras forage swards

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ABSTRACT

Disentangling the contribution of biodiversity to ecosystem processes is a central issue in ecology, especially given scenarios of global change in climate and biodiversity. Using experimental mixed grass-legume forage swards of the multisite experiment *Agrodiversity*, two trials were conducted, one in Ireland and one in Spain, to study the effects of plant diversity on arthropod communities. Although different in sampling period, sampling method and climate, similar patterns emerged. In both sites, there was a positive effect of plant diversity and evenness on arthropod species richness and abundance for the total arthropod responses as well as for the detritivore and parasitoid groups. This could provide evidence in support of cascading effects of diversity up the food chain. Possible yield effects are discussed.

Keywords: mixtures, diversity, evenness, species richness, arthropod abundance.

INTRODUCTION

In the field of agroecology, ecosystem functions associated with diversity have been tested in polycultures in empirical and experimental studies, and several advantages have been reported (Altieri 1999). Nevertheless, many questions remain unsolved, such as: the identification of mechanisms behind the observed properties; how to handle methodological shortcomings, such as separating evenness from identity effects (Lepš 2004), and how to deal with covarying variables which may mask real diversity effects (Hooper *et al.* 2005). Furthermore, there is a general consensus about the need to study the diversity effects across trophic levels (Hooper *et al.* 2005), including the consumer subsystems. In order to study diversity effects of legume-grass mixtures on the arthropod consumer subsystem, arthropod communities were surveyed in two sites within the COST 852 *Agrodiversity* experiment at Johnstown Castle (Ireland) and Gòsol (Catalan Pyrenees).

The objectives were to:

1) assess the effects of plant diversity and evenness on the total arthropod community and on the different trophic groups;
2) disentangle the effects of sward yield and sward diversity on the arthropod community;
3) assess whether patterns were consistent in both sites.

METHODOLOGY

The establishment of mixed swards of systematically varying proportions (i.e. evenness) of two grasses and two legumes followed the Agrodiversity experimental design (Kirwan *et al.* in press). Arthropod sampling took place before harvesting of the plant communities. At Johnstown Castle, arthropods were collected in summer and autumn 2004 and 2005 with a Vortis suction sampler (Arnold 1994). At Gòsol, pitfall traps were used over three weeks in autumn 2005, with weekly replacement. Collected arthropods were classified into families, genera, species or morphospecies. They were further sorted into trophic groups, namely herbivores, detritivores, predators and parasitoids. The arthropod response variables (abundance and species richness of the total arthropod community and of each trophic group) were modelled using generalized linear models (Dobson 2002) and following the methodology in Kirwan *et al.* (in press). A first model tested and estimated effects of plant evenness on arthropod response by entering them as a linear extension to a null model which included the identity effects of sown proportions of the plant species. Arthropods may respond to yield and/or plant diversity of mixtures. To investigate these separate effects we first introduced yield to the null model. Evenness effects were further examined in a final model which included both yield and evenness. Due to colinearity between yield and evenness, coefficients were not estimated.

F-ratios were calculated (Dobson 2002) to test:

1) the addition of sward yield to the null model and

2) the addition of evenness to the latter model.

RESULTS AND DISCUSSION

Sward yield showed a general positive relationship with sown evenness. This relationship, however, was not evident in Gòsol in the last harvest, which is when arthropods were sampled and may have influenced the observed yield effects on arthropod response. Compared with monocultures, mixtures in both sites showed enhanced arthropod total species richness and abundance, and the same response was found for the detritivore and parasitoid groups (*Figure 1*). Overall there was a positive effect of plant diversity on arthropod communities (*Figure 1*). This is contrary to other studies (Koricheva *et al.* 2000, Perner *et al.* 2005), and despite differences in sampling methods, sampling periods, and climatic conditions between the two sites. This positive effect was significant for total arthropod species richness and abundance as well as for the detritivore and parasitoid groups, for both response variables and in both sites. Furthermore, a significant diversity effect was also found for predator abundance in Gòsol. If evident at larger spatial scales, effects of plant diversity on upper trophic levels could affect ecosystem services provided by parasitism and predation.

In Gòsol yield did not influence arthropod response (*Table 1*), probably because of yield decreases at the time of arthropod sampling. In Johnstown Castle, when yield effects had been accounted for in the models (*Table 1*), we found that evenness explained a part of the variability not explained by yield for the total and parasitoid species richness and abundance, as well as for predator species richness and detritivore abundance.

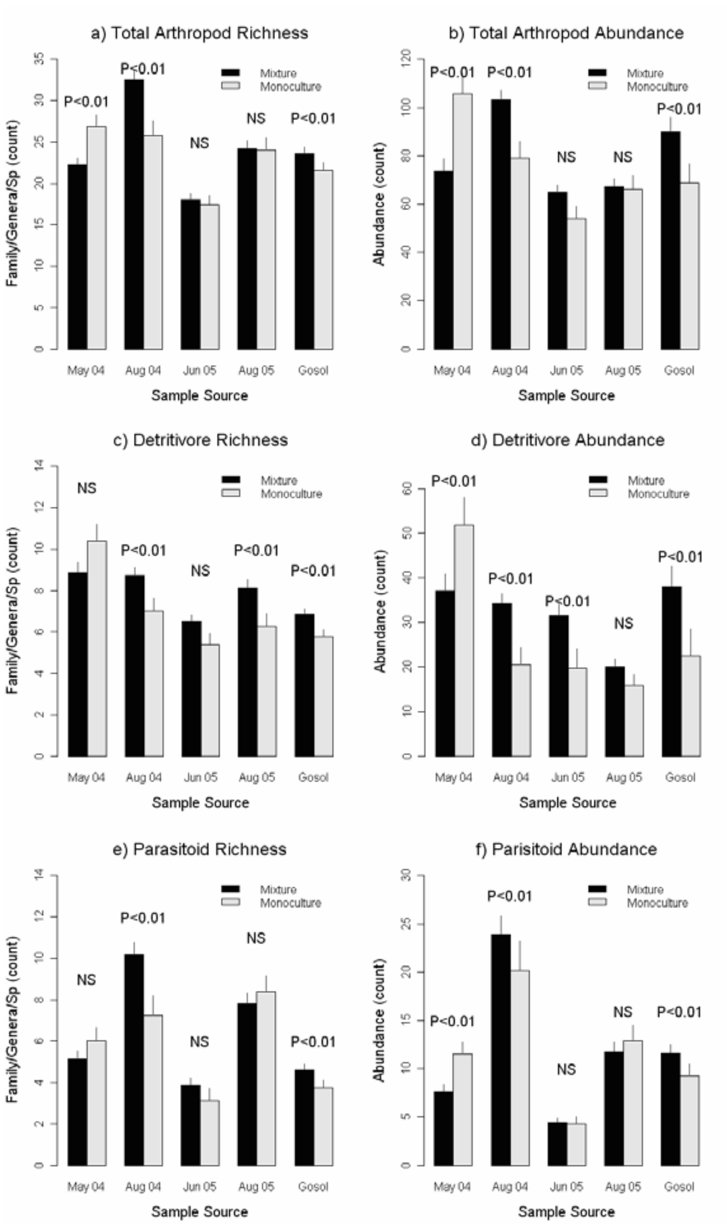

Figure 1: Arthropod species richness and abundance (average counts ± se) on four sampling occasions at Johnstown, Ireland and on one occasion at Gòsol. The bars are averages from monocultures and mixtures for global arthropod data (a, b), and for the detritivore (c, d) and parasitoid (e, f) trophic groups.

Table 1: Effects of sward yield and diversity on arthropod response variables. A significant F-ratio in the 'yield' column indicates an effect of yield on the arthropod response; in the 'yield + Div' column it indicates an effect of diversity beyond that attributable to yield. wj: total arthropod abundance without juveniles.

	Johnstown Castle		Gòsol	
	yield	yield + Div	yield	yield + Div
Sp. Richness	F (P-value)		F (P-value)	
Total	5.20 (P<0.001)	6.07 (<0.001)	1.79 (0.21)	3.49 (0.09)
Herbivore	0.72 (0.579)	1.89 (0.122)	0.003 (0.95)	1.04 (0.33)
Detritivore	6.22 (P<0.001)	2.16 (0.083)	2.99 (0.11)	6.46 (0.03)
Predator	1.45 (0.225)	2.91 (0.028)	1.39 (0.26)	1.22 (0.30)
Parasitoid	2.62 (0.041)	3.58 (0.011)	1.55 (0.24)	2.69 (0.13)
Abundance				
Total	10.80 (P<0.001)	9.18 (<0.001)	1.623 (0.231)	11.957 (0.007)
Total (wj)	7.66 (P<0.001)	6.01 (<0.001)	-	-
Herbivore	6.46 (P<0.001)	1.97 (0.108)	0.172 (0.687)	1.060 (0.330)
Detritivore	3.74 (0.008)	5.80 (<0.001)	0.999 (0.341)	5.141 (0.050)
Predator	0.46 (0.763)	1.83 (0.132)	0.738 (0.217)	3.260 (0.104)
Parasitoid	2.57 (0.044)	2.55 (0.048)	1.501 (0.249)	3.148 (0.110)

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Productivity and persistence of species in the legume/grass swards of the COST 852 experiment in Lithuania

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Keywords: grasses, legumes, yield, mixtures, monocultures

ABSTRACT

It is important to choose correct combinations of grasses and legumes and their varieties in order to exploit the complementary growth, balance of species, quality of herbage and productivity of swards. The current study was set up in order to acquire more information on the factors affecting the survival and productivity of different legume species and cultivars under Lithuanian conditions, according to the COST 852 Common protocol of the Agrodiversity Experiment. Perennial ryegrass, cocksfoot, red clover and white clover of Lithuanian varieties, with contrasting functional characteristics, were grown on a loamy Endocalcari-Epihypogleyic Cambisol in Dotnuva (55°24' N 23°50' E) at two sowing densities in varying proportions in mixtures and also in monocultures: the basic 30 plots were grown with nitrogen fertilization and the additional 18 plots without nitrogen. The advantages of pure cocksfoot and its mixtures over perennial ryegrass were demonstrated over three harvest years. Low sowing density had a negative effects on the sward yield in monocultures. The N fertilization had a positive effect on sward yield in all mixtures and monocultures, however negative effect on persistence of legumes was observed.

COST 852 - Results of Common experiment under contrasting conditions in Bulgaria

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ABSTRACT

A field trial was carried out according to the common methods of COST 852 (www.cost852.com) to determine the advantages of grass-legume mixtures in two different regions of Bulgaria. There was no significant mixture advantage at either site. In Pleven red clover was the most productive species but this was not observed in Troyan. Mixtures had lower infestation of unsown species at both sites but the levels of infestation were much higher in Troyan.

Keywords: grass/legume mixtures, dry matter yield, sowing density

INTRODUCTION

To obtain a higher yield of dry matter with better quality, as well as to decrease infestation of unsown species, grasses are grown in mixture with legumes. Legume/grass mixtures are more resistant to invasion and often perform better than pure swards (Mannetje 2006). The choice of proper combinations of grasses and legumes and their varieties is important to ensure a persistent and balanced mixture (Peeters *et al.* 2006). Legume based systems are known to contribute to sustainable, environmentally friendly agriculture (Porqueddu *et al.* 2003). The aim of this study was to assess legume based mixture swards in the environmental conditions in Central North Bulgaria.

MATERIALS AND METHODS

Experiments with 30 plots, within the common protocol of COST Action 852, were established in 2003 at Pleven and Troyan in Central North Bulgaria. Pleven is situated in a plain region at 168 m altitude (leached chernozem soil type), and Troyan is situated at the foot of the Balkan Mountains at 362 m altitude (gray forest soil type). Pure swards and eleven mixtures of two grass (*Lolium perenne* L. and *Dactylis glomerata* L.), and two legume (*Trifolium pratense* L. and *Trifolium repens* L.) species were sown under high (local recommended sowing rate) and low (60% of the local recommended sowing rate) densities. The eleven mixtures consisted of four dominated (70%) by each species in turn, six dominated by each pair of species (40% of each) and one in which all species were equally represented (25% of each) at sowing. The plots were arranged in two parallel lines, each with 15 plots, for the high and the low density, respectively. The plot size was 2 x 3 m, and before each cut a strip, 0.5 m wide, of each edge of the plot was cut to avoid the border effect. Samples for botanical composition were taken from a fixed quadrate (0.5 x 0.5 m) situated at the centre of each plot. The swards were cut at 5 cm height. One cut in the first year (2003) was obtained for the conditions in Pleven and Troyan (data not shown). Four cuts in 2004 and 2005 were obtained for

Pleven, and three and two cuts for Troyan, respectively. Dry matter yields and botanical composition data were recorded.

RESULTS AND DISCUSSION

There was no significant increase in dry matter yield between monoculture and mixtures of either medium or high evenness at either Pleven or Troyan (*Table 1*). Monoculture yield was not significantly different from average mixture yield at either site. There was no effect of density at either site nor was there an evenness x density interaction.

Table 1: Total annual yield (kg ha^{-1}) at three levels of evenness and two densities for Pleven and Troyan sites averaged over harvest years 2004 and 2005. The levels of evenness are L: monoculture, M: mixtures dominated (70%) by one species and H: mixtures dominated by two species. (40% of each and a mixture with each species equally represented). Densities are High: local recommended sowing rate and Low: 60% of the local recommended sowing rate.

Evenness	L	M	H	LvM p value	L v H p value	M v H p value	Mono vs Mixed p value
Pleven	9071	11260	11207	0.173	0.135	0.97	0.109
Troyan	5474	6075	5354	0.222	0.779	0.102	0.723
Density	Low	High	p value				
Pleven	10384	10919	0.643				
Troyan	5859	5298	0.122				

Total yields in plots with legumes exceeded the yield of monoculture grass plots for Pleven ($p<0.001$) but not Troyan (*Table 2*). Most of the yield in plots with legumes was from red clover in Pleven (*Figure 1a*) but this was not as marked in Troyan (*Figure 1b*). For plots with legumes the yield was not related to legume content for either site. There was no legume content x density interaction at either site.

Table 2: Total annual yield (kg ha^{-1}) at five levels of legumes in sown mixture for two sites

Legume %	Pleven	Troyan	P value for comparisons	Pleven	Troyan
100	13027	6014	100 vs 0	0.000	0.152
80	11198	5652	100 vs 50	0.239	0.354
50	11370	5438	50 vs 0	0.000	0.417
20	11015	5877			
0	5114	4935			

In Pleven red clover dominated in all mixed swards, with white clover contributing only a small amount and grasses only about 25% across mixtures (*Figure 1a*). The proportion of unsown species was about twice as high in grass and legume monocultures as in mixtures. In Troyan, legumes contributed somewhat less than grasses across mixtures and neither red nor white clover dominated the legume component (*Figure 1b*). Unsown species contributed about 50% in grass and legume monocultures and about half that across mixtures (*Figure 1b*).

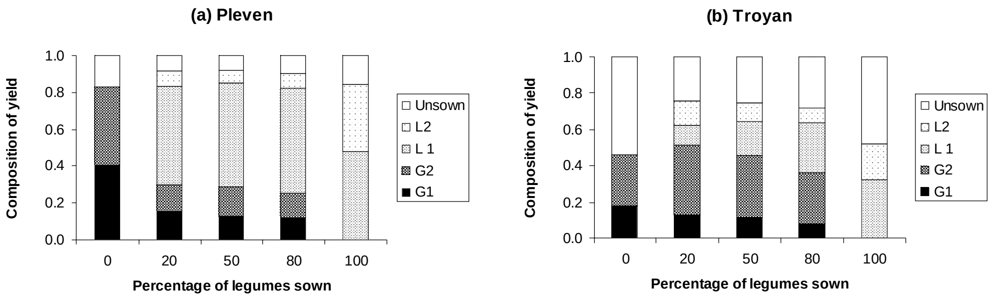

Figure 1: Botanical composition of the swards at Pleven and Troyan (av. 2004-2005) plotted against sown percentage of legumes. G1 Ryegrass, G2 Cocksfoot, L1 Red clover, L2 White clover

CONCLUSIONS

For the conditions of Pleven, red clover was the highest dry matter productive species and dominated the yields of plots with clover. Mixtures did not outperform average monocultures at either site. In both sites weed infestation was less in mixture than monoculture but the overall level of unsown species was much greater in Troyan than Pleven.

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Detection of effects driving the evolution of the swards in the COST 852 experiment fertilised at different N levels

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ABSTRACT

In the COST 852 experiment, the contribution of different functional groups to the stability of grass clover mixtures had been tested. At the Swiss experimental site, three levels of nitrogen (N) fertilisation were established in addition to the common experiment. Plots received either 10, 30 or 90 kg N after each cut, were cut five times annually and species proportions were determined during three years. Species identity, a negative feedback mechanism, positive interactions between grasses and legumes, and the level of N fertilisation were found to be the most important effects driving the evolution of the swards. Whereas *T. repens* proportion was strongly reduced by a higher N fertilisation, *T. pratense* was only slightly affected. Thus, clover proportions decreased towards the end of the experiment.

Keywords: interaction effects, negative feedback, RGRD method, species identity

INTRODUCTION

In the COST 852 common experiment, the contribution of different functional groups (grasses vs. legumes, fast vs. slowly establishing species) on grassland stability had been tested in a field experiment across a wide range of environmental conditions. The level of nitrogen (N) supply is expected to strongly affect yield and species composition due to changes in N availability to grass species (Luscher and Aeschlimann 2006). Therefore, at the Swiss experimental site, three levels of nitrogen fertilisation were established in addition to the common experiment to investigate the effects of an increasing N fertilisation on yield and shift of species composition.

MATERIAL AND METHODS

Pure and mixed stands of *Lolium perenne* L., *Dactylis glomerata* L., *Trifolium pratense* L. and *Trifolium repens* L. were established in 2002 according to the common protocol of the COST action 852 experiment. In addition, we established (i) three levels of N fertilisation after the first year's first harvest (10, 30 and 90 kg N ha^{-1} after each cut) and (ii) extreme dominant stands (sowing proportion of 90% of one species, and 3.3% of the others) and binary mixtures (50% of two species) at the intermediate N level. The swards were cut five times annually. The yield of the sown species was determined at the first harvest in 2003, 2004, 2005 and 2006. We applied the relative growth rate (RGRD) method (Connolly and Wayne 2005) to evaluate the effects of the species' identity, species interactions and N fertilisation on shift in species composition.

RESULTS AND DISCUSSION

Species identity

The evolution of one species' proportion was strongly dependent of its identity. In spring 2003 (year 1), the species expected to be fast establishing (averages of 59% and 16% for *L.*

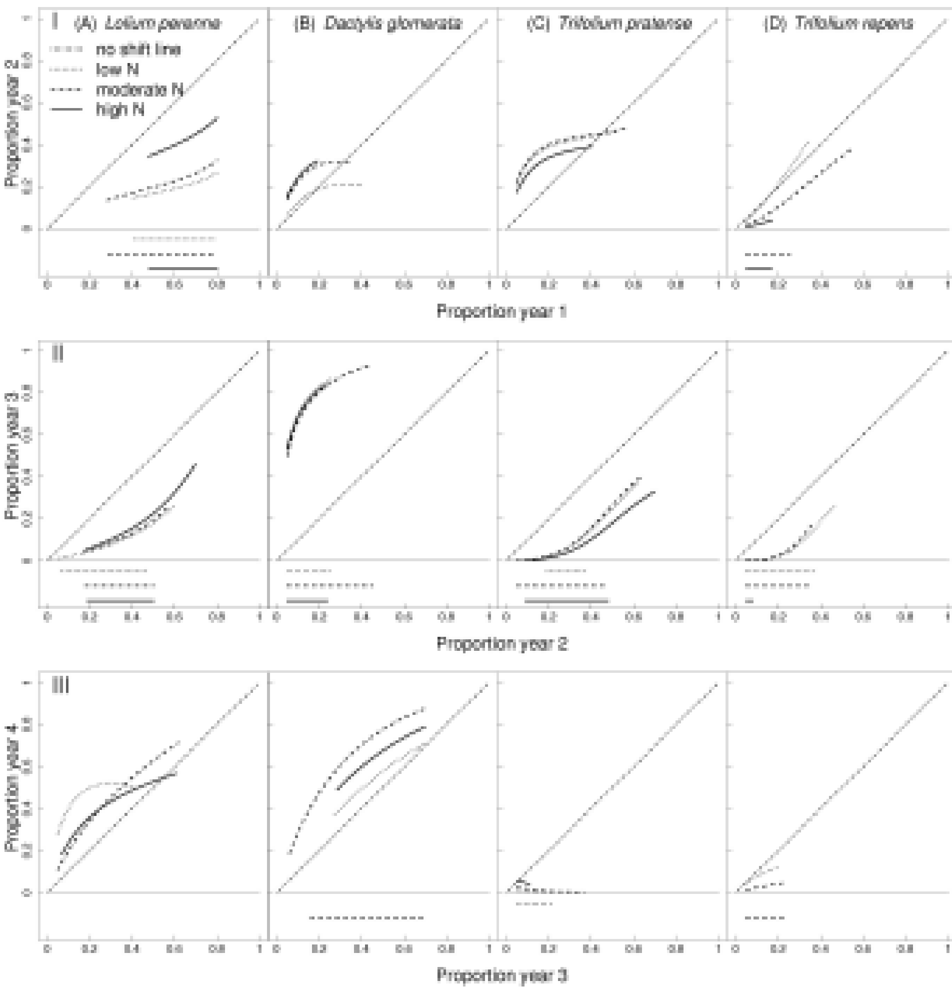

Figure 1: Final proportions of a species depending on increasing initial proportions of this same species at the three nitrogen fertilisation levels. The graphs show the fitted proportions *y*, which were calculated based on RGRD models (*Table 1*), for the years (I) 1 to 2, (II) 2 to 3 and (III) 3 to 4. Horizontal lines below the curves indicate the range of values for which vertical differences between the no shift line and the fitted proportions are significant (P ≤ 0.05). Missing bars indicate no significance over the whole range.

perenne and *T. pratense* respectively) were present at higher proportions than the species expected to be slowly establishing but more persistent (averages of 8% and 11 % for *D. glomerata* and *T. repens*, respectively). *L. perenne* and the clover proportions generally decreased from the first to the fourth year, while *D. glomerata* became increasingly dominant (*Figure 1*). These shifts are also indicated by the regression coefficients (*Table 1*, only shown for the first year): for instance, all three intercepts for the RGRD between *L. perenne* and the other species were significantly negative (-0.648, -1.336, -0.977), which means that *L. perenne* generally lost against all other species from the first to the second year. While *L. perenne* stabilised from the third to the fourth year, the loss of the clover species continued, resulting in generally low clover proportions at the end of the experiment. The decrease in *T. repens* proportions was probably due to the cutting frequency that might have been not intensive enough for white clover at this productive site.

Negative feedback

The intraspecific interaction effect most important in our experiment was caused by a negative feedback mechanism. An increasing biomass of *D. glomerata* and *T. pratense* resulted in a relative decrease of their own final proportions (*Figure 1*). For example, with respect to T. pratense, this effect is indicated by the coefficients of *T. pratense* for the RGRD between itself and all other species (1.337, 1.108 and -0.694). The detected negative feedback indicates that the species used different niches. Such behaviour has been found in various habitats (e.g. Suter *et al.* 2006), and our results suggest that niche differentiation between species may also be important in intensive grassland systems.

Table 1: Regression coefficients for the effects of increased species biomass and nitrogen fertilisation on the relative growth rate differences (RGRD) according to Connolly and Wayne (2005) for the comparison year 1 to 2. A negative intercept indicates that the 1st species generally lost against the 2nd species for the $RGRD_{1-2}$ and vice versa (for example, the intercept for the $RGRD_{Lp-Dg}$ (= - 0.648) indicates that *Lp* generally lost against *Dg*. Positive species coefficients or N coefficients indicate that an increase of a species' biomass or N fertilisation favoured the first over the second species for the $RGRD_{1-2}$ and *vice versa*. *Lp* = *L. perenne*, *Dg* = *D. glomerata*, *Tp* = *T. pratense*, *Tr* = *T. repens*, N_{mod} = moderate N, N_{high} = high N, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

year 1 to year 2	RGRD					
	Lp-Dg	*Lp-Tp*	*Lp-Tr*	*Dg-Tp*	*Dg-Tr*	*Tp-Tr*
Intercept	**- 0.648** **	**- 1.336** ***	**- 0.977** **	- 0.687	- 0.329	0.359
Lp	0.066	- 0.325	0.159	**- 0.391** *	0.093	**0.485** *
Dg	**1.433** ***	**- 1.208** *	**1.564** **	**- 2.642** ***	0.131	**2.772** ***
Tp	0.229	**1.337** ***	**0.644** **	**1.108** ***	**0.414** **	**- 0.694** **
Tr	**0.881** **	**2.636** ***	**1.578** **	**1.755** **	0.696	- 1.058
*Tr*²	**- 0.852** **	**- 1.496** **	- 0.627	- 0.644	0.225	0.869
Lp x Dg	0.241	**-0.958** *	**1.069** **	**- 1.200** **	**0.828** **	**2.028** ***
N_{mod}	- 0.291	0.119	**0.843** *	0.410	**1.134** ***	0.724
N_{high}	0.282	**0.920** *	**2.223** ***	0.639	**1.941** ***	**1.302** *

Species interactions

We found positive interactions between different species to be important. Increased initial biomass of *D. glomerata* favoured *T. pratense* over all other species. Increased initial biomass of clover promoted grasses (*Table 1*). The positive interactions between species in our system indicate that not only the presence of clover is beneficial for grasses, but also that the presence of grasses may be beneficial for clover.

Increased N fertilisation

Higher N fertilisation favoured both grass species over the clover species in the first year (*Table 1*), but not in the subsequent years. *T. repens* was consistently suppressed, whereas *T. pratense* was only slightly affected by a higher N fertilisation in all three years. We suggest that the disadvantage of *T. pratense* vs. grasses, when competing for light, was smaller compared to *T. repens*, due to the erect morphology of *T. pratense*.

CONCLUSIONS

The level of N fertilisation strongly affected the evolution of the sward composition. Furthermore, negative feedback and positive interactions between species significantly contributed to a stabilisation of the system, avoiding the swards becoming pure stands of *D. glomerata* within three years. Future research to improve the stability of clover proportion should consider cutting frequency, adjusted N fertilisation and species/cultivar persistency.

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Modelling reproductive allocation in *Sinapis arvensis*

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ABSTRACT

In this paper we study the reproductive allocation of the annual forb *Sinapis arvensis*. Linear regression has often been used to describe the relationship between reproductive allocation (RA) and biomass. Many plants in the experiment (c.a. 40%) did not reproduce. To deal with these zero responses we developed a mixture model variant of truncated regression to estimate the relationship between RA and aboveground biomass. We also examined the size of individual plants relative to the average size of plants in its stand and elevated CO_2 as possible determinants of RA. We found that elevated CO_2 enhanced RA but that biomass had little effect on it. The position of a plant within the size hierarchy had a large effect on RA; plants smaller than their neighbours allocated a smaller proportion to reproduction.

Keywords: reproductive allocation, truncated regression, zero values, mixture model, CO_2

INTRODUCTION

The annual forb *Sinapis arvensis*, native to Eurasia, is an important agricultural weed in the mid-western regions of the USA. (Fogg 1950). In this paper we study its reproductive allocation.

MATERIALS AND METHODS

Seeds of *S. arvensis* were sown in 25 cm diameter pots at 6 densities, 1, 2, 4, 8, 16 and 32 plants per pot and at CO_2 levels of 350 µL L^{-1} (ambient) or 700 µL L^{-1} (elevated). The experiment was structured as three blocks; density x CO_2 combinations were replicated twice within each block except for those at the lowest density, which were replicated four times, giving a total of 84 pots with 704 measurable individual plants. The aboveground biomass of each plant and its reproductive biomass were measured at harvest. Ln-ln linear allometric regression has often been used to describe the relationship between reproductive allocation (RA, defined here to be the ratio of reproductive biomass to aboveground biomass) and aboveground biomass. Many plants in this experiment (c.a. 40%) did not reproduce (*Figure 1*). This has previously been dealt with using truncated regression (Schmid *et al.* 1994). However, the circled values in *Figure 1* suggest that there are values that would not fit well with this method. We propose a modelling approach that allows for two possible groups within the RA responses. The first group contains all reproducing and some non-reproducing plants, but we assume all these plants had the ability to reproduce. The second group contains the remaining non-reproducing plants and we assume they could not have reproduced under their experimental conditions. We developed a mixture model in which the proportions of the two groups

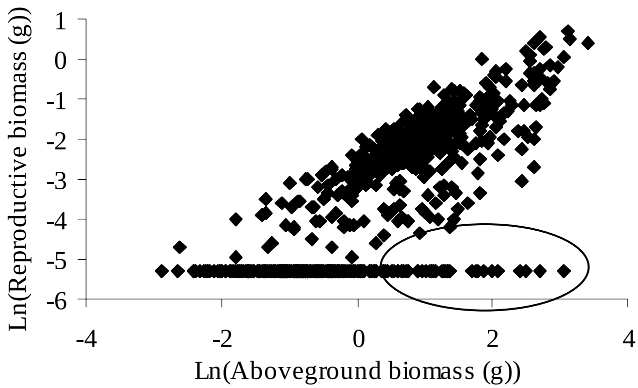

Figure 1: Ln (Reproductive biomass) vs Ln (Aboveground biomass). Note: The logarithm of non-reproducing plants is defined to be = -5.299.

were (1-*p*) and *p* respectively (Mc Lachlann and Peel 2000). For the first group we used a variant of truncated regression to model the relationship between the RA and aboveground biomass and CO_2. In the second group we modelled the proportion *p* as a logit function of aboveground biomass, CO_2 and Ratio (size of an individual plant relative to the average size of plants in their pot). Model parameters were estimated using maximum likelihood. Relevant interactions were also tested.

RESULTS

Conditional on being from group 1, we found that RA decreased as biomass increased but it increased under elevated CO_2, (*Table 1*). The proportion of plants from group 2 decreased under increasing biomass and Ratio and under elevated CO_2. There was a significant interaction between biomass and ratio in group 2. Plants smaller than the average size of plants in its stand (Ratio < 1) had a high proportion of plants in group 2 (*Figure 2*). Predictions from the joint distribution of the two groups are in *Figure 3*.

Table 1: Parameter estimates for the model conditional on being from group 1

Parameter	Estimate	S.E.	P-value
Intercept	-3.13	0.074	<.001
Ln(Biomass)	-0.11	0.041	0.010
CO_2	0.15	0.081	0.067

DISCUSSION AND CONCLUSIONS

We found that CO_2, aboveground biomass and position in size hierarchy affected the RA of *S. arvensis*. There was strong evidence for a second population of non-reproducing plants that did not follow the truncated allometric relationship. Overall, elevated CO_2 enhanced RA and plant biomass did not have a major effect on RA. The position of a plant within the size hierarchy of a stand had an impact on its reproductive allocation, the smaller a plant was relative to its neighbours, the less allocated to reproduction. The range of density in the expe-

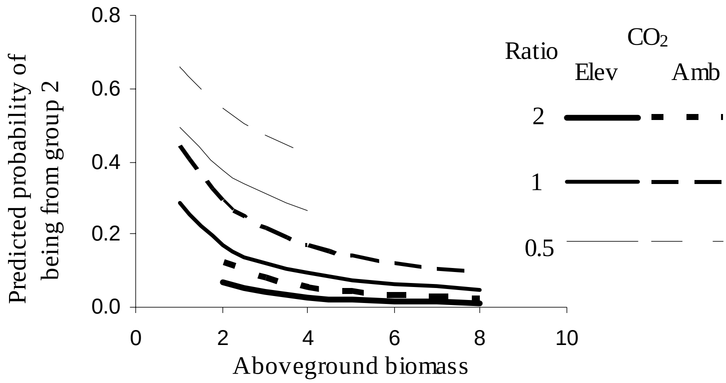

Figure 2: Predicted proportion of plants in group 2 versus biomass (g) for different combinations of CO_2 and ratio

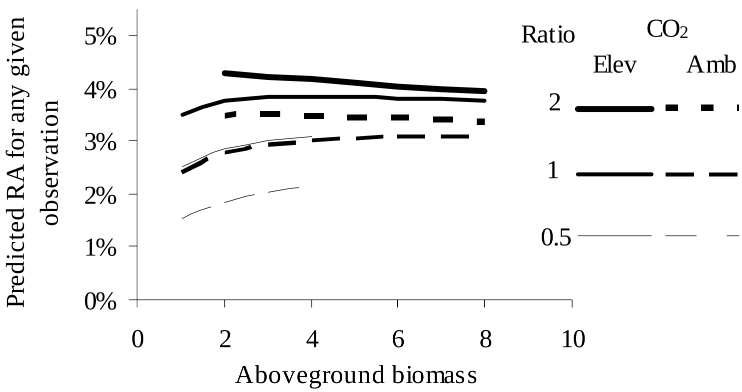

Figure 3: Predicted RA from the joint distribution of the two groups versus biomass (g) for different combinations of CO_2 and ratio

riment design was included to give a large range of plant sizes. It is not directly included in the model but is included implicitly through the size variable.

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The effect of the condensed tannins on the feeding behaviour of lactating sheep grazing a monoculture of sulla (*Hedysarum coronarium* L.)

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Keywords: Feeding behaviour, sheep, condensed tannins, PEG

ABSTRACT

The concentration of condensed tannins (CT) in sulla (*Hedysarum coronarium* L.) is moderate and overall regarded as beneficial. However, the intake of this forage can reduce diet digestibility, particularly during flowering phase, and negatively affect the dietary preference of sheep for this legume. An experiment was run to assess the effect of CT on feeding behaviour of sheep rotationally grazing sulla during flowering phase. Twenty-four late-lactating sheep were blocked in two homogeneous groups and submitted to the following treatments: i) PEG, dosed twice daily with 200 g/d of a 50% w/v water solution of polyethylene glycol; ii) C, (control), dosed twice daily with 200 g/d of water. The feeding behaviour (3 sheep per group) was continuously monitored for 24 hours in 6 weeks using the IGER behaviour recorders. On average, PEG group tended to have longer total grazing ($P < 0.07$) and eating time ($P < 0.09$). PEG group showed higher number of daily grazing meals ($P < 0.01$) and shorter interval between grazing meals than control group ($P < 0.01$). Control sheep offset the increasing amount of CT consumed by a reduction of total time devoted to grazing and an increase of the inter-meal intervals relatively to the PEG-dosed sheep.

INTRODUCTION

Sulla (*Hedysarum coronarium* L.), a short-lived perennial legume well adapted to most of Sardinian arable soils, has the potential to improve the nutrition and performance of sheep basically fed on grassland (Molle *et al.* 2003). The concentration of condensed tannins (CT) in sulla is moderate and overall regarded as beneficial for i) enhancing N utilisation in the gastro-intestinal tract, due to an effective ruminal by-pass of dietary protein, and ii) increasing parasite burden resilience. Previous research reporting the diet preference of Sarda dairy ewes given a choice of sulla and annual ryegrass (*Lolium rigidum* Gaudin), during the vegetative phase of pasture, showed a partial preference for sulla (Rutter *et al.* 2004). There was a clear decreasing trend of preference for sulla throughout the observation period probably due to the presence of condensed tannins. The concentration of CT tends to rise in sulla during flowering phase (Molle *et al.* 2003). An experiment was then undertaken on late-lactating sheep to assess the effect of grazing a monoculture of sulla at flowering phase on the feeding behaviour of sheep exposed or not to CT effect.

MATERIALS AND METHODS

The experiment was run during spring 2004 (April-June, flowering phase of sulla) at the Bonassai research farm (NW Sardinia, Italy, 33 m a.s.l., mean annual rainfall 582 mm). Two experimental plots (0.8 ha) of sulla (cultivar Grimaldi) were sown in October

2003 (35 kg ha^{-1}) and subsequently were rotationally grazed by twenty-four lactating sheep (grazing cycle = 14 days). The sheep were blocked in two homogeneous groups on the basis of live weight (48.3 ± 1.54 kg, means ± SEM) and milk yield (1946 ± 75 ml) and submitted to one of the following treatments: PEG, dosed twice daily with 200 g/d of a 50% w/v water solution of polyethylene glycol; C, (control), dosed twice daily with 200 g/d of water. The sheep groups were grazed as a flock all day with exception of milking time (two milkings daily). No supplement was offered. Herbage mass and sulla herbage DM proportion, were measured at beginning of each grazing period cutting twelve 0.5 m^2 quadrats per plot to ground level and partitioning herbage samples into sulla and other species. The feeding behaviour was continuously monitored for 24 hours in 6 weeks in 3 sheep per group. The IGER behaviour recorders (IBR, Ultrasound advice, London, UK; Rutter *et al.* 1997) were used. The records were analysed using the software Graze 0.8 (Rutter 2000), that computes eating time (ET, total of biting and chewing times), grazing time (GT, the sum of ET and any inter-meal time lower than 360 s), ruminating time (RT) and idling time (IT). The number of meals and meal duration was also calculated setting the minimum meal length to 60 s and the maximum intra-meal interval to 360 s. Hand-plucked samples of herbage were collected every fortnight and stored at -20°. The samples were then freeze-dried prior to analyse for DM, and CT expressed as leucocyanidin equivalent (by Butanol-HCl method). Feeding behaviour data were averaged per group and analysed with paired t-test to assess the effect of PEG supplementation.

RESULTS AND DISCUSSION

The pasture was featured by high herbage mass and proportion of sulla throughout the experiment (*Figure 1*). CT concentration of sulla hand-plucked samples ranged between 2 and 4% DM along with the trial, in line with previous results (Molle *et al.* 2003). On average, PEG group showed a trend towards longer total grazing ($P < 0.07$) and eating time ($P < 0.09$, *Figure 2*). No difference was found between treatments for total idling and ruminating times ($P > 0.2$). PEG group showed a higher number of daily grazing meals ($P < 0.01$) and shorter interval between grazing meals than control group ($P < 0.01$, *Table1*).

Overall this study shows that, despite the relatively low level of CT found in sulla samples, the sheep exposed to CT effect (control sheep) tended to graze less and in particular lengthened their inter-meal intervals probably trying to cope with the accumulation of CT in their

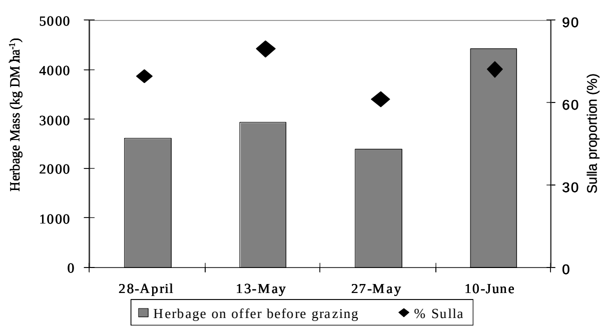

Figure 1: Herbage mass on offer (kg DM ha $^{-1}$) and percentage of sulla before grazing

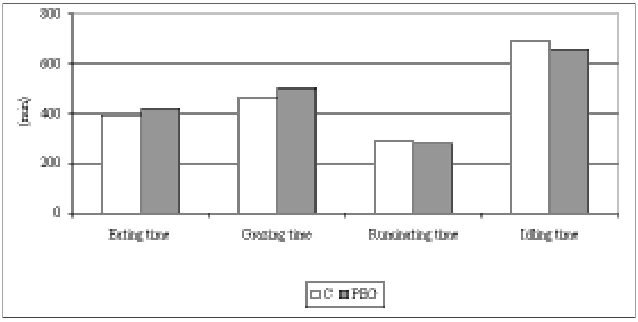

Figure 2: Effect of PEG on behavioural activities of grazing ewes

Table 1: Number and duration of meals in ewes supplemented (PEG) or not (C) with PEG

	C	PEG	SEM	P<
Inter-meal time (min)	56	43	1.98	0.01
Grazing meal duration (min)	27	23	0.58	0.01
Number of grazing meals	19	24	1.13	0.01

gastro-intestinal tract. In heterogeneous pastures, ruminants usually try to alternate the intake of tannic with non-tannic plants in order to dilute the dietary tannin concentration as observed by Villalba and Provenza (2002) in lambs. They found that when tannin ingestion increased, the satiation on the tannin-containing food likely encouraged them to explore alternative sites to get non-tannic feedstuffs.

CONCLUSION

Sheep grazing CT-containing Sulla (C group) grazed for a shorter time and had longer inter-meal intervals than the counterparts not exposed to CT effect (PEG group). The effect of CT on feeding behaviour was less evident than expected probably due to factors such as: relatively low level of condensed tannins in the Sulla pasture; high quality and quantity of herbage mass on offer which probably encouraged a selection against the most tannin plant components; possibly a social facilitation effect.

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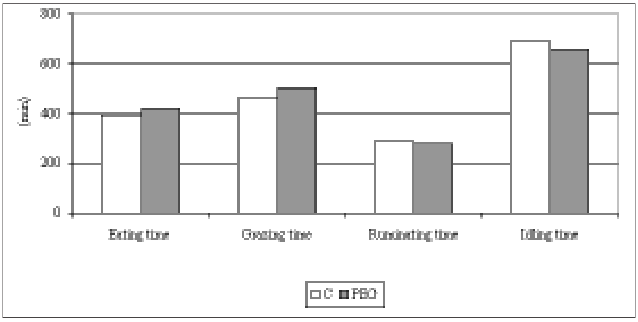

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Differences between red clover and ryegrass silages with possible consequences on PUFA-content of dairy products

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Keywords: Dairy, Silage, Lipolysis, PUFA, Red clover

ABSTRACT

The objective of the study was to determine differences between red clover and ryegrass silages that could influence PUFA-content of dairy products. Therefore labscale silages (pure ryegrass (RG), RG/red clover (RC) (70/30)) were made and kept for 8 weeks (n = 4). After mowing, wilting and ensiling the total amount of free fatty acids was determined. A significantly lower degree of lipolysis occurred in silages containing RC, compared with RG silages. Inhibition of lipolysis in silages with RC could be part of the explanation for the higher PUFA content found in milk from dairy cows fed RC silage. In a second experiment lipase activity of crude extracts of RG and RC with and without removal of phenols, was measured after 48 h incubation. The objective of this experiment was to look for a possible explanation for the lower degree of lipolysis, found in RC silages. Lipase activity in RC extracts with phenols was approximately 5 times lower than in the other two extracts (RG and RC minus phenols). The RC extract minus phenols presumably was lacking the substrate to express PPO activity. This makes PPO a possible lipase inhibitor with phenols as a substrate.

INTRODUCTION

Jones *et al.* (1995) reported that in alfalfa silages up to 80% of the nitrogen occurs in the non-protein-nitrogen (NPN) fraction, whereas in RC silages this was only 40%. The lower proportion of NPN in RC silage would be due to inhibition and/or obstruction of the plant proteases due to binding of quinones, formed from diphenols as a result of polyphenol oxidase (PPO) activity, to proteins and enzymes. Besides proteases, plant lipases also have been shown to be active during feed storage, wilting or ensiling. Due to lipase activity, fatty acids are liberated. These free fatty acids (FFA) are possibly more prone to oxidation prior to ensiling and/or digestion and biohydrogenation by rumen microbes. Thus the lipids of silages will be more saturated in the rumen than lipids of fresh forage, due to the higher proportion of FFA in silage. This could explain the difference in FA composition of milk from fresh grazing or silage fed dairy cows. Feeding RC silage instead of grass silage also has been found to induce higher milk polyunsaturated fatty acids (PUFA) (Dewhurst *et al.* 2003). This could possibly be due to in silo inhibition of lipase due to binding with quinones, formed by PPO activity. The aim of this study is to evaluate differences in lipolysis during ensiling between RG and RC by making labscale silages and asses the role of PPO in lipase inhibition.

MATERIALS AND METHODS

For the determination of in silo lipolysis, labscale silages (± 3kg fresh material) (pure RG, RG/RC (70/30)) were made and kept for 8 weeks (n = 4). RC (cv. 'Merviot') and RG (cv. 'Merlov') were mowed separately at the end of September 2004. The RG had a regrowth of 14 days, the RC of 50 days. The forages were wilted on a grid with airflow underneath, to accelerate drying. After 24 hours, part of the RG was ensiled; the other part was ensiled after mixing with the RC (70/30 RG/RC fresh matter ratio), further named mixed RC. No additives were used. Samples were taken after mowing, wilting and ensiling to determine the total amount of FFA and DM-content. A lipid extract was made by weighing 5 g of fresh material and using chloroform:methanol (2:1) extraction according to Folch *et al.* (1957). Thin layer chromatography (TLC) was used to separate lipid classes (Demeyer *et al.* 1978), after extraction from the silica gel, fatty acid were determined using gas chromatography (GC) after methylation (Raes *et al.* 2001).

In the second experiment crude extracts were made from 3 g of fresh material in a phosphate buffer (pH = 7.0). After ultraturraxing, incubation for 48 h, centrifugation and filtration, the lipase activity was measured by adding 100 µl of crude extract to 3.8 ml of phosphate buffer (pH = 7.0) and 100 µl of a methylumbelliferyl oleate (MBF) solution, fluorescence was measured. Before ultraturraxing, polyvinylpirrolidone (PVP), a phenol binding agent, was added or not to the RC extract. Therefore, there were 3 treatments.

RESULTS AND DISCUSSION

The DM contents of the forages at ensiling were 277 g/kg and 155 g/kg for the RC and RG, respectively. It seems there was a problem with the drying system as the ryegrass did not dry in contrast with the red clover. This implicated a difference in DM at ensiling which could have significant effects on silage fermentation rate and thus also on the lipase activity. All silages were of good quality.

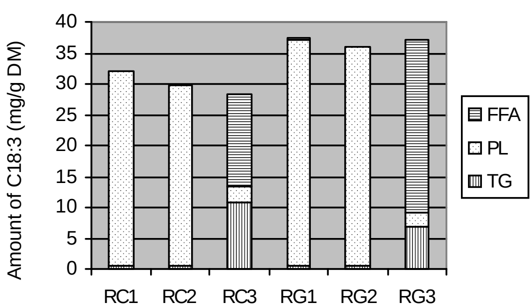

Figure 1: Amount of linolenic acid as free fatty acids (FFA), in polar lipids (PL) or as triacylglycerols (TG) after mowing (1), wilting (2) and ensiling (3) of mixed red clover (RC) and ryegrass (RG). (n = 3 for treatments 1 and 2 and n = 4 for treatment 3)

The amount of linolenic acid (C18:3) in the different fractions is reported in *Figure 1*. A decrease of linolenic acid during wilting was observed for RG and RC silage. This could be explained by oxidation of linolenic acid.

Almost no shift is observed in lipid fraction distribution after wilting. The majority of the lipolysis occurred during ensiling, resulting in a major shift in lipid fraction composition. Not only were fatty acids liberated due to lipase activity, in addition the amount of TG increased significantly, probably due to a stress activated enzyme called diacylglycerol acyltrasferase (DGAT). This enzyme mediates the last step in the formation of TG from monogalactosyl and digalactosyl diglyceriden (Kaup *et al.* 2002).

The amount of linolenic acid that was still present in the esterified fraction (TG or PL) in RC silages was, both proportionally as absolutely, higher compared to RG silages. Although it cannot be excluded that the DM content of the ensiled forage has a significant effect on the lipolysis in silo, it can be stated that the lower lipolysis was at least partially due to the inhibition of lipase in RC. Quinones, which are mostly formed by PPO activity, may be the main cause of this inhibition. This inhibition could be part of the explanation of the higher milk PUFA found in organic milk.

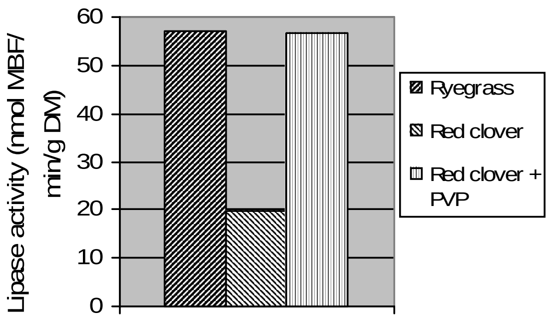

Figure 2: Lipase activity measured in ryegrass (RG) extracts and red clover extracts with (RC + PVP) and without (RC) polyvinylpirrolidone (PVP)

Figure 2 shows the results of the lipase activity measurement on a RG extract, RC extract with and RC extract without PVP, performed in a second experiment. The measured activity in RC extracts with added PVP (without free phenols) was equal to lipase activity in RG extracts. Lipase activity in RC extracts with phenols (without added PVP) was approximately 3 times lower than in the other two extracts (RG and RC minus phenols). The increase of RC lipase activity through PVP addition suggests phenols to be involved in a lipase inhibitory process. Phenols represent the substrate of polyphenol oxidase. PPO in RG has been reported to be considerably lower than PPO activity in RC. This makes PPO a possible lipase inhibitor, because PPO causes cross linking in proteins with phenols as a substrate. Cross linking in proteins could cause inhibition of enzymes (e.g. protease and lipase) (Jones *et al.* 1995).

CONCLUSIONS

Lipase activity in RC extracts with phenols was significantly lower than in RG extracts, presumably due to binding with quinones, formed by PPO activity. A lower lipolysis was also found in silages with RC, as suggested from the higher amount of esterified linolenic acid after ensiling. This could be part of the explanation for the higher PUFA content found in milk from dairy cows fed RC silage.

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Bioactive substances in *Trifolium pratense* and *Trigonella foenum-grecum*

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Keywords: red clover, trigonella, flavonoids, HPLC analysis

INTRODUCTION

Flavonoids are widely distributed in the Leguminosae family and they are usually found in plants as glycosides. Red clover (*Trifolium pratense* L.), is a biennial plant which is grown worldwide and is an important forage for ruminants and a health food for humans due to its antioxidative, antiviral, anticancerogenic, antispasmodic, estrogenic and expectorant properties (Leung and Foster 1996). Most of these properties are connected with isoflavones, which are found as red clover components. Isoflavones comprise a subgroup of flavonoids and from a chemical point of view they are structured by a chromane skeleton provided with a phenyl substituent at the C3 position (Rijke *et al.* 2001). In red clover, the isoflavones found are mainly formononetin and biochanin A, with smaller concentrations of daidzein and genistein, while formononetin and biochanin A are precursors of daidzein and genistein, respectively (Wu *et al.* 2003). Several methods to determine the content of isoflavones in red clover using high-performance liquid chromatography have been reported, where some of them are qualitative and other quantitative (Krenn *et al.* 2002, Klejdus *et al.* 2001, Rijke *et al.* 2001, Wu *et al.* 2003). However, in those studies, isoflavones were determined either in the leaves, flowers, stems or roots, mainly collected at the flowering stage.

Trigonella (*Trigonella foenum-graecum*) has been widely cultivated in Asia, Africa and Mediterranean countries for the edible and medicinal properties of the seeds. It has been reported that the seeds of trigonella have many pharmacological activities such as hypoglycaemic, cardiotonic and antihypertensive (Srinivasan 2006, Kaviarasan *et al.* 2006). In the aerial part of fenugreek the flavonols kaempferol, quercetin and the isoflavone formononetin as well as several glycosides of these substances have been found.

The purpose of the present study was to quantify the four most important flavonoids in the aerial part of red clover and trigonella, not only in samples collected at the flowering stage but also in samples collected at the vegetative and fruiting stage and to establish a faster and more sensitive analytical method than the ones already reported.

MATERIALS AND METHODS

Pure genistein, daidzein and kaempferol compounds were obtained from Alexis Biochemicals (Switzerland), biochanin A from Sigma-Aldrich (USA), formononetin from Extrasynthese (France) and quercetin from ICN Biomedicals (USA). Hydrochloric and phosphoric acids and all solvents used throughout the study were obtained from Merck (Germany). The purity of all organic reagents was checked by HPLC prior to their use.

Samples of the aerial part of red clover (variety Salino) and of Trigonella leaves were collected from plants grown in the field of the Agricultural University of Athens during vegetative, flowering and fruiting stage. 10 mg of the red clover dried - pulverized tissue were mixed with 700 ì l methanol, 100 ì l HPLC-grade water and 100 ì l concentrated HCl (37%) and the mixture was refluxed at 90°C for 1 hour. After filtration and dilution of the extract in HPLC-grade water (1% H_3PO_4 v/v), 100 ì l of the mixture were injected in an HPLC Spectra system P4000, equipped with an NPS column (53 mm × 4.6 mm, ID 1.5ì m). A gradient elution programme was used for 15 minutes with diluent Â: HPLC grade water with 1% H_3PO_4 (v/v) and diluent Â: acetonitrile with 1% H_3PO_4 (v/v), flow rate 0.5 ml/min.

Similarly one g, of the Trigonella dried and pulverized leaves, was extracted in an ultrasonic bath for 60 min at room temperature with 40 ml methanol-water (4:1, v/v). Subsequently, 10 ml concentrated HCl (37%) were added and the mixture was refluxed at 90ºC for 2 hours. After filtration and dilution of the extract in HPLC- grade water (1% H_3PO_4 v/v), 100 µl of the mixture were injected in a Spectra system P4000, equipped with a C18 column (125 mm × 2 mm, ID 5 ì m). A gradient elution programme was used for 18 minutes with diluent Â: HPLC water with 1% H_3PO_4 (v/v) and diluent Â: acetonitrile with 1% H_3PO_4 (v/v), flow rate at 0.5 ml/min. Determination of linearity, repeatability, accuracy, LOD and LOQ tests in samples and standards were performed.

RESULTS

The two forage crops grew satisfactorily, though trigonella grew faster and produced more forage dry matter than red clover (*Table 1*).

Table 1: Productivity of the two forage crops at different phonological stages and the dates these were obtained.

	Vegetative	Flowering	Seeding
Red clover	May 12th 136,3 g DM m^{-2}	June 20th 181,8 g DM m^{-2}	July 14th 209 g DM m^{-2}
Trigonella	April 24th 297,2 g DM m^{-2}	May 25th 388,3 g DM m^{-2}	June 12th 380 g DM m^{-2}

The greatest concentration of flavonoids was detected in red clover tissues collected at the vegetative stage. The aerial part of red clover at this stage contained 12.80 mg isoflavones g^{-1} dry matter, at the fruiting stage 12.42 mg isoflavones g^{-1} dry matter and at the flowering stage 9.76 mg isoflavones g^{-1} dry matter (*Table 2*). Formononetin and biochanin A were the principal isoflavones in red clover (formononetin found in higher concentration than biochanin A, in all studied stages), while daidzein and genistein were in low concentrations.

None of the trigonella samples in any of the phenological stages (vegetative, flowering and fruiting stage) contained formononetin (*Table 3*). The greatest concentration of flavonoids was detected in *Trigonella* leaves cut in the flowering stage, whereas at the fruiting stage total flavonoids were markedly lower. Moreover, the leaves contained more kaempferol than quercetin in any of the studied stages.

Table 2: Concentration of flavonoids in the aerial part of red clover at three phenological stages

Phenological stages	**Daidzein** mg g^{-1} DM	**Genistein** mg g^{-1} DM	**Formononetin** mg g^{-1} DM	**Biochanin A** mg g^{-1} DM	**Total flavonoids** mg g^{-1} DM
vegetative	0,23	0,22	8,24	4,11	12,80
flowering	0,63	0,11	6,20	2,82	9,76
fruiting	1,70	1,07	6,20	3,45	12,42

Table 3. Concentration of flavonoids in trigonella leaves at three phenological stages

Phenological stages	**Quercetin** mg g^{-1} DM	**Kaempferol** mg g^{-1} DM	**Formononetin** mg g^{-1} DM	**Total flavonoids** mg g^{-1} DM
Vegetative	4,80	6,42	0	11,22
flowering	5,46	7,08	0	12,54
fruiting	2,46	5,10	0	7,56

CONCLUSIONS

The developed assay has been shown to be accurate, sensitive, precise and reliable. The time of each analysis was much shorter (only 15 minutes) compared to the time analysis of previous studies (61 min in Krenn *et al.* (2002), 40 min in Wu *et al.* (2003) and 42 min in Rijke *et al.* 2001)), mainly due to the use of a considerably shorter column, which proved also to have great separation capacity and, combined with the specific elution program, yielded clear chromatograms.

Trigonella crop grew faster and more productive than red clover. Concentration of total flavonoids in the aerial part of red clover (biochanin A, formononetin, daidzein and genistein) was different at the studied phenological stages as it was also in the leaves of trigonella (quercetin, caempferol). It is concluded that further research is necessary if these forages are to be used for their bioactive substances.

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Mineral content of chicory (*Cichorium intybus*) and narrow leaf plantain (*Plantago lanceolata*) in grass-white clover mixtures

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Keywords: mineral content, chicory, narrow leaf plantain, grass clover

ABSTRACT

Organic dairy farmers in The Netherlands seek for system solutions to reduce the dependence on external minerals for supplementation of their dairy cows. In general herbs are known for a higher mineral content than grass. The objective of this study is to investigate the mineral content of chicory (*Cichorium intybus*) and narrow leaf plantain (*Plantago lanceolata*) in grass-white clover (*Trifolium repens*) mixtures. On five organic dairy farms on sandy soil and three farms on clayey soil, a mixture of grass (*Lolium perenne*), white clover, chicory and narrow leaf plantain were established in each of three years. From each mixture one fresh sample was taken at cutting stage in three following years. From the fresh samples the different plant species in the mixtures were separated and analysed on mineral content. Chicory had higher mineral content than grass and white clover. Most significant differences in mineral content between chicory and grass and white clover were for sulphur, copper and zinc. However, before chicory is recommended for increasing the mineral status, possible negative aspects of this herb such as lower DM intake by the animal, bitter milk taste and reduced absorption of copper through interaction with sulphur should be taken into account.

INTRODUCTION

Herbs can be included in grass clover mixtures for different reasons. For example a group of Danish dairy farmers, who are delivering their milk to a certain factory, grow a mixture of grass-clover with herbs for sector image purposes. Another reason for including herbs is enhancing functional biodiversity. For example, through the deep rooting system, chicory is able to utilize sub-soil N which is not used by ryegrass (Høgh-Jensen *et al.* 2006). Another form of functional biodiversity is the better production from narrow leaf plantain under drought conditions (Van Eekeren, unpublished results). On the other side it is also important that the dry matter production of the mixtures with herbs is not negatively influenced. The same goes for animal intake. For a lot of herbs it is known that they are very palatable for livestock. However, in the Netherlands it is the experience that goats avoid chicory when they are given a choice. Looking at milk production, Eriksen *et al.* (2006) could not find significant differences between cows grazing on grass clover with or without chicory. However, they found a barny and bitter taste of the milk from cows grazing on chicory. The presence of plants secondary metabolites (e.g. aucubin, a natural antibiotic in plantain, and condensed tannins and sesquiterpene lactones in chicory) may have positive effects on livestock health. In addition to the above benefits, herbs are known for a higher mineral content than grass. In the literature chicory and narrow leaf plantain are mentioned as potential herbs for inclusion in grass clover mixtures with a higher content of potassium, sodium, calcium, sulphur, boron, manganese, zinc, copper cobalt and selenium (Crush and Evans 1990, Younie *et al.* 2001). Organic dairy farmers in The Netherlands seek for system solutions to reduce the dependence on external minerals for supplementation of their dairy cows. The objective of this study is to investigate the mineral content of chicory and narrow leaf plantain in grass white clover mixtures on two soil types in the Netherlands.

MATERIAL AND METHODS

On five organic dairy farms on sandy soil and three farms on clayey soil, a mixture of grass (*Lolium perenne*), white clover (*Trifolium repens*, cv. 'Alice' or 'Riesling'), chichory (*Cichorium intybus* cv. 'Forage Feast') and narrow leaf plantain (*Plantago lanceolata*, local cultivar) were sown over the years 2002 to 2004. From each mixture one fresh sample was taken at the time of harvesting. From the fresh samples the different plant species in the mixtures were separated and analysed for mineral content with the AES-ICP method (Atomic Emission Spectrometry-Inductive Coupling Plasma technique). Only selenium was analysed with the AFS method (Atomic Fluorescent Spectrometry technique). Data for sandy and clayey soils were statistically analysed separately with a one way ANOVA, in which farms were treated as randomised blocks.

RESULTS

White clover, chicory and plantain had higher calcium content than grass on both soil types (*Table 1*). On sandy soils, chicory had significant higher contents of sodium, potassium, sulphur, copper and zinc, compared to grass and white clover. On clayey soils the mineral con-

Table 1: Mineral contents of the different crops on sandy and clayey soils

	Sand (5 farms)				Clay (3 farms)		
	Grass	White clover	Chicory	Plantain	Grass	White clover	Chicory
g kg^{-1} DM							
Crude protein	161.6ab	244.9c	179.2b	136.2a	141	235	158
Crude fibre	271	229	239	206	281	276	306
Ash	102.1a	102.6a	153.6b	113.8a	124.7	106.0	130
Calcium	4.67a	11.93b	13.30b	13.24b	6.87a	17.80b	15.10b
Sodium	0.94a	1.94ab	3.78b	1.04a	4.0	4.6	9.1
Phosphorous	4.91b	3.76a	5.76b	4.78ab	5.13b	3.43a	4.60ab
Potassium	36.9a	34.5a	54.6b	39.2a	34.6	25.4	30.6
Sulphur	3.53b	2.38a	5.32c	4.04b	3.97b	2.13a	4.33b
Magnesium	2.45a	3.53b	3.70b	3.20ab	2.42	3.20	3.80
mg kg^{-1} DM							
Iron	177.6c	140.1bc	131.6b	75.2a	148	113	178
Copper	8.85a	7.62a	19.76b	9.74a	8.97a	9.00a	11.97b
Zinc	52.4a	40.2a	229.2b	74.0a	29.7a	22.3a	47.0b
Manganese	101.4ab	77.1ab	117.0b	66.6a	34.8	26.7	29.7
µg kg^{-1} DM							
Cobalt	102	55	114	81	43.3	48.3	82.7
Selenium	43.3	28.9	33.4	42	123	126	220

Note: Values denoted with the same letter on the same line are not significantly different ($P < 0.05$).

tent of chicory was only significantly different from grass and white clover for sulphur, copper and zinc.

DISCUSSION AND CONCLUSIONS

Chicory had significantly higher contents of sulphur, copper and zinc than grass and white clover on both soil types. Therefore, it can be concluded that chicory has potential to be used on farms with a mineral deficiency and more specific a copper or zinc deficiency. Here, the question arises how much the intake of chicory has to be for covering a mineral requirement. For example, on an organic dairy goat farm no external minerals for supplementation were used, and Smolders *et al.* (2005) reported a copper deficiency. Using the daily ration of this farm of 1,5 kg DM grass clover and 1,0 kg DM grain per lactating goat, 40% chicory in the grass clover herbage DM would be necessary to meet the recommended requirement (10 g copper kg DM^{-1}). In this calculation it is assumed that 100% of the copper in the chicory is absorbed. Results of Youni *et al.* (2001) showed that part of the extra copper in chicory is absorbed. In their research the mineral status, including copper, of lambs grazed on 100% chicory was increased in comparison to grass. At the other side Jongbloed *et al.* (2005) argue that copper in the rumen can be bound to sulphur. Since the content if sulphur is also increased with chicory it is not expected that all copper in chicory is absorbed. All together this means that before chicory is used in practice for increasing the mineral status, possible negative aspects of this herb such as lower DM intake by the animal, bitter milk taste and reduced absorption of copper through an interaction with sulphur should be taken into account.

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Two years experiments with grass-legume mixtures in Wageningen

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Keywords: Legumes, Perennial ryegrass, Mixtures, Nitrogen

ABSTRACT

Pure perennial ryegrass (*Lolium perenne*; *Lp*) and binary mixtures with *Lotus corniculatus* (*Lc*), *Medicago sativa* (*Ms*), *Trifolium pratense* (*Tp*) and *Trifolium repens* (*Tr*) were sown in three replicates on a clay soil in Wageningen. Plots were cut four times in 2004 and five times in 2005. Annual yields of the mixtures ranged from 8.2 to 11.5 (t ha^{-1}) in 2004 and from 8.2 to 16.3 (t ha^{-1}) in 2005. The mixture with *Lc* yielded always lowest, the mixtures with *Tr* and *Tp* yielded highest in 2004, in 2005 mixtures with *Tp* and *Ms* yielded highest. The legume proportion increased during the year, except for the last cut in October. The N concentration in *Lp* was always lower than in any legume. The N concentration in *Lp* was positively related to the proportion of legume in field, and the total yield. So in 2004, the N concentration of *Lp* in the mixtures with *Tr* and *Tp* was highest, and in 2005 in mixtures with *Tp* and *Ms*. There was an increase in N concentration in *Lp* in monoculture and all mixtures throughout the year.

INTRODUCTION

In the search for quality legume-based forage systems in contrasting environments and in the context of the COST action 852 an experiment was established in 2003 with *Lolium perenne* and four legume species. The yield, botanical composition and N concentration were measured during three years, the results of 2004 and 2005 are reported, here.

MATERIAL AND METHODS

Pure perennial ryegrass (*Lolium perenne*; *Lp*; cv. Fennema) and binary mixtures with *Lotus corniculatus* (*Lc*; cv. Rocco), *Medicago sativa* (*Ms*; cv. Daisy), *Trifolium pratense* (*Tp*; cv. Pirat) and *Trifolium repens* (*Tr*; cv. Klondyke) were sown in 3 replicates on a clay soil in Wageningen, The Netherlands, in September 2003. Plot size was 3 by 8 meters. P and K, but no N fertilizer was applied and the plots were weeded. The establishment was good and plots were cut 4 times in 2004 and 5 times in 2005. Plots were harvested using a forage plot harvester (Haldrup, Løgstør, Denmark). Fresh matter was weighed on the field and two samples were taken. First, a sample for botanical composition was taken by collecting five hand plucks. In the lab, a subsample of 250 g was taken, this was hand-separated into grass, the accompanying legume and other species. Second, a sample of 250 g for dry matter percentage was taken with a grass core. All samples were oven dried at 70°C for 48 hours. The botanically pure perennial ryegrass and legume samples of the three last cuts of 2004 and all cuts of 2005 were ground with a 1 mm mill (Peppink, Deventer, the Netherlands). They were then sent to the Christian Albrechts University in Kiel, Germany, where they were analysed with NIRS for N concentration. Results were analysed for each year individually as a replicated random block design with legume treatment as main factor and block as covariate.

RESULTS

Annual yields

The annual yield of the different treatments differed significantly in both years as shown in *Figure 1*. Pure perennial ryegrass always yielded lowest, although in both years the mixture with *Lc* was not different ($P > 0.05$) from the monocultures. In 2004, the mixtures with *Tp* and *Tr* yielded highest ($P < 0.001$), whereas in 2005, the mixtures with *Ms* and *Tp* yield highest ($P < 0.001$). The yields of 2005 were much higher (> 3 t ha^{-1}) compared to 2004. This was not found for the pure stands of *Lp* and the mixture with *Lc*, which yielded similar in both years. In 2004, there was no difference among treatments in the yield of grass ($P = 0.078$), all yielding around 6 t ha^{-1}. In 2005, the yield of grass was highest ($P < 0.001$) in mixtures with *Tr* and lowest in *Ms*.

On average, the yield of grass was slightly higher in 2005 (ca. 6.5 t ha^{-1}), this was mainly due to the extra yield in the mixtures with *Tr*. In both years, yields of the legumes differed ($P < 0.001$). *Lc* yielded lowest in both years (1.8 t ha^{-1}). In 2004, *Tp* yielded highest with almost 6 t ha^{-1}. In 2005, *Ms* yielded almost 12 t ha^{-1}, also *Tp* yielded high (almost 9 t ha^{-1}). *Tr* yielded around 4.4 t ha^{-1} in both years.

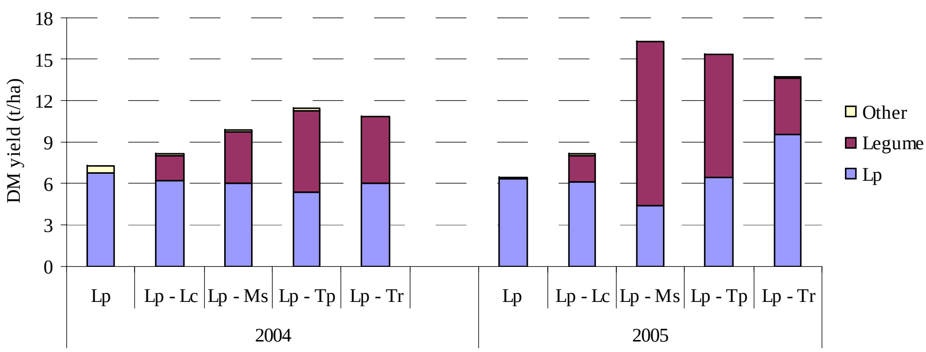

Figure 1: Yields of *Lolium perenne* (*Lp*) and binary mixtures with *Lotus corniculatus* (*Lc*), *Medicago sativa* (*Ms*), *Trifolium pratense* (*Tp*) and *Trifolium repens* (*Tr*) in 2004 and 2005 divided into *Lolium perenne* (grey bar), legume (dark bar) and other species (light bar).

Botanical composition

The botanical composition of all treatments at each cut during the two years is shown in *Figure 2*. In both years, the first cut had the highest ($P < 0.001$) proportion of *Lp*. During the growing season the proportion of legumes increased. Especially in mixtures with *Tp* and *Ms* this effect was very prominent. In 2005, these two species contributed < 50% to the total yield in May, whereas they contributed > 90% to the total yield in July. *Lc* increased its proportion mainly in July. In the final harvest the proportion of *Lp* increased in all mixtures. The infestation with weeds was generally low, also because the plots were weeded. The proportion of weeds never exceeded 10% of the total yield.

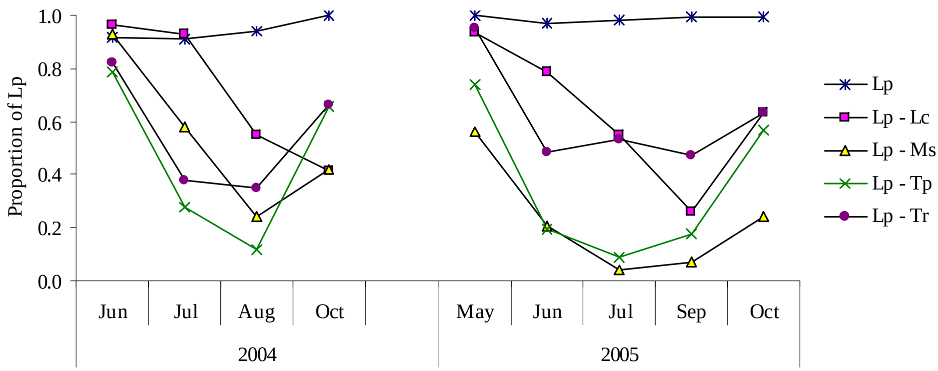

Figure 2: Proportion of *Lolium perenne* (*Lp*) in plots with pure *Lp* stands and binary mixtures with *Lotus corniculatus* (*Lc*), *Medicago sativa* (*Ms*), *Trifolium pratense* (*Tp*) and *Trifolium repens* (*Tr*) in 2004 and 2005. The proportion of legume (not shown) is complementary.

Nitrogen concentration

The N concentration in *Lp* and accompanying legumes in plots with pure perennial ryegrass and binary mixtures with legumes are presented in *Figure 3A* and *3B* respectively. The N concentration of *Lp* was always lower than of the legumes.

Lp had the lowest N concentration in pure stands; however the levels did not significantly differ from the *Lp* in mixture with *Lc*. The concentration of N in *Lp* was positively related (R > 0.95) to the biomass produced by the accompanying legume within each year. Thus, productive legumes also resulted in a higher N concentration in the accompanying *Lp*. The N concentration of the legumes was higher ($P < 0.001$) in 2004 than in 2005 (41 vs 39 g kg^{-1} DM). The N concentrations of all legumes were almost similar, however, in 2004, *Tp* (39 g kg^{-1} DM) and in 2005, *Lc* (36 g kg^{-1} DM) had a slightly lower ($P < 0.01$) N concentration than the other legumes.

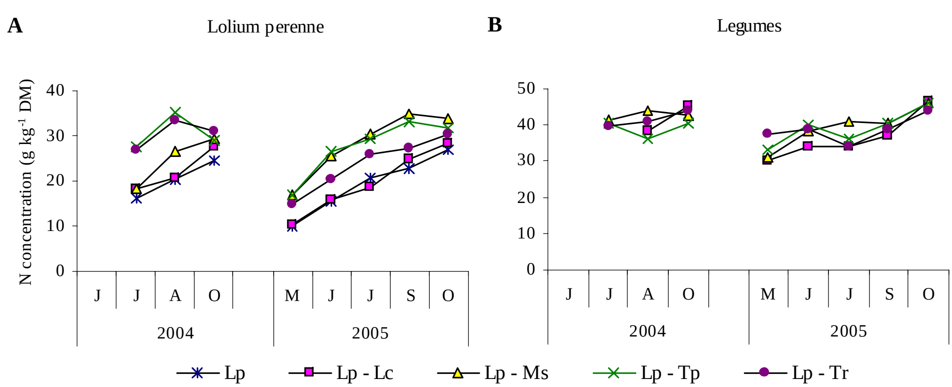

Figure 3A: The Nitrogen concentration of *Lolium perenne* (*Lp*) in plots with pure *Lp* stands and binary mixtures with *Lotus corniculatus* (*Lc*), *Medicago sativa* (*Ms*), *Trifolium pratense* (*Tp*) and *Trifolium repens* (*Tr*) in 2004 and 2005.
Figure 3B: The N concentration of the accompanying legume in the same plots of *Figure A*.

The N concentration in *Lp* increased during the year (*Figure 3A*). This was especially visible in 2005 (15 g kg^{-1} DM in May to 30 g kg^{-1} DM in October). All treatments showed a similar trend, so even pure stands of *Lp* increased in N concentration during year. Also the N concentration of the legumes showed a seasonal increase (*Figure 3B*), with the lowest levels in May and the highest in October, although the increase was less pronounced than in *Lp.*

DISCUSSION

The results of this experiment are consistent with other cutting experiments (Heichel and Henjum 1991, Elgersma and Hassink 1997) with grass-legume mixtures. *Lc* is not promising: yields were low and N was apparently hardly transferred to the grass, finally the stands were vulnerable to invasion of weeds. *Tp* and *Ms* yielded very well under the cutting regime applied in this study. Both species did transfer the fixed atmospheric N, to *Lp*, increasing both the N concentration as the yield of the grass.

However, these positive results in this cutting experiment cannot directly be transferred to a grazing situation. Brummer and Moore (2000) showed that *Tp* and *Ms* do not perform well under grazing, whereas *Tr* could perform well under both cutting and grazing conditions (Schils *et al.* 1999).

CONCLUSION

In systems with low inputs of inorganic N fertilizer, grass-legume mixtures can still produce relatively high yields. Especially mixtures with *Tp*, *Ms* and *Tr* are promising.

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How grass species and N-fertilisation influence the proportion and quality of different legume species

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ABSTRACT

Lucerne, red clover, white clover, perennial ryegrass and *Festulolium* were grown alone or in mixed swards with one legume and one grass species. Red clover and *Festulolium* showed a high yield potential both in pure and mixed swards. The leaf:stem ratio in legumes was not affected by mixing with grass. The proportion of legumes was considerable lower in mixtures with *Festulolium* than with perennial ryegrass. For most minerals the concentrations was higher in legumes than in grasses. All three legumes were less competitive for a great part of the minerals when growing together with a grass. Opposite, the grasses had a high competitive strength. Changes in mineral concentration corresponded approximately to the effect of decreasing or increasing the N supply by 120 kg N ha^{-1} for the legumes and grasses, respectively.

Keywords: legumes, grass, mineral concentration, cutting

INTRODUCTION

The proportion of grasslands with a cutting-only regime is increasing primarily because a decreasing number of herds in Denmark are grazing. Therefore species as red clover, lucerne and *Festulolium* are becoming more used in grass-leguminous stands. The expectation is that the yield potential is higher and that the herbage quality is lower in these species compared to the common used species, i.e. white clover and perennial ryegrass. Knowledge is however lacking on the possibilities for mixing species for optimizing both yield and quality. The aim of the experiment was to study the interactions between sward composition and quality and growth of the individual species in the mixtures. In this paper, yield, botanical composition and mineral content in spring growth are reported.

MATERIALS AND METHODS

On a sandy clayey soil, white clover (*Trifolium repens*, c. Milo), red clover (*Trifolium pratense*, c. Rajah), lucerne (*Medicago sativa*, c. Pondus), perennial ryegrass (*Lolium perenne*, c. Mikado) and *Festulolium* (x *Festulolium*, c. Perun) were established in pure stands and in mixtures with one legume and one grass with two replicates in 2005. All pure and mixed swards were fertilized on two yearly N levels; pure legumes at 0 and 120 kg N ha^{-1}, mixed swards at 120 N and 240 N and pure grass at 240 N and 360 N. In spring, the N supply levels were 0, 42, 84 and 126 kg N ha^{-1}, respectively. In excess potassium was applied (150 kg K). Spring growth of 2006 was examined by harvesting plots with one-week interval (May 17, 23 and 31). Botanical composition was examined by hand separation. The species were separated into leaves, including laminae and petiole, and stems, including floral parts. The samples were dried, grinded in a titanium-coated mill, digested and analyzed by ICP-MS (Agilent

7500c, Agilent Technologies, UK) for the following elements: B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Zn and Al. Al was used as a control for soil dust contamination.

RESULTS AND DISCUSSION

Yield and botanical composition

Swards with *Festulolium* yielded higher than swards with perennial ryegrass, and *Festulolium* competed stronger than perennial ryegrass, as the proportion of legumes was lower in *Festulolium* swards (*Table 1*). The leaf:stem ratio in the legumes were neither affected by grass in the sward (P = 0.93) nor by the N-level (P = 0.81). On the other hand, lucerne had an effect on the leaf:stem ratio in both grasses. The stem content in the grasses was higher, when growing together with lucerne than together with white or red clover (P = 0.04). The yield potential in red clover was higher than swards with white clover and lucerne, and the N-response was lower in red clover, as the yield increase was lower with increasing N-rate.

Minerals in the species

The legumes had a higher concentration than the grasses for most minerals: Mg, Ca, Cu, B, Co, Cu, Zn and Mo. For the rest there were only small differences. For nearly all minerals the concentration was considerable higher in red clover stem than in lucerne stem (*Table 2*). This resulted in a higher amount of harvested minerals in red clover than in lucerne, both because of the higher concentration and because of the higher yield. Generally the concentration of the single minerals in *Festulolium* were lower than in perennial ryegrass in both leaf and stem component. This can both be due to higher herbage mass in *Festulolium* and to a species difference.

Effect of competition and N-rate on mineral composition

The concentration of Mg, P, S, K, B, Cr and Cu decreased in all legumes in mixed swards compared with pure swards, indicating a low competitive strength of the legumes. The results

Table 1: Dry matter yield, proportion of legumes in the mixed crops and proportion of leaf of the species. Mean of three harvest times

	Herbage yield ($t\ DM\ ha^{-1}$)				Proportion of legume (% of DM)		Leaf proportion (% of species DM) (in mixed crops : legume/grass)			
	0N	120N	240N	360N	120N	240N	0 N	120N	240N	360N
WC	1.6^{c}	1.7^{f}					100	100		
WC + RG		2.2^{e}	2.6^{d}		26^{d}	18^{c}		100/65	100/64	
WC + FL		2.7^{cd}	3.2^{b}		9^{e}	4^{d}		100/52	100/49	
RC	3.0^{a}	3.1^{ab}					83	81		
RC + RG		2.9^{bc}	3.1^{bc}		69^{a}	56^{a}		81/66	82/64	
RC + FL		3.3^{a}	3.4^{ab}		39^{c}	23^{c}		79/56	79/52	
LU	2.2^{b}	2.5^{d}					56	54		
LU + RG		2.7^{cd}	2.9^{bc}		54^{b}	37^{b}		55/56	57/56	
LU + FL		3.1^{ab}	3.6^{a}		21^{d}	17^{c}		56/47	55/47	
RG			2.4^{d}	2.7^{b}					65	60
FL			3.1^{bc}	3.8^{a}					49	49

WC: white clover, RC: red clover, LU: lucerne, RG: perennial ryegrass, FL: festulolium
Different characters: significant difference (P < 0.05) within columns

Table 2: Concentration of different minerals in plant components. Mean of three harvest times. Results from mixed swards fertilized with 240 kg N

	K g kg^{-1} DM		Mg g kg^{-1} DM		Ca g kg^{-1} DM		S ppm		Cu ppm		B ppm	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
WC	41^{b}		2.0^{b}		13^{b}		4.3^{c}		11^{b}		19^{c}	
RC	36^{c}	58^{a}	2.6^{a}	3.3^{a}	15^{ab}	9^{a}	4.1^{c}	3.8^{a}	17^{a}	16^{a}	23^{b}	22^{a}
LU	31^{d}	46^{b}	2.2^{b}	1.8^{b}	16^{a}	8^{b}	5.1^{a}	3.0^{b}	11^{b}	8^{b}	29^{a}	13^{b}
RG	44^{a}	37^{c}	1.5^{c}	1.0^{c}	8^{c}	3^{c}	4.6^{b}	3.4^{b}	11^{b}	7^{b}	4^{d}	2^{c}
FL	41^{ab}	34^{c}	1.4^{c}	1.0^{c}	7^{c}	3^{c}	4.1^{c}	3.1^{b}	8^{c}	6^{b}	3^{d}	2^{c}

WC: white clover, RC: red clover, LU: Lucerne, RG: perennial ryegrass, FL: *Festulolium*
Different characters: significant difference (P < 0.05) within columns

for Ca and Mg are shown in *Table 3*. Pure legumes could be compared with mixed swards at an N-rate of 120 N. Only Fe, Ni and Al concentrations increased in the legumes when growing in mixed compared with pure. In general, the opposite was the case for the grasses,

Table 3: Concentration in leaf/stem and harvested amount of Ca and Mg at the last harvest time in the single species. Mixed sward compared with pure crop and effect of N-rates

		0 N		120 N		240 N		360 N		0 N	120 N	240 N	360 N
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem				
		Concentration of Ca (g kg^{-1} DM)								Harvested Ca (kg ha^{-1})			
Pure	WC	17.4		16.4						38	37^{x}		
Mix	WC			17.3		15.9					10^{y}	8	
Pure	RC	17.4	9.4	20.6	11.5					61	74^{x}		
Mix	RC			17.7	10.8	19.0	11.9				39^{y}	27	
Pure	LU	16.8	7.3	23.3	9.3					33	51^{x}		
Mix	LU			18.0	8.8	22.0	8.4				19^{y}	13	
Pure	RG					6.8^{xb}	1.8^{xb}	10.9^{a}	2.5^{a}			15	25
Mix	RG			7.8^{b}	2.2^{b}	10.5^{ya}	2.8^{ya}				9	17	
Pure	FL					5.0^{x}	1.6^{xb}	10.3	2.3^{a}			8^{x}	26
Mix	FL			6.9^{b}	2.6	10.4^{ya}	2.2^{y}				11	19^{y}	
		Concentration of Mg (g kg^{-1} DM)								Harvested Mg (kg ha^{-1})			
Pure	WC	1.9		2.3						4.1	5.3^{x}		
Mix	WC			2.0		2.5					1.0^{y}	1.2	
Pure	RC	2.8	2.9	3.5	3.6					11.4	14.5^{x}		
Mix	RC			2.7	3.2	3.4	4.0				7.2^{y}	6.0	
Pure	LU	2.0	1.2	2.9	2.1					4.4	7.8^{x}		
Mix	LU			2.1	1.5	3.1	1.9				2.3^{y}	2.2	
Pure	RG					0.6^{xb}	0.1^{xb}	1.9^{a}	1.1^{a}			1.3^{x}	5.8
Mix	RG			0.9^{b}	0.4^{b}	1.9^{ya}	1.2^{ya}				1.2	4.0^{y}	
Pure	FL					0.8^{x}	0.2^{xb}	1.9	1.1^{a}			1.3^{x}	
Mix	FL			0.7^{b}	0.2^{b}	1.9^{ya}	1.1^{ya}				1.0	5.2^{y}	7.1

WC: white clover, RC: red clover, LU: Lucerne, RG: perennial ryegrass, FL: *Festulolium*, Pure: pure stand, Mix: mixed sward with one legume and one grass. Different characters (a and b): significant difference (P < 0.05) between the same component (leaf or stem) in the same row. Different characters (x and y): significant difference (P < 0.05) between Pure and Mix in the same column within the same species.

where the effect of mixing with legumes could be examined at 240 N. The concentration of Mg, P, S, K, Ca, Cr, Mn, Co, Cu, Mo and Al increased in the grasses in mixed compared with pure sward. An increased N supply generally increased the concentration of minerals both in pure swards and in mixed swards (*Table 3*). The decrease in mineral concentration in legumes due to mixing with a grass was approximately corresponding to a decrease in the N supply by 120 kg N. Parallel for the grasses, the increase in mineral concentration due to mixing with a legume approximately corresponds to an increase in N supply by 120 N (*Table 3*).

CONCLUSIONS

The ongoing experiment has shown how the choice of grass species and N-fertilisation influence the proportion and quality of different legume species. Mixing the legumes with grasses reduced the total supply of most minerals in the harvested herbage due to a low competitive strength of the legumes and to a lower concentration in the grasses than in the legumes.

Effects of various feeding strategies upon utilization of nutrients from red-clover silage based diets - a simulation study

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Keywords: legumes, nitrogen, nutrient utilization, dairy cow, simulation models

ABSTRACT

A dynamic dairy cow simulation model was used to study the effects of various feeding strategies upon utilization of nutrients, especially nitrogen, for different legume-based diets. In general, legume proteins are rapidly degraded. In order to maximize the utilization of N from legumes for ruminal microbial protein production, rapidly degradable carbohydrates must also be available. The dynamic model used in this study, the Nordic Dairy Cow Model Karoline, combines current knowledge on digestion dynamics and dairy cow metabolism. The study is an example of how this type of a model can be used to get an overview of the likely effect of different feeding strategies upon nutrient utilization from red clover silage. From simulations of six diets it was concluded that high intakes of red clover silage results in poor N utilization. A better feeding strategy would be to substitute part of the red clover silage with low N hay and straw, and select supplementary feeds/concentrates with low N level, low N degradation rate and high amount of WSC and starch.

INTRODUCTION

The protein requirements of ruminants are met by degraded CP for microbial protein synthesis and protein that escapes ruminal degradation. An efficient microbial protein synthesis requires a good balance in the supply of energy and protein to rumen microbes, but is also positively affected by increased rumen passage rates, which are positively correlated with digestibility and feeding level. Excessive ruminal protein degradation may be the most limiting nutritional factor in many forage legumes (Broderick 1995). This problem can be avoided to some extent by choosing varieties and forage conservation practices that limit the rate and extent of ruminal protein degradation. However, the ration formulation is the final step in all efforts to maximize nutrient utilization. Important parameters to match the high degradability of legume proteins are the degradation characteristics of the various carbohydrate fractions of the diet, as well as the protein from other sources than the legume. Because of the complex relationships between various factors discussed above, feeding trials that only test few treatments at a time have their limitations while studying the effects of ration formulation upon utilization of nutrients. Mathematical models of ruminant nutrition can be used for these purposes, both as research tools and also in practical feed evaluation. In the current study, the dynamic model Karoline (Danfær *et al.* 2006a) is used. Karoline combines current knowledge on digestion dynamics and dairy cow metabolism. The purpose of the study is to show an example of how this type of a model can be used to get ideas about the likely effect of several different feeding strategies upon nutrient utilization from legumes. The study concentrated on the distribution of feed nitrogen into urea, feaces and milk. It is not expected that all answers are a complete description of reality, but evaluation results (Danfær *et al.* 2006b) of the Karoline model indicate that it is suitable for the purpose of this study.

Table 1: Chemical composition and rumen degradation of feeds used for simulations

		g/kg DM of:			Rumen degradation:	
Feed	CP	NDF	Starch	WSC	EFD 0.04	EPD 0.08
Red clover silage (RC)	205	353	0	208	0.41	0.78
Timothy hay, late cut (H)	101	686	0	132	0.30	0.54
Straw (S)	70	761	0	100	0.32	0.82
Concentrate (C)	207	309	155	226	0.39	0.39
Maize (M)	88	121	713	19	0.63	0.42
Barley (B)	112	178	609	50	0.42	0.74
Sugar beet pulp (SBP)	108	370	5	350	0.68	0.88

CP = crude protein, NDF = neutral detergent fiber, WSC = water soluble carbohydrates, EFD 0.04, EPD 0.08: see above

MATERIALS AND METHODS

To run the model, a detailed description of individual feeds and diet composition is needed, as described by Danfær *et al.* (2006a). This description takes too much space to be listed in this paper. However, *Table 1* reports some of the most important characteristics of the feeds used in the simulations. The data for the red clover silage is based on a study of Bertilsson and Murphy (2003), but for the other feedstuffs the data is from feed tables established in connection with the Karoline modeling project, based on various data from experiments within the Nordic countries (Danfær et al., 2006a). To give an idea of the degradation characteristics, two values are reported: EFD 0.04/h: effective fiber (NDF) degradability assuming NDF rumen passage rate of 0.04/h; EPD 0.08/h: effective protein degradability assuming CP rumen passage rate of 0.08/h. The model also needs some information about the animal, namely its stage of lactation and initial body weight. The simulations reported here were made with a 600 kg cow in a middle lactation (week 20). An index to estimate likely feed intake is incorporated into the model. This index (Huhtanen *et al.* 2002) is based on silage characteristics (digestibility, total acids, ammonia N). The model estimates likely forage intake from this index combined with corrections for concentrate intake. It should be noted that the ECM production as simulated by the model and reported in *Table 2* could be a slight overestimation, based on the following test: The legume-based diets in the study of Bertilsson and Murphy (2003) were simulated by the Karoline model, using all the feed and animal inputs as given in their paper. The average observed values for the 10 diets reported were 29.6 kg ECM/d but for the same diets simulated by Karoline the average yield was 30.5 kg ECM/d.

RESULTS

Table 2 lists the results of six simulations, as briefly described below: Diet 1: High intakes (13 kg DM) of red clover silage with fixed amount (7 kg DM) of high-fiber concentrate lead to poor N utilization. Diet 2: When RC silage is reduced to 5 kg DM, substituted with low N hay and straw, N utilization is improved but ECM yield is reduced considerably, and body balance becomes negative. Diet 3: Reducing the straw and adding 3 kg barley improves ECM

Table 2: Simulation results: energy corrected milk (ECM), energy balance and nitrogen utilization

Composition of diet #	ECM kg/d	Energy bal. MJ/d	Nitrogen excretion (% of intake) Urine	Faeces	Milk
1: 7 kg C, 13 kg RC	27.7	2.39	55	21	24
2: 7 kg C, 5 kg RC, 5 kg H, 2.8 kg S	25.7	-0.99	44	28	28
3: 7 kg C, 3 kg B, 5 kg RC, 5 kg H, 1.4 kg S	29.0	3.18	44	28	28
4: 3 kg C, 7 kg B, 5 kg RC, 5 kg H, 1 kg S	29.2	4.10	41	29	30
5: 3 kg C, 7 kg M, 5 kg RC, 5 kg H, 1 kg S	30.3	5.21	36	31	33
6: 3 kg SBP, 7 kg M, 5 kg RC, 5 kg H, 1 kg S	30.2	5.32	32	32	36

See *Table 1* for abbreviations of feeds

yield and body balance but N utilization is unchanged. Diet 4: Further increase of barley, now at the expense of the high fiber concentrate, improves N utilisation and has small positive effect on ECM yield. Diet 5: Maize instead of barley - improves N utilisation and ECM yield. Diet 6: Sugar beet pulp instead of high-fiber-concentrate - improves N utilisation but ECM yield is similar.

CONCLUSIONS

- High intakes of red clover silage result in poor N utilisation
- According to the present simulations a better feeding strategy is to substitute part of the red clover silage with low N hay and straw, and select supplementary feeds/concentrates with: (i) low N level, (ii) low N degradation rate and (iii) high amount of WSC and starch
- Karoline is a useful tool for investigating likely effects of different feeding strategies and results can be used to suggest treatments in animal feeding trials

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Variation in polyphenol oxidase (PPO) activity in red clover under different sward management systems

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Keywords: Red clover, polyphenol oxidase activity, seasonal effects, sward management

ABSTRACT

Several studies show that polyphenol oxidase (PPO) in red clover reduces proteolysis and increases nitrogen use efficiency of the ruminant, but not much is known about factors affecting PPO activity in red clover plants. PPO activity was analysed in red clover plants from legume-grass mixtures subjected to three different management systems. The cutting frequency (3 respective 5 cuts) caused only minor differences in PPO activity, whereas a significant increase in PPO activity could be proved for the rotational grazing system, compared to the simulated grazing system with equal use frequency. Results indicate a metabolic adaptation of the red clover plants due to the grazing process itself, leading to a control factor which has to be considered in case of the red clover's nutritional properties.

INTRODUCTION

Forage legumes containing secondary compounds, such as condensed tannins and polyphenol oxidase (PPO), may offer considerable benefits in terms of an increased N utilisation for ruminants. Red clover contains high amounts of PPO and also small soluble phenolics, responsible for the enzymatic browning reaction: if plant tissue is damaged, PPO is released and reacts with phenols in the presence of oxygen to form reactive quinones. These quinones react with proteins and additional phenolics to form quinone-protein complexes, resulting in partially protected proteins which will be slowly degraded in the rumen. In fact, findings of several studies comparing red clover to alfalfa with similar protein contents but different contents of PPO show that red clover reduced proteolysis in the silo and in the rumen as well (Sullivan and Hatfield 2006, Jones *et al.* 1995), which was related to its PPO activity. Consequently, a more efficient nitrogen utilisation by the animal was observed, as proved by changes in the proportion of N excreted in milk and faeces, and concentrations of milk and blood urea nitrogen (Broderick *et al.* 2001).

It has to be assumed that varying red clover PPO contents will cause different effects on protein utilisation, but the relationship between PPO activity and extent of protein degradation in the rumen has not yet been quantified, and there is a lack of knowledge about factors influencing PPO activity in red clover, e.g. cultivar, environmental and seasonal effects. PPO genes are expressed differently in individual plant compartments; therefore it seems to be obvious that PPO activity of a plant differs during its developmental stages in regard to the actually dominating plant organ. There is no data available so far for a quantification of PPO activity in red clover under different management systems.

MATERIALS AND METHODS

A field trial was established in 2004 at the experimental site „Lindhof“ in Northern Germany, with the experimental design carried out according to the common protocol of WG 3 from the COST Action 852. In 2005, red clover samples were taken from the grass-legume plots of the three management systems 'simulated grazing' (**SG**; 5 cuts, cut every 30±3 days), 'rotational grazing' (**RG**; 9 animals were allowed to graze plots of a size of 1500 m² for 3-5 days at intervals of 30±3 days), and 'silage cutting' (**SC**; 3 cuts, cut every 50±5 days), with three replicates each. In the rotational grazing system, samples were taken the day before animals turned out to the pasture plots. Fresh plants were harvested with a cutting height of 5 cm, separated into leaves and stems and fractions immediately frozen and stored at -27°C. Analysis of PPO activity was carried out according to Escribano *et al.* (1997). For the extraction of PPO, frozen leaves were homogenised in liquid N_2 with pestle and mortar. Phosphate buffer (pH 7.3) was used as extraction medium, containing proteinase inhibitors. After several centrifugation steps, PPO activity was analysed in the extracts by a spectrophotometric assay at 400 nm for 12 minutes, using caffeic acid as substrate. Protein contents of the plant extracts were determined by Bradford assay allowing the measured PPO activity to be expressed on a protein basis, given as IU per protein (mg/g DM). Data were evaluated statistically using ANOVA and Student's t-test and corrected by the Bonferroni-Holm procedure.

RESULTS

Within the three systems, PPO activity increased during the year with highest activity levels at the final cut (*Figure 1*). Interestingly, the cutting frequency caused only minor differences between the PPO activity measured in red clover from the RG and SC systems, compared to the differences between the systems SG and RG with five utilisations each. Starting at equal PPO activity at the 1st utilisation, plants from the 2nd to the 5th utilisation of the RG system gained up to more than twofold higher activity levels than plants of the SG system. The differences observed between the RG and the SG system were statistically significant at P<0.05.

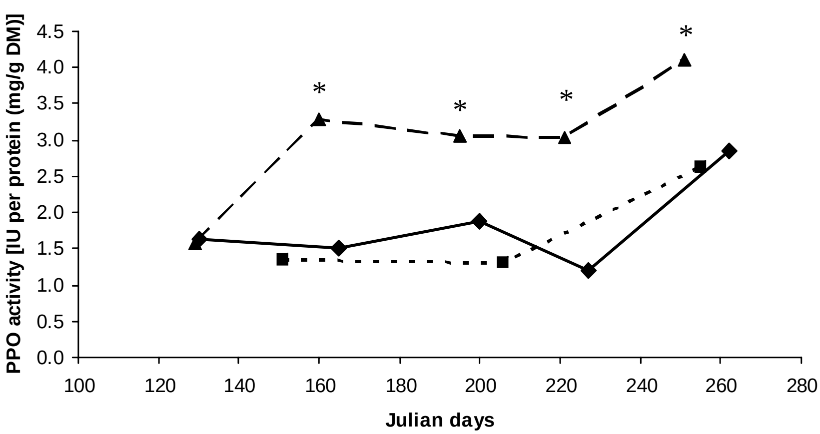

Figure 1: Influence of the management systems rotational grazing (RG▲), simulated grazing (SG ◆) and silage cutting (SC■) on PPO activity in red clover leaves (* differ significantly from the corresponding SG values (*P* < 0.05), SE = 0.22).

DISCUSSION

While the observed differences in PPO activity between the SG and the RG system cannot be explained by larger deviations between the phenological stages of the red clover plants (data not shown), it may be assumed that the grazing process itself has an impact on red clover plants. As the assumed function for pathogen defence of PPO in plant organs as well as contents of other plant components may influence PPO activity, the results observed may reflect changes in the plant's biochemical behaviour. In general, under stress and with higher N supply plants are stronger affected by herbivores, consequently producing more substances for defence. Greater damage of red clover tissue due to the grazing animals, trample damages, and N input due to faeces and urine as well during the 3-5 days of grazing may have resulted in higher PPO contents in plants of the RG system, compared to the SG system where plants were only cut. Further, an induced metabolism of phytoalexins, like the increase in the contents of chlorogenic acid or caffeic acid, is known to occur e.g. in potato plants if damaged or infected by pathogens (Nicholson and Hammerschmidt 1992). As these substances act as substrates for PPO in red clover, a comparable reaction of this plant and therefore increasing substrate concentrations in red clover may have resulted in a measurable increase in PPO activity. Even quinone derivatives are built in some plants as a mechanism for defence.

The fact that the increase in activity is persistent and measurable even after four weeks of time lag between the cutting respective grazing dates indicates a stress-induced metabolic adaptation of the red clover plants due to the grazing process.

CONCLUSIONS

Restricted to only one year's data, conclusions are limited by the short term observation period, but our findings may give interesting indications for further research activities. Results of the present study show that the phenological stage has a minor effect on red clover PPO activity, whereas different sward management systems have a considerable impact and possibly induce a systemic adaptation. In practice, extended knowledge about influencing factors on PPO activity may contribute to realisable regulation mechanisms for an optimised utilisation of red clover as a forage plant for ruminants.

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Eating behavior of dairy cows offered fresh or ensiled ryegrass, white clover, and red clover

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Keywords: forage, legumes, feeding pattern, choice diet, cattle

ABSTRACT

Two experiments were conducted to study the feeding behavior of dairy cows when offered white clover, red clover, and ryegrass either in fresh (experiment 1) or in ensiled form (experiment 2). Both experiments were carried out by a similar protocol in a free-stall barn. In each experiment, 18 dairy cows were assigned to three experimental groups. Cows were allowed to select either between white clover and ryegrass (WG), or between red clover and ryegrass (RG), or between all three forage species (WRG). Total dry matter intake was not affected by treatment across experiments. In experiment 1, the number of meals, meal size, and the overall eating rate of the diet were not affected by treatment, but the eating rate was increased with individual forages that were preferred, and found significant ($P < 0.05$) in case of white clover versus ryegrass when offered simultaneously. In experiment 2, the number of meals, meal size, and the overall eating rate of the diet were also not affected by treatment. The eating rate was significant increased with white clover silage that was preferred over the other offered forages in WG ($P < 0.05$) and WRG ($P < 0.01$) treatments. However, the eating rate was similar with red clover silage and ryegrass silage when offered simultaneously. Overall, in each study, preference for or against a forage type was generally reflected by the eating rate, and difference in eating behaviour on individual forages disappeared when overall eating behaviour of the diets were compared.

INTRODUCTION

An understanding of the eating behavior of dairy cows provides insight into how forage species and characteristics affect voluntary intake of cattle, and assist in clarifying their diet selection when they are offered different forage types to select from. The objective of this study was to investigate the feeding behavior of dairy cows offered white clover, red clover, and ryegrass either in fresh (experiment 1) or in ensiled form (experiment 2).

MATERIALS AND METHODS

Perennial ryegrass (*Lolium perenne* L., cultivar 'Fennema'), white clover (*Trifolium repens*, 'Klondike'), and red clover (*Trifolium pratense*, 'Pirat') were used in the two experiments, which were carried out by a similar protocol in a free-stall barn. In each experiment, 18 dairy cows (Brown Swiss and Holstein; allocated in balanced proportions to the experimental groups) were assigned to three experimental groups. Cows were allowed to select either between white clover and ryegrass (WG), or between red clover and ryegrass (RG), or between all three forage species (WRG). Individual forages were offered in separate troughs whereby access to feeding was controlled through electronic identification (responders), and intake per access was registered through electronic feed balances. Individual forage types were allocated to different troughs every three days to counteract memory effects. Besides these experimental forages, cows were fed barley (according to milk yield) and hay each morning after milking (on average 1.4 kg/d dry matter (DM)). Troughs were refilled with the forages seven times a day, with the first feeding (only hay at that time as a prevention measure against acidosis and bloat) at 6:30 h and the last at 20:00 h. Criteria were applied to evaluate the eating behavioural data: min. meal size, 50 g; min. meal duration, 1 min; min. intermeal interval, 8 min. Eating rates below 10 g DM/min and over 125 g DM/min were excluded from the analysis. Obtained data was subjected to analysis of variance (GLM from SAS) regarding diet type (three diets). The MIXED procedure, including a repeated statement with animal as repeated subject, was used to test for differences among forage types within each dietary treatment.

RESULTS

Table 1 presents the nutrient contents of the experimental forages used in experiments 1 and 2. In experiment 1, across dietary treatments, the majority of the daily intake was consumed during daytime, and feeding activity characteristically peaked following feeding. The dry matter intake (DMI) of the experimental forages and total DMI were similar across dietary treatments (on average 17.3 and 21.3 kg/d). In case of the WG diet, 38% white clover and 62% ryegrass were selected, with RG, 47% red clover and 53% ryegrass, and with WRG, 30 % white clover, 31% red clover, and 39% ryegrass were selected. The average number of meals per day (on average 10.6), the meal size (1.7 kg DM/meal), as well as the eating rate (47.1 g DM/min), were not affected by diet type. In case of the WG diet, ryegrass was eaten much faster ($P < 0.001$) than white clover (54.6 vs. 38.6). The forages in the other treatments (RG and WRG) were eaten equally fast (on average 50.6 and 39.2 g DM/min, respectively).

Table 1: Nutrient contents of the experimental forages

	Ryegrass		White clover		Red clover	
Nutrient (g/kg dry matter (DM))	Fresh	Ensiled	Fresh	Ensiled	Fresh	Ensiled
DM[a]	177	454	103	243	127	267
Crude protein	192	113	230	212	224	179
Neutral detergent fiber	457	603	272	294	333	398
Acid detergent fiber	263	383	252	291	242	318

[a] g/kg fresh weight

In experiment 2 feeding the ensiled forages, the majority of the daily intake across treatments was consumed during daytime, and feeding activity characteristically peaked following feeding as in experiment 1. The dry matter intake (DMI) of the experimental silages and the total DMI was similar across dietary treatments also in the second experiment (on average 12.4 and 18.8 kg/d). Feeding silages, with the WG diet, 73% white clover and 27% ryegrass were selected; with RG, 69% red clover and 31% ryegrass; with WRG, 60% white clover, 18% red clover, and 23% ryegrass. No difference between dietary treatment was found in the average number of meals per day (on average 11.3), in the meal size (1.2 kg DM), and in the eating rate (51.8 g/min). However, different eating rates were obtained for the different forage types offered within the WG, RG, and WRG diet. A tendency for a higher eating rate ($P < 0.1$) on white clover silage compared to ryegrass silage (61.8 and 39.5 g DM/min) was observed for cows on the WG diet, and cows on the WRG diet had a clearly higher ($P < 0.01$) eating rate for white clover silage compared to red clover silage and ryegrass silage (54.6 vs. 42.3 and 32.2 g DM/min). The eating rates of ryegrass silage and red clover silage in the RG treatment were similar (41.3 and 52.4 g DM/min).

DISCUSSION

In general, across the experiments, the average number of meals, eating duration and eating rate were comparable to the values reported for dairy cows by Dürst *et al.* (1993), who had used the same housing and feeding facilities. Furthermore, in both experiments, cows exhibited diurnal feeding patterns, which are commonly observed for cattle under grazing conditions (e.g. Penning *et al.* 1991) as well as in group housing conditions (e.g. Dürst *et al.* 1993). Additionally, the results from both experiments illustrate that preference for a forage type is associated with an increased eating rate, rather than with changes in the number of meals or meal size. Therefore, in experiment 2, liking of clover silages and disliking of the ryegrass silage, which was of lower nutritional quality than the clover silages, may have subsequently increased and decreased eating rate. However, the similar eating rates in case of red clover silage and ryegrass silage, when red clover silage was preferred, may be explained by a reduced handling time (prehension, mastication, ruminating) by cows in case of red clover silage compared to ryegrass silage. Schubiger *et al.* (1998) namely discussed that red clover contains proportionately more cell wall than white clover due to the larger proportion of fibrous stems in red clover compared to the proportion of less fibrous stolons in white clover, which may have required more mastication. The smaller meal size and the lower overall intake by cows of the ensiled forages in experiment 2 compared to the fresh forages in experiment 1 is most likely to be explained by the higher fiber content, which probably increased mastication and rumination time. Additionally, the higher fiber contents of the ensiled forages should have caused for rumen to fill more quickly than the fresh forages, thus explaining the shortened overall eating duration.

CONCLUSIONS

Our results show, from the variables that were analyzed, that the general preference for fresh ryegrass over clover in experiment 1, and the preference for clover silages (especially white clover silage) over ryegrass silage in experiment 2 were reflected by eating rate, indicating that, apart from the physical structure of the forages, nutrient quality may be involved. Furthermore, this study illustrates that differences in eating behaviour of individual forages are compensated by contrasting differences in other diet ingredients thus leaving the overall eating behaviour rather unchanged.

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In-vitro digestibility and energy concentration of different legumes - results from the COST 852 experiments in Austria

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Keywords: legume quality, digestibility, energy concentration, alpine site

ABSTRACT

According to the common protocol of working group 3 within COST 852 two field experiments have been carried out at HBLFA Raumberg-Gumpenstein, Austria. Comprehensive recordings and analyses have been made during 3 years focussing on yield productivity, forage quality and botanical composition of the established mixtures. Special attention was given to different legume species and on their function for grassland ecosystems. Considerable differences between legume species concerning yield, competitiveness, forage quality and energy yield were found.

INTRODUCTION

For most of the Austrian grassland and dairy farmers home-grown forage from meadows and pastures is a substantial element of sustainable farming management. Different measures, aiming at the improvement of forage quality, are therefore of great interest for practice and research. Especially on extensive grassland farming systems, organic farms and integrated farms, biological N-fixation plays an important role in terms of nutrient fluxes and nitrogen budget. Regarding the importance of legumes for agriculture and the insufficiently explained effects of climatic conditions, as the main topic of COST 852, field experiments have been established at the Federal Research and Education Centre for Agriculture at Raumberg-Gumpenstein located in the mountainous region of Austria.

MATERIAL AND METHODS

The field experiment design at Gumpenstein mainly followed the common protocol of working group 3 (*Figure 1*). Both unfertilised field trials were established in 2002, using the below-mentioned seed amounts and kept for three vegetation periods from 2003 to 2005. Besides phenological observations, the proportion of grasses, legumes and herbs have been estimated and measured by separation for each growth. Yield data (fresh mass and dry matter), content of crude nutrients, digestibility of organic matter and energy content have been analysed for all treatments.

The separated forage samples have been analysed for digestibility of organic matter by the two-stage technique for in vitro digestion of forage crops (Tilley and Terry 1963). Energy concentration was predicted on the basis of DOM by means of regression equations.

RESULTS AND DISCUSSION

There were significant differences between the treatments concerning dry matter production within the two cutting systems (*Figure 2*). In the pure grass stands the average yield amoun-

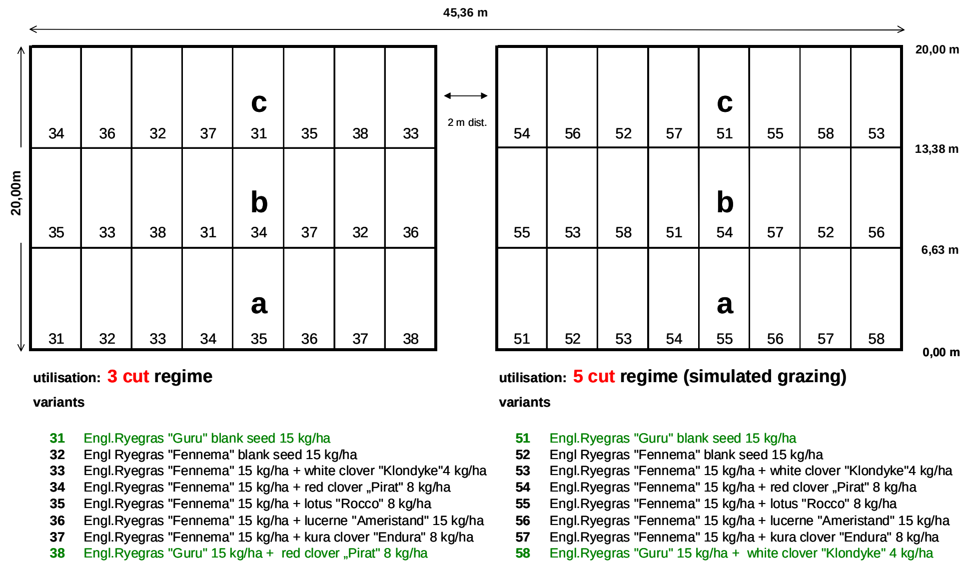

Figure 1: Design of the COST 852 field experiment at HBLFA Raumberg-Gumpenstein

ted to 47 resp. 49 dt dry matter per ha and year which represents the natural potential of the site. The effect of legumes as an important companion plant for grasses is impressively demonstrated by much higher yields for the combination of ryegrass with white clover, red clover and bird´s foot trefoil. The most productive and stable combination during the whole period was ryegrass with white clover both in the 3 cut and in the 5 cut experiment. Bird´s foot trefoil performed sufficiently in the 3 cut system, whereas lucerne showed a disappointing performance at all, mainly caused by a low pH-value (5.0) and by insufficient soil phosphorus content (4 mg P per 100 g fine soil). With the exception of ryegrass in combination with red clover and bird´s foot trefoil, which are sensitive to higher utilisation frequency, there were no significant differences in the yield production between the two cutting regimes.

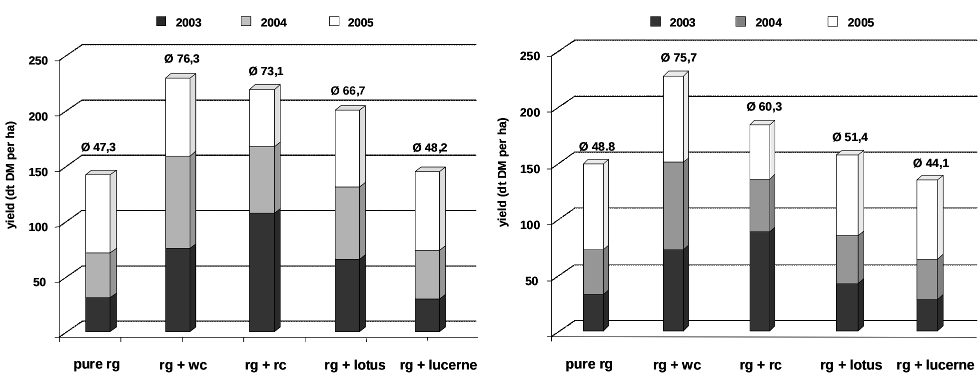

Figure 2: Yield productivity during the observation period (left chart = 3 cut system, right chart = 5 cut system)

These results indicate the limitation of yield production by the site conditions, which in practice lead to a typical 3 cut system with possibly one additional grazing activity in favourable years.

Figure 3 is showing the proportion of grasses, legumes and herbs within the different treatments of the 3 cut regime during the whole observation period. Generally the proportion of herbs decreased in all treatments, whereas grasses and legumes developed variably. In the unfertilised pure grass stand there was an increasing invasion of white clover over the years, which certainly has positively influenced yield production. The treatment with ryegrass and white clover showed a very stable botanical situation during the years, whereas red clover and bird´s foot trefoil declined. The proportion of lucerne increased but there was also a stronger invasion of white clover. Generally the proportion of legumes in the observed treatments has been reflected in the yield productivity of the different mixtures.

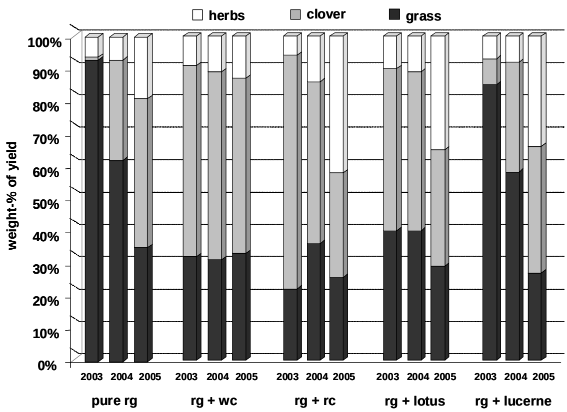

Figure 3: Proportion of grass, legumes and herbs during the observation period from 2003-2005

Table 1 and *2* include the results of the different legumes and of ryegrass (both from the blank seed and from the mixtures) for the years 2003 and 2004.

In both years the digestibility of organic matter of the forage samples was low but with the exception of white clover within the range of the data given in the German feeding value table (DLG-Futterwerttabelle 1997), which is also used in Austria for feeding calculations. This is mainly caused by the relatively long growth period, which leads to higher contents of hardly digestible or indigestible substances in plants. Bird´s foot trefoil showed significantly lower digestibility values than all other legumes, which might have been caused by a higher concentration of condensed tannins. There was a significant impact of the companion legumes on the quality of ryegrass, which once more underlines the importance of legumes for agriculture.

Table 1: Digestibility of Organic Matter (DOM %) - average of three cuts/year

	white clover	red clover	bird´s foot trefoil	lucerne	ryegrass (blank seed)	ryegrass (from mixtures)
2003	71.4	68.6	57.8	65.0	70.5	71.5
2004	72.4	66.0	57.4	62.8	72.4	74.0
DLG	80-81	61-79	n.a.	57-75	68-83	n.a.

Table 2: Energy concentration (MJ Net Energy Lactation/kg DM) - average of three cuts/year

	white clover	red clover	bird´s foot trefoil	lucerne	ryegrass (blank seed)	ryegrass (from mixtures)
2003	5.8	5.5	4.3	5.1	5.8	5.9
2004	6.0	5.2	4.3	4.9	6.0	6.3
DLG	6.5-7.1	5.0-6.9	n.a.	5.1-6.3	5.5-7.1	n.a.

The energy concentrations for the analysed samples are low compared with DLG data and show great differences within the legumes (*Table 2*). Again the positive impact of legumes on the quality of the companioned grass is visible by higher energy concentration especially in 2004.

The product of dry matter yield and energy concentration results in the energy yield (MJ NEL/ha), which is presented in *Figure 4*. The pure, unfertilised grass stand amounted to app. 15,000 MJ NEL/ha and year, which is comparable with the data of extensively managed alpine and mountainous grassland in Austria. The highest energy yield resulted from the mixture of ryegrass and red clover in 2003 with more than 55,000 MJ NEL/ha, which is within the range of ley farming areas and intensively used grassland. Mixtures with ryegrass and white clover or red clover showed the best overall productivity for the two years period followed by mixtures with ryegrass and bird´s foot trefoil or lucerne.

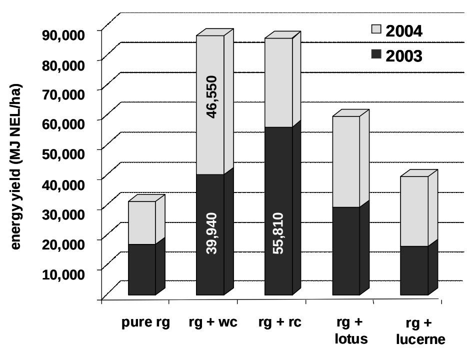

Figure 4: Quality yield (MJ NEL/ha) during the observation period from 2003-2004

CONCLUSIONS

The results of the Austrian field experiment within COST 852 clearly demonstrate the function and importance of legumes for grassland ecosystems. There were considerable differences between legume species concerning dry matter yield, competitiveness, forage quality and energy yield production. All these aspects have to be considered for the selection of legumes and for their usage in seed mixtures. Concerning the relatively low digestibility and energy concentration, grass and legume cultivars have to be chosen, which are well adapted to the site conditions.

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The potential feeding value of grass-legume mixtures in dry Mediterranean conditions

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ABSTRACT

The paper discusses the feeding value per unit area of different grass-legume mixtures sown within the common activity of the COST Action 852, as calculated on the basis of forage nutritive value and forage yield. The species used in the experiment, *Lolium rigidum*, *Dactylis glomerata*, *Medicago polymorpha* and *Medicago sativa*, belong to four functional groups: grass, legume, fast-establishing and slow-establishing species. Herbage samples, taken in the second year of the trial on 5 dates, were submitted to NIRS analysis to determine CP and IVDMD of sown and unsown species. Mixtures showed a higher yield per hectare of CP and IVDMD than the average of the monocultures, but lower values than lucerne monoculture and lucerne-dominated mixtures. A strong seasonal fluctuation of CP and digestible DM on offer occurs for the simoultaneous drop in forage quantity and quality during summer, reducing accordingly the number of sheep units that can be maintained.

Keywords: grass-legume mixtures, IVDMD, Net Energy, forage quality

INTRODUCTION

Forage legumes, adapted to a wide range of soil types, climatic conditions and management systems, will become increasingly important components of sustainable agricultural production systems in Europe. In Mediterranean regions, the pasture seed mixtures usually marketed consist of a small number of annual legume species, few subclover varieties. They usually include annual grasses and sometimes perennial grasses for areas with high rainfall. Aspects like herbage quality and nutritive value of Mediterranean mixtures has been relatively overlooked in literature.

Indeed annual grasses are prone to an abrupt quality fall, during the onset of the reproductive phase in spring. This can be successfully counterbalanced by grazing legume monocultures such as sulla (Molle *et al.* 2003) or burr medic, or potentially by the utilisation of grass-legume mixtures (Rochon *et al.* 2004). The aim of the study was to evaluate the effect of functional group mixing (grass/legumes, fast/slow establishing species) on their potential feeding value (Digestible Dry Matter and Crude Protein) and its seasonal variation within mixed swards in Mediterranean conditions.

MATERIALS AND METHODS

The experiment investigates forage quality of different grass-legume mixtures within the common activity of the COST Action 852. Thirty plots (3 x 3 m) were sown in October 2002 at the Ottava Research Station (Sardinia, Italy), with average annual rainfall of 550 mm, calcareous soil (pH = 7.5) and a typical Mediterranean climate. The species used in the experiment represent four functional groups: grass (G), legume (L), fast-establishing (subscript 1) and slow-establishing (subscript 2) species. The sown species were *Lolium rigidum* cv Nurra (G_1), *Dactylis glomerata* cv Currie (G_2), *Medicago polymorpha* cv Anglona (L_1) and *Medicago sativa* cv Mamuntanas (L_2). The plots included pure stands and 4-species combinations, ranging from dominance by one or by two species to total evenness (centroid = equal contribution of all mixture components), following a 'simplex design' duplicated at two sowing densities (high density = 35 kg ha^{-1}, low density = 60% of high density). Herbage samples taken in the second year of the trial on 5 dates (31/03/04, 31/05/04, 14/07/04, 14/09/04, 16/11/04) were analysed by NIRS analysis, and refer to % crude protein (CP) and in vitro dry matter digestibility (IVDMD) of each component of the mixture. Net energy (NE) was estimated according to Van Soest (1994). Total NE and CP availability per hectare were then calculated multiplying DM yield per energy and CP concentration. Sheep unit day per hectare was also estimated dividing total NE and CP availability by energy (NE) and protein (CP) requirements of sheep (AFRC 1985). For example 2000 sheep unit day can be interpreted as the energy/protein needed to maintain 200 ewes for 10 grazing days per hectare.

RESULTS AND DISCUSSION

Considering total CP and IVDMD (*Table 1*), the centroid gave better responses than the average of the monocultures but not than the L_2 pure stand. L_2 dominated plots gave similar responses to the centroid, with an increased digestibility. G_2 pure stand showed the poorest forage quality. The higher performance of the centroid and L_2 plots are due to their good quality associated to the best biomass production.

Table 1: Potential yield of CP and IVDMD (kg/ha) for each mixture type. Duncan test ($P < 0.05$)

Pure stand/Mixture type	Total CP	Total IVDMD
G1 pure stand	536 h	3751 f
G2 pure stand	543 h	2544 g
L1 pure stand	857 g	4172 ef
L2 pure stand	1628 a	5849 ab
Centroid	1226 be	4722 bf
Dominance by G1	1006 eg	4640 bf
Dominance by G2	1061 dg	4174 ef
Dominance by L1	1142 cf	5572 ac
Dominance by L2	1474 ab	6242 a
Dominance by G1 and G2	995 eg	4320 df
Dominance by G1 and L1	874 fg	4355 cf
Dominance by G1 and L2	1359 bc	5622 ab
Dominance by G2 and L1	1245 be	5317 ae
Dominance by G2 and L2	1262 be	4864 bf
Dominance by L1 and L2	1309 bd	5522 ad
Average Pure stands	891	4079
Standard Error	*83*	*363*
% change av. mono vs centroid	38	16
% change best mono vs centroid	-25	-19

The NE availability (*Figure 1*) of the centroid mixture was high in late winter (March) and spring (May) although legume pure stands generally tend to prevail. The availability of CP (*Figure 1*) was also high in the centroid but always lower than L_2 (lucerne). The availability of nutrients was overall better spread in the centroid and L_2 pure stand than in the other treatments. These two treatments showed a ratio between crude protein (g/kg DM) and metabolisable energy (ME, Mcal/kg DM) always higher than what recommended for sheep (NRC 1985). The seasonal average number of sheep unit day on NE basis per hectare reached the highest values in spring (May), as expected in Mediterranean rainfed pastures (*Figure 2*). This was particularly evident in L_2 and G_2 monocultures. The centroid and L_2 were higher throughout the year in terms of sheep unit day on CP basis, with very high levels in mid-autumn also due to the lower sheep requirements usually found in this period. This trend confirms the good performance of grass and legume mixtures during all the year in terms of NE and CP.

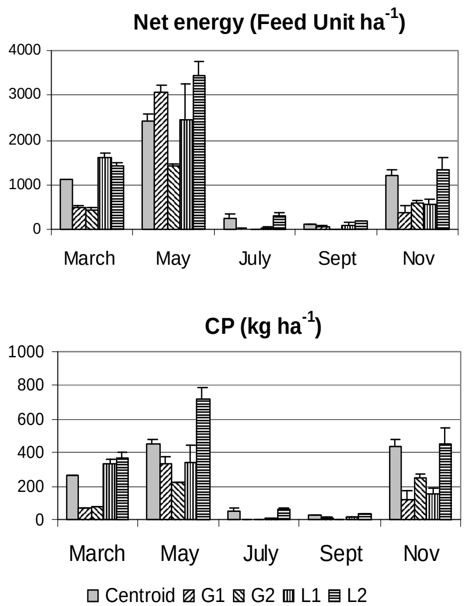

Figure 1: Comparison of Net Energy and Crude Protein availabilities per unit area in pure stands and the centroid along the seasons.

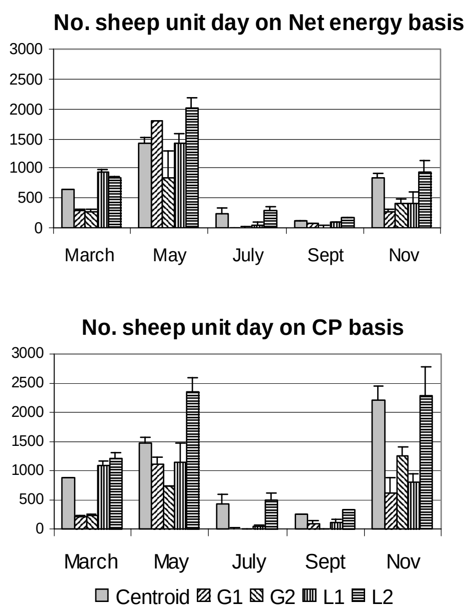

Figure 2: Potential carrying capacity in pure stands and the centroid along the seasons

CONCLUSIONS

On the basis of these preliminary results, the use of mixtures of species of different functional groups seemed to give a higher potential yield of nutrients compared to grass and annual legume pure stands but not to perennial legume pure stand, assuring also a more seasonally-balanced quality of herbage with respect to pure stands.

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Clover species in grass-clover silages affects milk fatty acid composition

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Keywords: *Trifolium pratense*, *Trifolium repens*, silage, dairy cow, linolenic acid

ABSTRACT

The objective of this experiment was to investigate the effect of clover species in grass-clover silage and of concentrate supplementation on milk fatty acid (FA) composition. *Trifolium repens* (WC) and *Trifolium pratense* (RC) grown in mixture with grasses were preserved in round bales and fed to autumn calving dairy cows during the first 12 weeks of lactation. Milk produced on RC silage had a higher proportion of polyunsaturated FAs (3.09 vs. 2.84%, $P < 0.05$) of total FAs than milk from WC silage. In particular, the levels of linolenic acid were higher on RC than on WC, with 0.86 vs. 0.73% ($P < 0.001$), respectively. RC milk contained also a higher n-3/n-6 FA ratio, considered to be beneficial, than WC milk (0.91 vs. 0.83, $P < 0.05$). Concentrate supplementation increased the levels of linoleic acid (1.30 vs. 0.85%, $P < 0.001$) and reduced the proportion of linolenic acid (0.64 vs. 0.95%, $P < 0.001$) and the n-3/n-6 FA ratio (0.58 vs. 1.16, $P < 0.001$) compared to no supplementation. The effect of clover species was found both without and with supplementation. This means that, independent of concentrate supply, profit can be taken from the more beneficial FA composition of the milk with RC compared to WC silage.

INTRODUCTION

Pasture-based diets were shown to increase the content of n-3 fatty acids (FA) and conjugated linoleic acid (*cis*-9, *trans*-11 C18:2, CLA) in milk, whereas diets with a high proportion of grain-based supplements and preserved forages, e.g. silage, seems to reduce the content of these FA (Elgersma *et al.* 2006, Dewhurst *et al.* 2006). It has, however, been shown that feeding silage made of white (*Trifolium repens* L.) or red (*Trifolium pratense* L.) clover may yield milk with a high concentration of the beneficial FA; especially red clover has resulted in high concentrations of C18:3n-3 in milk (Dewhurst *et al.* 2003). However, in the experiments of Dewhurst *et al.* (2003) the silages were made of pure clover, fed either solely or mixed with pure grass silage when fed. It is known that the feeding values of clovers and of particular grasses may differ when grown in pure or mixed grass/clover stands (Lehmann *et al.* 1978). The effect of a plant species grown in pure stands on milk quality, for example, is thus not necessarily the same as when those species are cultivated in mixed stands.

The objective of the work reported was to examine the effect of white clover and red clover based silages, prepared from mixed grass clover leys, fed to cows without and with concentrate supplementation, on the fatty acid composition of milk fat.

MATERIAL AND METHODS

The experiment was carried for two consecutive years (2004 and 2005) at the University of Life Sciences, Ås, Norway, during the first period of each of two lactations (first 89 (s.d. = ± 16) days in milk starting 16 (s.d. = ± 11) days post partum), with 4 groups of dairy cows fed 2 types of grass clover silages without (- C) and with (+ C = 10 kg fresh weight per day and cow) standard concentrate supplementation. The silage treatments were: 1) grass/white clover (WC) round bales from the second and third cut; and 2) grass/red clover (RC) round bales from the same cutting times as in 1. The grass grown in mixture with the clovers consisted of a mixture of *Phleum pratense* L., *Festuca pratensis* Huds., and *Lolium perenne* L. in both treatments. Crop production and animal husbandry were managed according to organic standards. Each year 28 autumn-calving cows of the Norwegian Red Cattle breed were allocated to the 4 treatments per year, balanced for calving time and parity, leaving 7 cows per treatment. Milk samples were collected every 4 weeks for milk fatty acid analysis giving two samples per cow and year.

Milk lipids were separated, followed by transmethylation to produce fatty acid methyl esters (FAME) according to Feng *et al.* (2004). Analyses to separate FAME were made by gas chromatography with a 200 m CP-Select CB for FAME capillary column (Varian Inc.)

Analysis of variance was conducted with year (2004 or 2005), silage type (WC or RC), supplementation level (- C or + C), parity (1 or > 1 lactations), and lactation week as fixed effects, and animal within treatment and season as a random effect, accounting for co-variation within animal in an analysis of repeated measures using SAS (SAS 1999). Reported estimates are LS means.

RESULTS

The silages were well-preserved (*Table 1*). The clover proportion was higher in RC than in WC, but otherwise there were only small and mostly non significant differences in chemical composition (P > 0.05).

Average milk yield was not significantly affected by clover species (24.8 and 25.3 kg/d for WC and RC, respectively). Nor were there any significant effect of clover species on FA with less than 18 C atoms (*Table 2*). However, RC resulted in higher concentrations of linoleic-

Table 1: Chemical analysis of the silage

	2004		2005	
	WC	RC	WC	RC
Clover, g/100g DM	39[b]	57[a]	30[b]	40[a]
Dry matter, g/kg	313[a]	285[b]	268[b]	284[a]
Crude fat g/kg DM	171	163	150	147
NEL, MJ/kg DM	6.10	6.00	6.07	5.84
Ammonia-N, g/kg total-N	78.3[a]	67.4[b]	67.2	66.5
Lactate, g/kg DM	40.1	43.5	59.4	58.9
pH	4.64	4.57	4.64	4.76

WC = white clover - grass silage, RC = red clover - grass silage. Means on the same line within year carrying no common superscript are significantly different (P < 0.05).

acid, linolenic acid and a higher n-3/n-6 FA ratio than WC. Concentrate supplementation had a significant effect on most FA and increased the proportion of linoleic acid but decreased the concentration of linolenic acid relatively more and, consequently, the n-3/n-6 FA ratio was reduced (*Table 2*). There was no significant interaction between clover species and supplementation. The effect of clover species was thus similar at both supplementation levels.

Table 2: Effect of clover species (WC = white clover, RC = red clover) in grass clover silages without (- C) and with (+ C = 10 kg/cow and day) concentrate supplementation on the fatty acid (FA) composition of milk fat (% of total FA). Average over 2 years

	WC		RC			P-value	
Fatty acid (FA)	- C	+ C	- C	+ C	SED	S	C
Short chain fatty acids, (< 14)	13.6	15.0	13.1	15.4	0.46	NS	***
C14:0 (myristic)	10.6	11.1	10.2	11.0	0.31	NS	**
C16:0 (palmitic)	28.5	27.2	27.9	26.2	0.86	NS	*
C18:0 (stearic)	9.7	10.6	9.8	11.1	0.43	NS	***
C18:1 (oleic)	19.0	19.3	19.9	18.7	0.75	NS	NS
C18:1 (vaccenic)	2.00	1.48	2.01	1.61	0.106	NS	***
C18:2 n-6 (linoleic)	0.82	1.25	0.89	1.35	0.052	*	***
CLA cis-9, trans-11 + C:20	1.02	091	1.02	0.92	0.047	NS	**
C18:3 n-3 (linolenic)	0.87	0.58	1.03	0.69	0.040	***	***
Sum polyunsaturated	2.80	2.88	3.04	3.14	0.098	***	NS
n-3 / n-6	1.11	0.55	1.20	0.61	0.048	*	***

NS: not significant (P > 0.1), (*)P < 0.1, * P < 0.05, ** P < 0.01, P < 0.001, SED = Standard error of the difference, S = WC vs. RC. C = - C vs. + C

DISCUSSION

The reduction of linolenic acid and the increase in the linoleic acid proportion of total FA with concentrate supplementation is in agreement with Dewhurst *et al.* (2003). Dewhurst *et al.* (2003) also found a positive effect of clover silages relative to pure grass silage on some beneficial FA. They did not find a consistent effect of clover species on the milk's content of linolenic acid as in the present study, but in their study there was a much higher apparent recovery of linolenic acid from the diet in milk on red clover than on white clover silages. Dewhurst *et al.* (2003) explained this by less FA biohydrogenation in red clover than in white clover. In the present study, the effect of red clover might also have been due to less biohydrogenation. However, as we do not yet have the FA composition of the feed analysed, a higher supply of linolenic acid due to a higher content in red clover than in white clover silage cannot be ruled out as the total feed intake was on average the same (figures not shown). Additionally, part of the RC effect may have been actually a clover proportion effect. There was no effect of silage type on live weight change (figures not shown), so mobilisation of fat cannot explain the effect of clover species.

CONCLUSION

Feeding red clover-grass silage compared to white clover/grass silage resulted in a higher content of beneficial FA, particularly the n-3 linolenic acid, in milk. Concentrate supplementation reduced the content of beneficial FA.

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Overview over the WG 3 common protocol - influence of contrasting environments on forage quality of grass and legumes

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ABSTRACT

The objective of the present study was to characterize the influence of legume species and site on feed quality of forage legumes and a companion grass. Four participating sites evaluated a common protocol, where forage legume species growing in binary swards with perennial ryegrass were compared. At each site, swards were cut after 30±3 days of regrowth in the first production year. The forage legumes (white clover, red clover, alfalfa, and birdsfoot trefoil) and the companion grass (perennial ryegrass) were analyzed separately for metabolizable energy (ME) and crude protein (CP) contents. The results show variation of ME content between legumes and sites, whereas for the companion grass a stronger influence of site was observed, with Norway showing the highest ME contents. Birdsfoot trefoil based swards have the companion grass with the lowest CP content. The CP contents of legumes were lower in Norway and Germany. It was concluded that CP and ME contents of forage legumes and grass varied differently between sites and legume species.

Keywords: legumes, legumes, feed quality

INTRODUCTION

Although grassland based production systems account for half of the animal requirements for feed in Europe, large amounts of protein-rich feed concentrate are imported and the demand for high quality vegetable protein is still increasing. Forage legumes are valuable protein sources, providing alternatives for ruminant nutrition. They may also reduce the need for mineral nitrogen fertilization, and contribute to reduce nitrogen losses on the farm. However, performance and feed quality of forage legume based swards may differ between sites in Europe due to environmental constraints. The objective of the present study was to characterize the influence of legume species and site on feed quality of forage legumes and companion grass. The study is part of the EU COST 852 Action.

MATERIAL AND METHODS

White clover, red clover (*Trifolium pratense* L.), lucerne (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) were established at each site as binary swards with perennial ryegrass (*Lolium perenne* L.) in a randomized split-plot design with three replicates. The first cut of swards was at the point when the node of the grass was detectable with subsequent cuts after 30±3 days. Five cuts were harvested at each site. Only the first production year (the year after sward establishment) from each site was considered. Samples were separated into legume, grass and weed fraction. The feed quality analysis determinations comprised crude protein (CP) and metabolizable energy (ME) content of the grass and legume fraction separately. The CP content was calculated from the total N content of the sample and the ME content was estimated using the cellulase method, according to De Boever *et al.* (1988).

Data on a year basis were submitted to the analysis of variance and means were compared using Student's T-Test. The probabilities were adjusted using the Bonferroni-Holm test.

RESULTS

Table 1 shows the weather data for the participating sites from March 31st to October 1st of the first production year. Norway has the lowest global radiation and average temperature.

Table 1: Weather data of the first production year of each participating site

Site	Temperature, °C	Precipitation, mm	Global radiation, J/cm²
Norway	10.6	551	1274
Switzerland	15.3	722	1546
Netherlands	14.9	396	1568
Germany 2004*	13.8	418	1550
Germany 2005*	14.1	325	1551

* In Germany the experiment was sown each year.

The results of the ME content of companion grass and legumes are shown in *Table 2* to *4*. For the companion grass, the interaction site*sward type was significant (*Table 2*). The ME content of the companion grass was highest in Norway and lower contents were observed in Germany 04.

For the ME content of forage legumes only main effects were significant (*Tables 3* and *4*). White clover with 10.9 MJ ME/kg DM was the legume species with the highest ME content across sites. In Norway birdsfoot trefoil did not perform well and no samples were available.

Table 2: Metabolizable energy content of companion grass (perennial ryegrass)

Site/sward	White clover	Red clover	Alfalfa	Birdsfoot trefoil	Pure stand
Norway	11.8a	11.5a	11.6a	11.9a	11.9a
Switzerland	10.7b	10.4b	10.8b	10.4b	10.6bc
Netherlands	10.8b	10.8b	10.6b	10.5b	10.9b
Germany 04	10.9b*	10.7b*	10.5b	10.4b	10.3c
Germany 05	10.5b	10.8b	10.6b	10.4b	10.4bc

SE = 0.15, n = 73
* significant different to pure grass stand ($P < 0.05$) within sites
a,b significant differences between sites ($P < 0.05$)

Table 3: Metabolizable energy content of forage legumes - site effect

Site	ME, MJ/kg DM
Norway	10.5c
Switzerland	10.3c
Netherlands	10.9a
Germany 04	10.4c
Germany 05	10.7b

SE = 0.07, n = 45

Table 4: Metabolizable energy content of forage legumes - legume species effect

Species*	ME, MJ/kg DM
White clover	10.9a
Red clover	10.5b
Alfalfa	10.3b

SE = 0.05, n = 45
* Birdsfoot trefoil excluded due to missing values in Norway

The CP content of the companion grass is shown in *Table 5*. The interaction site*sward type was significant. Switzerland had the highest CP contents and Germany 05 showed the lowest contents. Grass growing with birdsfoot trefoil showed similar CP content as the pure grass stand. In Norway, only grass growing with red clover shows higher CP contents.

Table 5: Crude protein content of companion grass (perennial ryegrass)

Site	White clover	Red clover	Alfalfa	Birdsfoot trefoil	Pure stand
Norway	13.2c	14.2b*	12.4c	11.8c	11.8b
Switzerland	18.4ab*	17.7a*	19.4a*	15.9a	15.9a
Netherlands	19.7a*	18.6a*	16.8b*	14.2b	13.2b
Germany 04	17.4b*	15.0b*	15.6b*	13.2bc*	10.2c
Germany 05	10.5d*	11.3c*	10.2d*	8.5d	7.2d

SE = 0.60, n = 73
* significant different to pure grass stand ($P < 0.05$) within sites
a,b significant differences between sites ($P < 0.05$)

The CP content of the forage legumes is shown in *Table 6*. The interaction site*sward type was significant. Norway showed the lowest CP contents between sites for all legume species. Considering legume species, alfalfa has the highest CP contents between all sites and red clover the lowest. An exception was Germany, where no differences for legumes species were observed.

Table 6: Crude protein content (% DM) of forage legumes at different sites

Site	White clover	Red clover	Alfalfa	Birdsfoot trefoil
Norway	20.4cB	20.1cB	22.4cA	-
Switzerland	24.5bA	22.3bB	25.6bA	25.2
Netherlands	26.0aA	23.4bB	27.1aA	26.6
Germany 04	26.0aA	25.1aA	25.8abA	26.1
Germany 05	23.4bA	24.4abA	24.5bA	24.3

SE = 0.39, n = 45
a,b significant differences for sites within sward type
A,B significant differences for legumes within site

DISCUSSION

Based on the results presented for the cutting system, the influence of sites on forage quality of binary swards was most pronounced for the companion grass. For the companion grass, the influence of site was clearly influenced by the environmental conditions, as the grass growing in Norway had the highest ME contents. Although white clover was the legume species with the highest ME content, differences due to site were of minor magnitude. The CP of forage legumes ranged from 20.4 to 27.1% DM between sites and legume species. But differences in CP between species are of minor magnitude as well. The frequent cut each 30 days may be one of the reasons, as plants were always cut in an early development stage.

CONCLUSION

The observed differences between sites and legume species for ME and CP contents in legumes are of marginal relevance for ruminant nutrition. The results suggest that for contrasting environments any legume species could be used in binary swards and may produce forage of similar CP and ME contents when cut every 30 days. Especially in the northern Europe grasses have to be considered as they may influence forage quality of feed.

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Estimating dry matter yield of legume-grass swards by field spectral measurements using the vegetation index EVI

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ABSTRACT

A pot experiment was conducted under controlled conditions to investigate the potential of field spectral measurements for a non-destructive assessment of dry matter. Binary mixtures and pure swards of annual ryegrass (*Lolium multiflorum*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*) and lucerne (*Medicago sativa*) were grown in 0,16 cm² wood boxes. One day before harvest all swards were measured with a field spectrometer (ASD FieldSpec Pro® JR.) in the range of 350 to 2500 nm. For further data processing the Enhanced Vegetation Index (EVI) was calculated from the data of spectral measurements and related to the dry matter yield of the harvested swards. The results show significant relations between dry matter yield and vegetation index. Nevertheless different reflection characteristics from legumes and grass complicate dry matter yield detection of mixed swards. For an improved understanding regression analysis were conducted including the fraction of legumes (% of DM) and the vegetation index as explanatory variables.

Keywords: field spectroscopy, legume-grass swards, dry matter yield, Enhanced Vegetation Index

INTRODUCTION

The growth of legume-grass swards is affected by several parameters such as soil conditions, water availability and nutrient supply. The non-destructive detection of dry matter yield during regular work in the field would facilitate a more accurate management of nutrients, plant treatments or reseeding. The present article focuses on a non-destructive detection of dry matter yield of different legume-grass swards using field spectroscopy. To exclude external effects such as changing light conditions or a different nutrient supply, the experiment was conducted under controlled conditions in a green house.

MATERIALS AND METHODS

Sixteen different experimental swards were investigated in three replications and at four different growth stages. Beside pure swards of annual ryegrass, red clover, white clover and lucerne different binary legume-grass swards were sown (*Table 1*).

To compare swards at different growth stages the legume-grass-mixtures were sown every two weeks. After an early cleaning cut, which was conducted 9 weeks after sowing, all swards were harvested at a common date, so that 4, 6, 8, and 10 weeks old swards could be compared. The sowing was carried out manually with a distance between rows of 12 cm and at a sowing depth of 0.5 cm. The pots (40 x 40 x 20 cm) were filled with homogenised loamy soil. Soil

Table 1: Seeding ratios (kg/ha) of the experimental swards

	seeding ratio (kg/ha)			
Annual ryegrass (AR)	20			
Red clover:AR	2:20	4:20	6:20	8:20
White clover:AR	1:20	2:20	3:20	4:20
Lucerne:AR	4:20	8:20	12:20	16:20

analysis indicated optimum levels of phosphorus, magnesium and potassium and a pH-value of 6.7. No fertilizers were used. To determine the fraction of grass, legumes and unsown plants in the sward, total above-ground biomass was separated in its fractions. After the determination of all species the samples were dried at 65 °C for 30 h.

One day before harvest all swards were measured with a field spectrometer (ASD FieldSpec Pro® JR.) in the range of 350 to 2500 nm using a halogen lamp as light source. For further data processing the Enhanced Vegetation Index (EVI) (Heute and Justice 1999) was calculated from the data of the field spectral measurements and related to the dry matter yield of the harvested swards (M = Mean):

EVI = (((M 800nm-900nm)-(M 650nm-700nm))/((M 800nm-900nm)+6*(M 650nm-700nm)-7,5*(M 450nm-500nm)+1))*2

RESULTS AND DISCUSSION

The growth stages of the investigated swards ranged from tillering, stem elongation, head emergence and start of flowering depending on their date of seeding. As field spectroscopy measures the entire reflection signal of one pot, plant architecture and parts of visible soil have an important impact on the detected readings. All swards being in the stage of tillering had higher parts of visible soil in oversight. The pure legume swards in the more advanced growth stages showed a closed canopy without visible soil. In contrast the ryegrass with its erectophil position of leaves still had visible soil even if biomass yield was high (*Figure 1*).

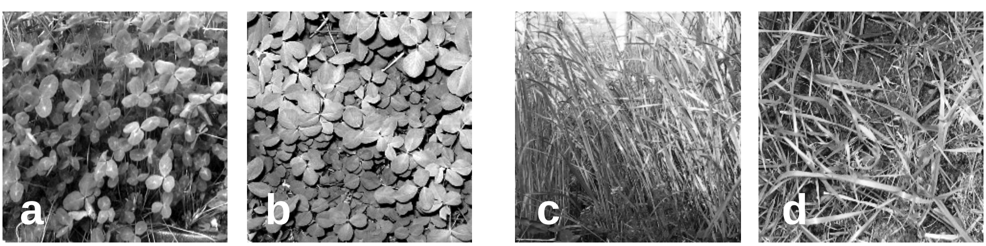

Figure 1: Legumes and ryegrass in a sideway view (a, c) and in oversight (b, d)

In *Figure 2* the dry matter yield of legumes, grass and mixed swards are mapped against the vegetation index EVI. It was thought that the influence of leave structure and parts of visible soil caused the irregular distribution of values. The annual ryegrass in pure sward showed relative small values of EVI, whereas legumes in pure swards held high values. The values of mixed swards were in between both pure swards. It seemed that in mixed swards the fraction of legumes affected the relation between EVI and the dry matter yield.

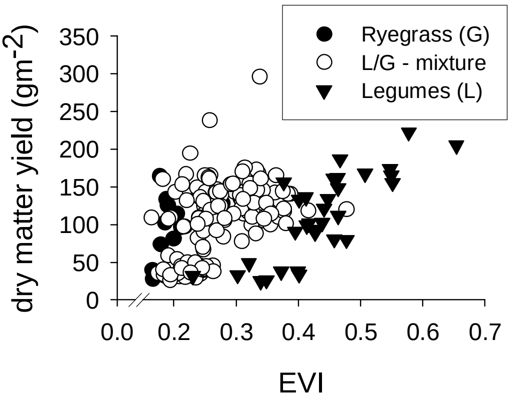

Figure 2: Dry matter yield of pure and mixed legume-grass swards depending on EVI

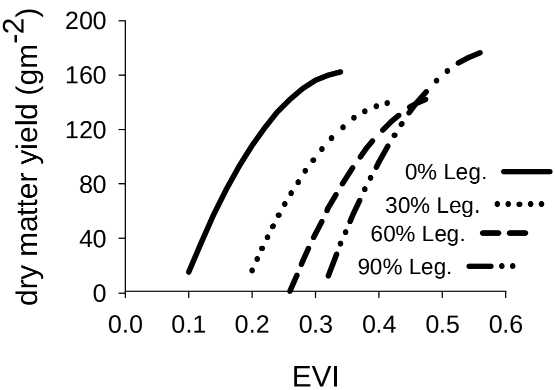

Figure 3: Dry matter yield depending on EVI (x_1) and the content of legumes (x_2)
$R^2 = 0.34$, s.e. = 40.2
$y = 1607{,}7x_1 - 2258{,}38x_1^2 - 5{,}42x_2 + 11{,}81x_1 * x_2 - 123{,}14$

For this reason based on all mixed swards legume contribution (% of DM) was included beside EVI in the calculation of dry matter yield (*Figure 3*). The result of the regression analysis shows a significant quadratic curve with a positive interaction between EVI and the legume content. Only the parts of the curve where yield data were available are displayed. Due to the positive interaction of EVI and the legume content the response curve moves to the right on the x-axis with an increasing legume contribution in the mixture. Thus, for the investigated swards an estimation of dry matter yield seems to be impossible if the fraction of legumes is not known.

Because of the planophil growth of legumes leaves, they could act as an indicator for the closure of the canopy and hence for the fraction of soil visible in oversight. With an increased legume contribution in the mixture the fraction of visible soil decreases. Thus, consideration of cover data e.g. from digital image analysis (Himstedt 2007) could be a promising approach.

CONCLUSION

The incident light is reflected by legumes and annual ryegrass in specific ways due to their distinct architectures. At the same level of biomass yield, swards with higher proportions of legumes in mixture show higher values of EVI than grass dominated swards. This positive interaction between EVI and the legume contribution complicates the estimation of dry matter yield.

Legumes, with their planophil growth habit, could indirectly be an indicator for the fraction of soil visible in oversight. In this investigation the annual ryegrass had a very fast growth so that it was tall and less densely tillered. Such swards (which are not common in practice) showed high parts of visible soil though they had a relatively high biomass yield. For this reason the cover of swards should be included for the estimation of dry matter yield in further investigations.

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Image analysis as a tool to evaluate legume proportions in legume-grass mixtures

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ABSTRACT

A pot experiment was conducted to estimate the dominance structure of legumes in legume-grass mixtures by image analysis. Pure swards and binary legume-grass mixtures of red clover, white clover, lucerne and perennial ryegrass were sown in two week intervals and harvested at the same date to compare swards at different growth stages. Digital images of all variants were taken one day before harvesting. Sward composition was detected by fractionating biomass into grass, legumes and unsown species as reference. A subset of eight images with varying legume content taken of the longest grown swards was selected to develop an optimized image analysis procedure with Optimas®. The resulting image analysis procedure was used for all treatments of all sward ages. The estimated dominances of legumes were compared to the references. Good results were detected using image analysis for estimating the dominance of legumes. A significant coherence between the detected legume proportion and the dry matter yield was found ($R^2 = 0.7$). Undesired legume identifications in pure grass swards can be caused by weeds with rounded leaves (*Stellaria media*) and visible ground. The next steps will be to verify a relationship in swards of different ages and to check further procedures of image analysis, in particular pattern recognition methods.

Keywords: Image analysis, legume-grass mixtures, legume proportion, dry matter yield

INTRODUCTION

Arable forage crops often grow in heterogeneous mixtures. This composition affects numerous and important performance parameters like nitrogen fixation and yield. In this context the fraction of legumes is of high importance. If the proportion of legumes in the sward is known, site specific treatments can be performed e.g. adapted fertilization, reseeding or a calculation of preceding crop effects.

An efficient, indirect and nondestructive method of determining the botanical composition of swards would be of great use in precision farming models over a fairly long-term period.

To devise an optimal image analysis method for detecting the canopy cover of legumes digital pictures of several legume-grass mixtures were taken. The first results of image analysis were correlated with both the visual classified canopy cover of legumes and the dry matter yield of the manually separated biomass to determine the accuracy of the image analysis method.

MATERIALS AND METHODS

A pot experiment lasting nine weeks, compared pure swards and binary legume-grass mixtures of red clover, white clover, lucerne and perennial ryegrass. To compare swards of different ages the legume-grass mixtures were sown every two week in 2004, calendar week (CW)

46, 48 and 50 and harvested 35, 49 or 63 days after sowing. All treatments were sown in four replications. Beside pure swards of perennial ryegrass (20 kg ha^{-1}), red clover (8 kg ha^{-1}), white clover (4 kg ha^{-1}) and lucerne (16 kg ha^{-1}) the following mixtures were sown: R8G (red clover 8 kg ha^{-1} + perennial ryegrass 20 kg ha^{-1}), R2G (red clover 2 kg ha^{-1} + perennial ryegrass 20 kg ha^{-1}), WG (white clover 4 kg ha^{-1} + perennial ryegrass 20 kg ha^{-1}) and LG (lucerne 16 kg ha^{-1} + perennial ryegrass 20 kg ha^{-1}). To determine the sward composition, total above-ground biomass was fractionated into grass, legumes and unsown species. The samples were dried for 18 h at 65 °C.

One day before harvesting digital pictures of the swards were taken. On the basis of four specific vertices the digital pictures were georeferenced with the program SAGA (System for Automated Geoscientific Analyses Version 2.0, 2005). For image analysis the computer program Optimas® of the Media Cybernetics® Company was used. The image analysis with Optimas® aquires the canopy cover of legumes in percent of the total picture area (Image analysis P_a).

Images of eight variants were selected as reference and legumes were classified visually. With the GIS-Program TopoL® (Version 6.5) legume covered areas were encircled and set as vector graphics. In a subsequent area analysis the exact area covered by legumes (visual image classification P_v) was calculated. The eight reference images were selected from the 63 days old swards containing pure swards of grass (G), red clover (R), white clover (W) and lucerne (L), two red clover-grass mixtures with 8 kg ha^{-1} (R8G) and 2 kg ha^{-1} (R2G) sowing rate of red clover respectively, one white clover-grass mixture (WG) and one lucerne-grass mixture (LG). A detailed description of the ascertained image analysis procedure with the program tools Erode und Delate (Optimas®) can be found in Himstedt *et al.* (2006).

RESULTS AND DISCUSSION

The legume canopy cover obtained by image analysis P_a (%) of the eight selected calibration images shows a significant linear correlation (R^2 = 0, 94, *Figure 1*) with the legume canopy cover ascertained by visual classification P_v. The question is, if this coherence can also be found in relation to the dry matter yield of the legume-fraction. Hence, the same image analysis procedure was used over all variants and an equation for the correlation of legume dominance P_a and dry matter yield of legumes of all 63 days old variants (n = 32) was calculated. A clear coherence between P_a and the dry matter yield exists (R^2 = 0.7, *Figure 2*). It is noticeable that red clover and white clover both pure and in mixtures show a closer relationship with the reference than lucerne and grass. Problems with legume identification in pure grass swards with image analysis can be caused by weeds with rounded leaves (*Stellaria media*) and visible ground. The dominance of lucerne determined by image analysis was lower then the measured dry matter yield (%). This likely results from the longish leaf shape, which was partly treated in a similar manner to the longish grass shape.

Comparing P_a (% canopy cover) and dry matter yield (%) of the pots, it must be considered that grass and unsown species growing underneath the visible leaves of legumes are not included in P_a, but affect the dry matter yield of the legumes considerably.

Using the dry matter yield calibration (*Figure 2*) the dry matter yield of legumes for all variants and ages was approximated and compared with the measured dry matter yields

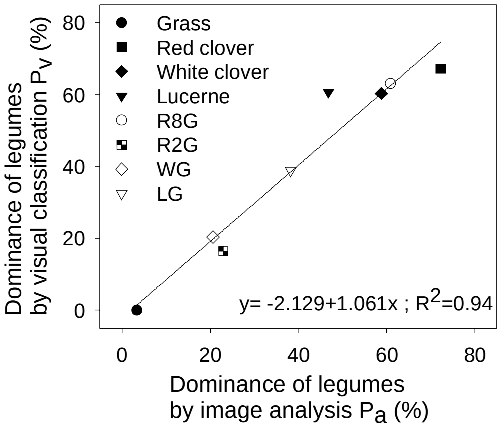

Figure 1: Relationship between the visually classified legume canopy cover P_v (%) and the dominance of legumes P_a (%) ascertained by image analysis of the eight calibration images. (R8G: Red clover/grass (red clover 8 kg ha^{-1}), R2G: Red clover/grass (red clover 2 kg ha^{-1}), WG: White clover/grass, LG: Lucerne/grass)

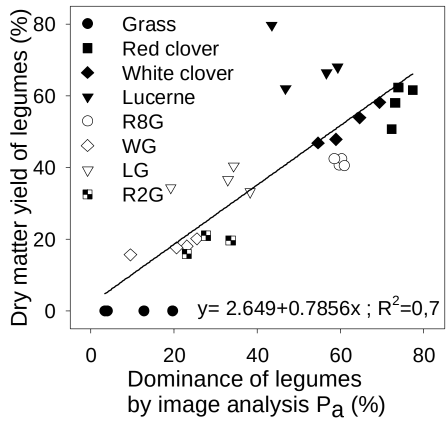

Figure 2: Relationship between legume dominance P_a and measured dry matter yield of legumes (%) of all 63 days old variants, each in 4 replications (n = 32).
(R8G: Red clover/grass (red clover 8 kg ha^{-1}), R2G: Red clover/grass (red clover 2 kg ha^{-1}), WG: White clover/grass, LG: Lucerne/grass)

(*Figure 3*). The younger the swards the less accurate are the calculations due to an increasing visibility of the ground.

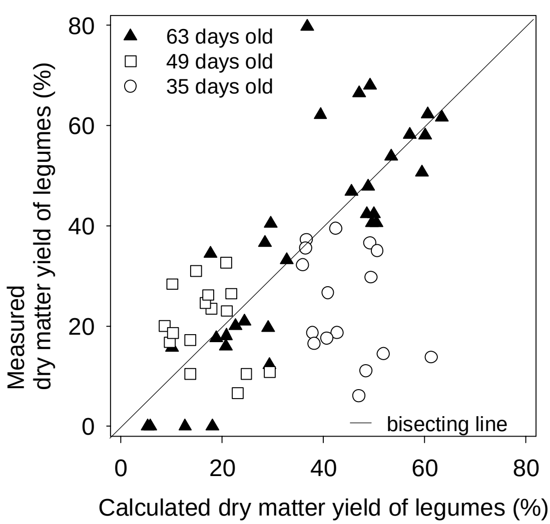

Figure 3: Comparison of calculated dry matter yield of legumes (%) (y = 2.649 + 0.7856x; *Figure 2*) with measured dry matter yield of legumes; legume-grass swards of different ages (n = 64)

CONCLUSIONS

Promising results were achieved by using an image analysis procedure for estimating the dominance of legumes. The dry matter yield of red and white clover can be expressed by image analysis ascertainment both for pure swards and mixtures. However, difficulties with the selected procedure can appear, caused by longish leaf shapes of lucerne, weeds with rounded leaves or visible ground. Although, not included in the procedure, age of the swards is likely to have an effect. The next steps will be to verify a relationship for swards of different ages and to involve complex classification criteria like extension and circularity.

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Variability for non structural carbohydrates in alfalfa

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Keywords: starch, alfalfa, carbohydrates, breeding, daylight period

ABSTRACT

Forage legumes are generally poor in non structural carbohydrates (NSC) and rich in degradable proteins. Our objectives were to determine the extent of genotypic variability for NSC concentration in alfalfa and to determine the effect of the length of the daylight period before cutting on alfalfa NSC concentration and on rumen fermentation as measured by gas production during incubation of alfalfa with rumen fluid. We concluded that extensive variability for NSC concentration exists within an alfalfa population and that increasing the daylight period up to 12 h before cutting favours the accumulation of starch and NSC in alfalfa and has a positive effect on rumen fermentation.

INTRODUCTION

Extensive degradation of proteins combined with insufficient readily fermentable energy in alfalfa results in poor forage N utilization by ruminants and substantial N losses to the environment. In the UK, an increased digestible dry matter intake in cows fed a ryegrass cultivar high in water soluble carbohydrates (WSC) led to increased milk yields, whereas increased efficiency of utilization of this ryegrass in the rumen resulted in a more efficient use of feed N for milk production and reduced N excretion (Miller *et al.* 2001). In the USA, research has shown that ruminants prefer alfalfa harvested at sunset because of its increased NSC concentration (Fisher *et al.* 2002). This increase in NSC, associated with improved digestibility and decreased fibre concentrations, has the potential to improve daily dry matter intake and animal responses (Burns *et al.* 2005). Significant variation for WSC amongst 17 alfalfa cultivars has been reported in fresh and wilted forage samples (Monteiro *et al.* 1998) but little is known on variability of NSC amongst alfalfa genotypes.

Our objectives were to determine the extent of genotypic variability for NSC concentration within an alfalfa population (Exp. 1) and to determine the effect of the length of the daylight period before cutting on forage concentrations of WSC, starch, and NSC, and on gas production during incubation of alfalfa with rumen fluid (Exp. 2).

MATERIALS AND METHODS

In Exp.1, nearly 500 genotypes from the alfalfa cultivar 'AC Caribou' were grown under controlled conditions and first sampled on 2 April 2004 on the third regrowth. These genotypes were subsequently transplanted in a field and a second forage sampling was done on

13 September 2004. At this harvest, 76 genotypes with a DM yield inferior to the means minus a standard error were eliminated. An additional sampling was done on 28 July 2005. All three samplings were done at the early flowering stage of development. Forage samples were dried at 55°C immediately after each harvest, ground to pass through a 1-mm screen, and scanned using NIR spectroscopy (FOSS North America Inc., FOSS NIRSystems 6500). Samples were analyzed by HPLC for soluble sugars (sucrose, fructose, raffinose, and pinitol) and by colorimetry for starch. The NSC concentration, estimated by the sum of soluble sugars and starch, was predicted using NIR spectroscopy.

In Exp. 2, 20 pots of alfalfa (cv. AC Caribou) with 10 plants per pot (14 cm in diameter) were placed in a growth chamber with air temperatures of 22°C/17°C (day/night) and a 16-hour photoperiod. Alfalfa from four pots was harvested after 0, 2, 4, 8, and 12 hours of light. Samples were dried and ground as in Exp. 1, and analyzed by HPLC for soluble sugars and by colorimetry for starch. Samples of 1 g were also placed in 160 mL bottles with 85 mL anaerobic medium (Longland *et al.* 1995) and 10 mL ruminal digesta. Pressure and volume of gases were measured at regular intervals over 142 h.

RESULTS

In Exp. 1, the NSC concentration varied from 5 to 170 mg/g DM (mean = 69, SD = 30.7) on the first sampling date and from 73 to 230 mg/g DM (mean = 136, SD = 26.7) on the second sampling date. Based on these results, 50 genotypes with high and 50 genotypes with low NSC concentrations were selected. An additional sampling was conducted on 28 July 2005 to validate these groupings. Although the ranking of genotypes varied with the three sampling dates, ten genotypes with consistently high (118, 171, and 101 mg/g DM) and ten genotypes with consistently low (47, 96, and 67 mg/g DM) NSC concentrations at each of the three sampling dates were identified (*Figure 1*).

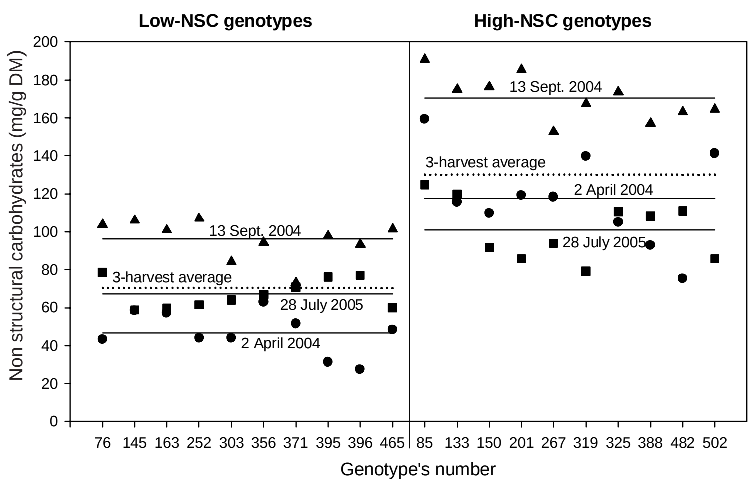

Figure 1: Concentration of non structural carbohydrates (NSC) at three different harvests (▲ = 2 April 2004, ▲ = 13 September 2004, ▲= 28 July 2005) in 10 alfalfa genotypes selected for low NSC and 10 genotypes selected for high NSC

In Exp. 2, forage WSC concentration was not affected by the treatments, but starch and NSC concentrations in alfalfa increased with the length of the daylight period and reached maximal values (104 and 185 mg/g DM) after 12 h (*Figure 2*).

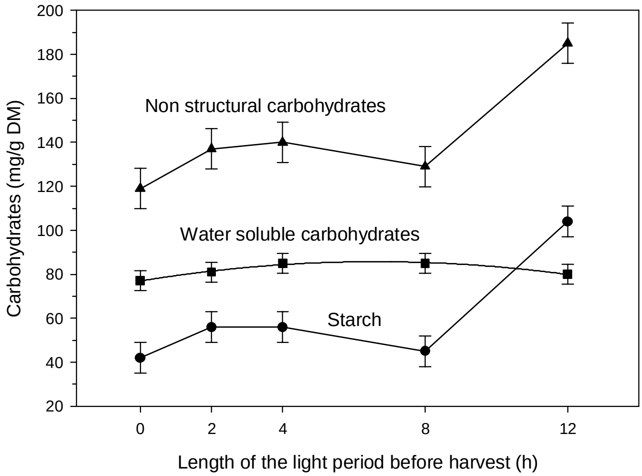

Figure 2: Carbohydrate concentrations of alfalfa harvested as a function of the length of the light period

Rumen fermentation, assessed by in vitro gas production from forage samples incubated in an anaerobic medium inoculated with rumen fluid, also increased with the length of the daylight period before cutting as indicated by the maximum values from the cumulated gas production curves (*Figure 3*).

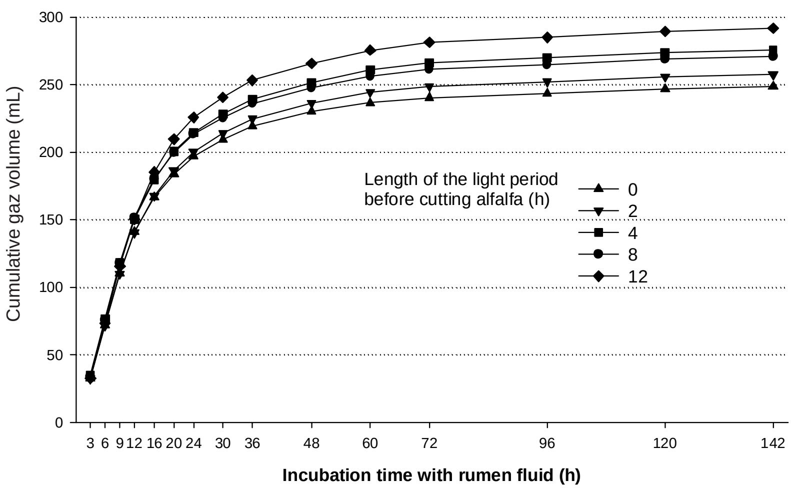

Figure 3: Cumulative gas production during fermentation of alfalfa harvested with increasing length of the light period and incubated in rumen fluid

DISCUSSION

Extensive variability for NSC concentration exists within an alfalfa population. Clones of the high- and low-NSC genotypes, and populations obtained by intercrossing genotypes within the low- and high-NSC groups are currently under evaluation. Increasing the daylight period up to 12 h before cutting favours the accumulation of starch and NSC in alfalfa and has a positive effect on rumen fermentation. This concept is now under evaluation in a field experimentation involving dairy cows.

CONCLUSION

We conclude that increasing NSC concentration in alfalfa might be possible either through breeding or a simple management practice such as late afternoon cutting.

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Spatial scale of plant heterogeneity and diet selection by grazing livestock: a review

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Keywords: Diet selection, heterogeneity, spatial scale

ABSTRACT

This paper reviews research into the influence of the spatial scale of plant heterogeneity on diet selection and intake by grazing livestock. Preference studies have shown that sheep and cattle grazing adjacent, separate monocultures of perennial ryegrass and white clover select mixed diets with a partial preference of 70% (+/- 10%) for clover. There is also a consistent diurnal pattern of preference, with the proportion of grass in the diet increasing towards the end of the day. In contrast, sheep and cattle grazing from intimately mixed grass/clover swards typically have a lower proportion of clover in their diet, which is attributed to the selection costs associated with searching through the mixture for their preferred herbage. Production studies show that cattle achieve higher herbage intakes and greater milk yields when grazing spatially separate grass clover swards compared to cows grazing intimately mixed swards. Research with heifers demonstrates that the critical scale of spatial separation lies between 12 and 36 cm. All these studies demonstrate the importance of the spatial scale of plant heterogeneity in determining diet composition and herbage intake in grazing sheep and cattle, and consequently the study and measurement of plant spatial heterogeneity in mixed swards is to be encouraged.

INTRODUCTION

Historically, sheep and cattle grazed from pastures that contained a mixture of plant species. Although the intensification of livestock production following the Second World War lead to increased use nitrogen-fertilised monoculture pastures of ryegrass, there is now a growing interest in incorporating legumes into grass swards. Whilst mixed grass/legume swards may have functional benefits which boost productivity (Kirwan *et al.* 2007), mixed swards also offer the animal a choice of plant species, enabling the animals to select their own diets. If we are to develop grazing management systems that optimise nutrient intake by livestock grazing mixed swards, we need to understand the factors that influence diet selection. This paper reviews research that investigates the role of the spatial scale of plant heterogeneity in diet selection in grazing herbivores.

GENERAL METHODS

A key concept in diet selection studies is the distinction between preference and selection. Preference is defined as 'what the animals select given the minimum of physical constraints' (Parsons *et al.* 1994), and is typically measured by giving the animals free choice to select their diets from adjacent, separate monocultures of different plant species e.g. white clover and perennial ryegrass. In contrast, selection is defined as 'preference modified by environmental circumstances' (Hodgson 1979) e.g. the need to search through an intimately mixed grass/clover sward for the preferred plant species imposes a constraint on the animal and is therefore an example of selection. In other words, preference is about what the animals 'want' to eat and selection is about what they 'end up' eating as a result of some constraint e.g. the cost imposed by having to select their diet from an intimate mixture of plant species. The main distinction between preference and selection studies is the spatial scale of heterogeneity of the plant species being offered to the grazing animals, and, consequently, this distinction allows the influence of the scale of plant heterogeneity on diet selection and intake to be investigated.

GENERAL RESULTS

A variety of studies (reviewed in detail by Rutter 2006) have demonstrated that both sheep (e.g. Parsons *et al.* 1994) and cattle (e.g. Rutter *et al.* 2004) have a partial preference of approximately 70% (+/-10 %) for clover i.e. given a free choice between adjacent ryegrass and white cover monocultures, clover forms approximately 70% of their diet. These studies also report a consistent diurnal pattern of preference, with a strong preference for clover in the early part of the day, with the proportion of grass in the diet increasing over the course of the day. Champion *et al.* (2004) found that sheep grazing an intimately mixed grass/clover sward had a lower daily intake than those grazing adjacent grass and clover monocultures. Whilst the animals grazing the mixed sward had a higher proportion of clover in their diet than was present in the sward, this was less than that in the animals grazing the adjacent monocultures. They attributed the lower intake to the cost of the animals selecting their preferred herbage (at that time of day) from an intimately mixed sward. Similarly, Nuthall *et al.* (2000) found that cattle grazing adjacent grass and clover monocultures had a higher daily intake (and a higher milk yield) than those grazing mixed grass/clover swards. Rutter *et al.* (2001) demonstrated that free choice was not necessary to achieve the intake and production benefits associated with separate swards, and that similar benefits could be achieved in dairy cattle using a treatment they called 'temporal allocation' which involved grazing clover (only) following morning milking and grass (only) following afternoon milking (i.e. following the natural diurnal pattern of preference). The critical spatial scale at which heterogeneity imposes selection costs was investigated by Rutter *et al.* (2005) using adjacent monoculture strips of grass and clover at different widths. They found that beef heifers selected a diet consisting of approximately 60% clover from strips of 36 and 108 cm width, but only approximately 40% clover from 12 cm wide strips and an intimate mixture of ryegrass and white clover. From this they concluded that the critical scale at which spatial heterogeneity imposes selection costs lies between 12 and 36 cm for beef heifers grazing perennial ryegrass and white clover.

DISCUSSION

The results from these studies show that both sheep and cattle have clear diet selection goals. In livestock grazing perennial ryegrass and white clover this includes aiming to eat a mixed diet with a partial preference of ~70% for clover and with a diurnal pattern of preference. When grazing intimate mixtures of plant species, both sheep and cattle will make a trade-off and reduce their intake rate as they attempt to eat their preferred herbage at that point in the day. As the spatial scale of heterogeneity increases (i.e. with increasing spatial separation), selection costs are reduced, resulting in higher daily intakes and increased production. These findings have important implications for those studying the complex interactions between plant species in mixed swards under grazing. Firstly, the proportion of a preferred herbage that is selected from a mixed sward can be higher than the proportion of that plant in the sward i.e. livestock do not graze at random, and the proportion of a plant in their diet will probably be different to the proportion of that plant in the sward. Consequently, preferential grazing of one (or more) species over one (or more) others is likely to be an important factor influencing the dynamic interactions and stability of those plant species, even in intimate mixtures. Secondly, the spatial scale of plant heterogeneity has an influence on the diet selection of grazing livestock, affecting their intake and level of production, which has obvious economic implications.

CONCLUSIONS

Diet preference and selection studies demonstrate the importance of the spatial scale of plant heterogeneity in determining diet composition and herbage intake in grazing sheep and cattle, and consequently the study and measurement of plant spatial heterogeneity in mixed swards is to be encouraged.

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Herbage and milk productivity and quality when grazing different legumes and herbs

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ABSTRACT

In order to evaluate how different legumes and herbs affects the quality of organic milk a grazing study was performed at the organic research station Rugballegaard with 48 dairy cows. Swards with lucerne (*Medicago sativa*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*) and white clover together with chicory (*Cichorium intybus*), respectively, were established in 2005 together with perennial ryegrass (*Lolium perenne*) in two replicates. The study was carried out in 2006 over three two-week periods during the season. Generally, the proportion of legumes and chicory was high in the four species mixtures. Sward composition highly affected grazing behaviour. Sward height measurements indicate that the white clover mixture was eaten without much selection, the red clover was partly avoided in some areas, and in contrast lucerne seemed to be eaten selectively. In chicory swards selection occurred in a patchy way. Milk yield and protein content was unaffected by treatment. Milk urea content was clearly lowest in the milk from the cows grazing chicory.

Keywords: legumes, herbs, grazing, sward structure

INTRODUCTION

It is known that the botanical composition of the fodder affects the composition of milk and subsequent the flavour (Collomb *et al.* 2002). Pasture contains high levels of α-tocopherols and carotenoids, and an on-going survey of the composition of organic milk in Europe (QLIF-project) indicates that it is possible to reach extremely high levels of conjugated linoleic acids, α-tocopherols and carotenoids, which are expected to contribute positively to the nutritional value, when dairy cows are entirely fed on pasture (Nielsen, pers. comm.).

The hypothesis of this work was that it is possible to produce milk with a distinct flavour and composition using feeding concepts with high levels of grazing. Specifically, it was the aim to analyse the effect on herbage production, composition, intake, selection and milk quality of large proportions of different legumes and herbs in the pasture. The results on herbage composition, productivity, selection during grazing and milk production are presented here.

MATERIALS AND METHODS

A grazing study was performed at the organic research station Rugballegaard with 48 dairy cows. Swards with lucerne, red clover, white clover and white clover together with chicory, respectively, were established in 2005 together with perennial ryegrass in two replicate paddocks. The study was carried out in 2006 over three, two-week periods during the season, where the herd were split into four groups before each period according to stage of lactation, parity and milk yield. The cows grazed the two replicates alternately with one day in each. The swards were unfertilized and irrigated at high drought stress. Before each period the sward biomass was determined and the grazing area adjusted to equal herbage allowance. Observations were made on herd and sward productivity, herbage quality, botanical composition and intake during grazing with special attention to selection. Sward height was determined before and after each grazing period by 50 and 100 measurement, respectively, using a rising-plate meter. Sward productivity was estimated indirectly in an area fenced off during the period. At the start and after one week of grazing the herbage mass and the botanical composition were determined in 0.5 m² samples in the grazed area and in the fenced area. Furthermore the grassland species were analysed in order to calculate the feeding value and for components affecting the quality of the milk including fatty acid composition, tocopherols and carotenoids and further for content of potential flavour compounds that can be transferred to the milk (not reported here). The cows were on pasture 20 hours daily and supplemented with 6.2 kg dry matter cow^{-1} day^{-1} (oats 82%, hay 16%, mineral mix 2%) fed restrictively twice daily after milking. Milk samples were collected three times during the last week of each feeding period.

RESULTS AND DISCUSSION

Herbage composition, productivity and selection during grazing

Generally, the proportion of legumes and chicory was high in the four species mixtures included in the study (*Table 1*). The exception was only 12% lucerne in the two-week period in June caused by too short a rest period from the previous period in May. Lucerne requires a longer rest period in-between grazing than the two weeks offered in this experiment.

Differences in sward structure indicated different grazing behaviour of cows on different sward types (*Figure 1*). Prior to grazing, sward height did not vary much except in the lucerne mixture. After two weeks of grazing the sward with white clover still had a relatively even height, lucerne was much more even than before grazing and both the red-clover and the chicory mixture were more variable in height than before. The chicory mixture, especially was very unevenly grazed. Altogether the height measurements indicated that the white clover mixture was eaten without much selection, the red clover was partly avoided in some areas, and in contrast lucerne seemed to be eaten selectively. In chicory swards selection

Table 1: Proportion of the main component of the species mixtures and herbage mass at the beginning of each period, and growth rate during the 2-week experimental period. SD in parenthesis.

Sward mixture	Proportion of main component (%) May	Jun.	Aug.	Herbage mass kg DM ha^{-1} May	Jun.	Aug.	Herbage growth rate kg DM ha^{-1} d^{-1} May	Jun.	Aug.
White clover	60(16)	46(14)	40(7)	1799 (340)	1670(306)	904(217)	134(36)	109(54)	43(56)
Red clover	69(18)	47(15)	41(15)	2383(155)	2178(194)	907(343)	82(80)	122(34)	57(29)
Lucerne	26(8)	12(5)	39(15)	2476(315)	1249(652)	1462(471)	106(90)	124(70)	11(17)
Chicory	73(14)	54(14)	41(19)	1738(230)	1609(297)	886(521)	123(71)	102(94)	62(35)

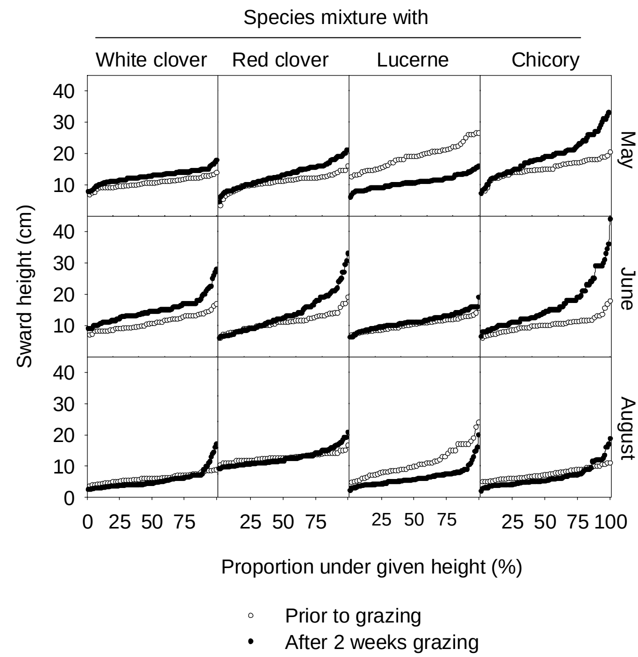

Figure 1: Sward structure previous to and at the end of each two-week grazing period. Percent of measurements below a given height is shown in relation to the height.

occurred in a patchy way in the May and June grazing periods as the sward was grazed down in some patches and untouched in others. We could not explain this pattern of selection within this species.

Intake and milk production

Milk yield was unaffected by treatment and was at a high level in all three periods (*Table 2*). The milk protein content was also similar between treatments, but with a reduction over the season. Milk urea content was clearly lowest in the milk from the cows grazing chicory, whilst the ranking of the other treatments changed during the season, with red clover being highest in the first two periods and white clover in the last period.

Table 2: Milk yield, protein- and urea content during the 2-week experimental period. SD in parenthesis.

Sward mixture	Milk yield kg milk cow^{-1} d^{-1}			Protein content g protein kg^{-1} milk^{-1}			Urea content mmol urea kg^{-1} milk^{-1}		
	May	Jun.	Aug.	May	Jun.	Aug.	May	Jun.	Aug.
White clover	33.3(6.4)	32.2(6.1)	31.8(6.2)	33.9(2.5)	33.6(3.4)	31.7(3.1)	3.9(1.0)	5.5(0.9)	6.8(0.9)
Red clover	30.3(6.8)	32.1(6.8)	31.4(4.1)	34.6(2.6)	32.7(3.5)	32.1(2.2)	4.4(1.2)	6.1(0.7)	5.8(1.1)
Lucerne	32.8(7.5)	32.4(6.8)	28.2(7.7)	33.3(3.6)	33.6(2.7)	32.7(5.0)	3.8(1.0)	4.9(1.1)	6.2(1.2)
Chicory	33.9(6.5)	31.3(6.0)	29.2(6.1)	33.4(2.3)	33.3(2.0)	30.9(2.7)	1.6(0.3)	2.4(0.7)	4.3(0.9)

CONCLUSIONS

We have presented some herbage and milk production results from an ongoing study on how grazing of different legumes and herbs affects the quality of organic milk. Generally, the proportion of legumes and chicory was high in the four species mixtures. Sward composition highly affected grazing behaviour: the white clover mixture was eaten without much selection, the red clover was partly avoided in some areas, and in contrast lucerne seemed to be eaten selectively. Milk yield and protein content was unaffected by species mixture whereas milk urea content was clearly lowest in the milk from the cows grazing chicory. Analysis of the quality of herbage and milk in this study are in progress.

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Effect of white- or red clover supplementation to a ryegrass-based diet on the concentrations of beneficial functional fatty acids in the milk fat of dairy cows

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ABSTRACT

The effects of white clover and red clover on the fatty acid composition of milk fat of cows fed fresh and ensiled ryegrass-based diets were compared in two experiments. In experiment 1, fresh ryegrass was mixed with white or red clover (60/40, on dry matter (DM) basis). Experiment 2 involved similar mixed diets in ensiled form, and two ryegrass silage diets, either without or with supplementary rumen-undegradable protein. All diets were included some hay and barley. Total DM intake, milk yield, and milk fat content were not affected by diet in both experiments. In experiment 1, higher concentrations of α-linolenic acid ($P < 0.05$) and *n*-3 fatty acids ($P < 0.1$) were measured in the milk fat from the white clover- compared to the red clover supplemented diet, although concentrations of C18:1*trans*-isomers and CLA, and the *n*-6/*n*-3 ratio were similar across diet types. In experiment 2, addition of clover silage to ryegrass silage increased the concentrations of *n*-3 fatty acids, inclusive of α-linolenic acid in milk fat, and reduced those of conjugated linoleic acids (CLA), C18:1*trans*-isomers, and the *n*-6/*n*-3 ratio, compared to the diets not supplemented with clover. In conclusion, white clover seemed to be slightly superior to red clover supplementation, but the effects of clover supplementation as such were generally larger. However, in the latter case beneficial (more *n*-3 fatty acids) and undesired effects (less CLA) were partially compensatory.

INTRODUCTION

Apart from grass, clovers are sources of α-linolenic acid, and only a small number of studies have reported milk of legume-fed cows to contain higher levels of *n*-3 polyunsaturated fatty acids (*n*-3 or ù -3 PUFA) than grass-fed cows (Dewhurst *et al.* 2003, Steinshamn *et al.* 2006). Since there is an enormous interest in the positive health aspects of animal products, the objective of this study was to compare the effects of white clover and red clover on the fatty acid composition of milk fat of cows fed fresh and ensiled ryegrass-based diets.

MATERIALS AND METHODS

Perennial ryegrass (*Lolium perenne* L., cultivar 'Fennema'), white clover (*Trifolium repens*, 'Klondike'), and red clover (*Trifolium pratense*, 'Pirat') were used in this study. The study consisted of two feeding experiments, which were carried out with twelve (experiment 1) and 24 dairy cows (experiment 2), respectively. In experiment 1, fresh ryegrass was mixed with fresh white (WF) or red clover (RF) (60/40, on dry matter (DM) basis). Experiment 2 involved similar mixed diets in ensiled form (WS and RS, respectively), and two ryegrass silage-based diets, without (GS) or with supplementary maize gluten (GS+). The experimental forages were always harvested at the early flowering stage for both fresh feeding parallel to silage making. Barley was supplemented according to requirements for milk production, and some extra hay was fed to each cow. Cows were of Brown Swiss and Holstein breed and were allocated to groups in a balanced manner. The experimental period lasted for 36 days in both experiments, with the last 8 days being reserved for measurements and sample collection. Fatty acids in feed and milk were quantified by use of gas chromatography. Data was subjected to analysis of variance (GLM) and contrast analysis (clover versus ryegrass) by SAS (version 8.02), with diet type and lactation stage as fixed effects, including covariates.

RESULTS

In experiment 1, voluntary dry matter intake (DMI) and consumption of α-linolenic acid (ALA), were not affected by diet type (on average 18.7 kg/d and 179 g/d). Furthermore, milk yield and milk fat content were similar for both diet types (on average 26.4 kg/d and 4.3 g/kg). In the milk fat, the concentration of ALA was significantly higher ($P < 0.05$) in case of WF compared to RF (10.9 vs. 9.2 g/kg of total fatty acids methyl esters (FAME)). Consequently, a tendency for a higher level of *n*-3 fatty acids ($P < 0.1$) on the WF compared to the RF diet was found (1.33 vs. 1.16% of total FAME). The *n*-6/*n*-3 ratio, the concentrations of C18:1*trans*-isomers and total CLA were not affected by diet type in experiment 1 (on average 1.98, 37.1 and 14.1 g/kg FAME, respectively).

In experiment 2, total DMI was similar across diets (on average 18.8 kg/d), but forage intake was significantly higher ($P < 0.01$) on the WS diet compared to the other diets (16.3 vs. 13.5 kg/d). The intake of α-linolenic acid was numerically higher for the cows on the WS diet (130 g/d) compared to the other diets (on average 117 g/d). Milk yield and milk, fat content were not affected by diet type in experiment 2 (on average 25.5 kg/d and 4.0 g/kg).

Clover supplementation to the ryegrass-based diet affected the fatty acid profile of the resulting milk fat more than type of clover, although white clover silage supplementation resulted in numerically higher concentrations of ALA and *n*-3 fatty acids compared to red clover silage supplementation (11.4 and 14.3 g/kg FAME vs. 10.3 and 12.8 g/kg FAME, respectively). The concentrations of *n*-3 fatty acids, ALA and the *n*-6/*n*-3 ratio in the milk fat from cows fed diets not supplemented with clover were on average 10.9 and 8.4 g/kg FAME, and 2.57, respectively. Addition of clover silage (white or red clover) increased concentrations of *n*-3 fatty acids (+25%, $P < 0.01$) and ALA (+30%, $P < 0.05$), and reduced the *n*-6/*n*-3 ratio in the milk fat (-26%, $P < 0.001$), compared to the unsupplemented diets. Furthermore, concentrations of C18:1 cis isomers, saturated fatty acids, and *c*9*t*11-CLA in the milk fat from cows on ryegrass silage diets were on average 203, 688, and 8.6 g/kg FAME, respectively. Supplementation of ryegrass silage diets with clover silage (white- or red clover) resulted in milk fat with lower levels of C18:1 *trans*-isomers (-19%, $P < 0.1$), higher levels of saturated fatty acids (+4.9%, $P < 0.001$), and a tendency for lower levels of *c*9*t*11-CLA (-22%, $P < 0.1$) than the unsupplemented diets.

DISCUSSION

In experiment 1, the white-clover supplemented diet resulted in milk fat with a higher concentration of ALA than the red-clover supplemented diet. A possible reason, although not measured, could have been that an increased passage rate with white clover, compared to red clover, caused for the increase in recovery of ALA (Dewhurst *et al.* 2003), while consumption of ALA was not affected by diet type in experiment 1. As a consequence, a higher concentration of total *n*-3 fatty acids was measured in the milk fat of the white clover supplemented diet compared to the red clover supplemented diet. However, this elevated level of *n*-3 fatty acids was not enough to result in a significant reduction of the *n*-6/*n*-3 ratio in the milk fat from cows on the WF diet. This ratio was, however, still far below the threshold of 5 seen critical to human nutrition (DACH 2000). Furthermore, the lack of difference in concentrations of C18:1*trans*-isomers and CLA with white and red clover supplementation indicates that ruminal biohydrogenation was similar.

In experiment 2, type of clover supplementation did not significantly affect the concentrations of total CLA, and *trans*-C18:1-isomers in the milk fat, which indicates that a similar level of biohydrogenation of C18 unsaturated fatty acids in the rumen took place on the white- and red-clover based diets. However, clover supplementation to ryegrass as such, was found to reduce the concentrations of total CLA and total C18:1 *trans*-isomers, which suggests that ruminal biohydrogenation was slightly reduced when clover silages were added to ryegrass silage. Our results support studies from Dewhurst *et al.* (2003), who also observed a reduced biohydrogenation of ALA when feeding clover silage compared to grass silage. This phenomenon may have subsequently allowed for more ALA and *n*-3 PUFA to be secreted by the milk, which was slightly more pronounced with white clover than with red clover supplementation. This is in contrast to results from other studies, where the opposite was found (Dewhurst *et al.* 2003, Steinshamn *et al.* 2006). The present results imply that, with respect to the *n*-6/*n*-3 ratio, clover-silage supplemented ryegrass diets seem to be more beneficial to human health (Simopoulos 2002) than ryegrass-only diets. However, this could be compensated by less desired effects (less CLA).

CONCLUSIONS

Supplementation of clover, favoring white clover slightly over red clover, to a ryegrass-based diet, compared to a non-supplemented diet, alters the concentrations of functional beneficial fatty acids in the milk fat, resulting in a milk that may differ in benefits for human health to a certain degree.

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Reseeding into established grassland

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Keywords: direct ground injection, ley, reseeding, *Trifolium pratense*

ABSTRACT

A ley seed mixture was sown into an established grass sward on a morainic silt loam in Ås, Norway (59.7°N) using a slurry application equipment for direct ground injection (DGI). The seeds of timothy, perennial ryegrass, and red clover were mixed into the slurry tank and spread together with the slurry by a pressurised injection equipment. Seed rates of the three species were 0.7, 0.7, and 0.3 g m^{-2}, respectively. The injection was carried out in early May in a third year sward of pure meadow fescue, and seedlings emerged satisfactorily. Frequent cutting increased their light exposure, but only clover established sufficiently to make up at most some 25 percent of the total plant stand in the following growing season. This maximum was reached only after severely reducing growth of the old fescue stand, either by reducing the N-supply or by spraying with glyphosate before seed injection. Possible improvements of the method under practical conditions are considered.

INTRODUCTION

Under Norwegian conditions, the persistence of legumes is inadequate for their survival as a satisfactory proportion of the sward till the end of a normal ley period. Red clover (*Trifolium pratense*), the most used legume for short-term leys, normally survives for at most two seasons after the establishing year. Only under scanty N-fertilisation and lenient harvest regimes, a sufficient number of plants will survive to make a satisfactory stand in the third ley year. The experiment was carried out to try whether the direct ground injection system (DGI) with seeds added to the slurry might be a possible method for reseeding of red clover and other desirable species into an established grassland.

MATERIALS AND METHODS

The experiment was carried out on a 3rd year ley sown as a pure stand of meadow fescue (*Festuca pratensis* L.). In the first ley year the sward had been used for an N-fertilisation experiment designed as a split plot with small plots for weekly samplings and DM yield determinations, and six rates from 0 to 30 g N m^{-2} $year^{-1}$ on the main plots. The second ley year the field was continued as a mere N-fertilisation experiment of the same design, but with a normal three harvest regime. Before the start of the present experiment, four of the small plots were sprayed with glyphosate at a 'normal' rate for weed killing, 140 mg m^{-2}, and in dilutions to give ½, ¼, and ⅛ of this rate, respectively.

The working principle of the most vital part of the DGI equipment used in this experiment, the pressurised slurry (and seed) injection organ, is shown in *Figure 1* (Morken and Sakshaug 1998). An amount of 2.5 kg of a seed mixture of timothy (*Phleum pratense* L. cv. 'Grindstad'), perennial ryegrass (*Lolium perenne* L. cv. 'Tove'), and red clover cv. 'Nordi' was added

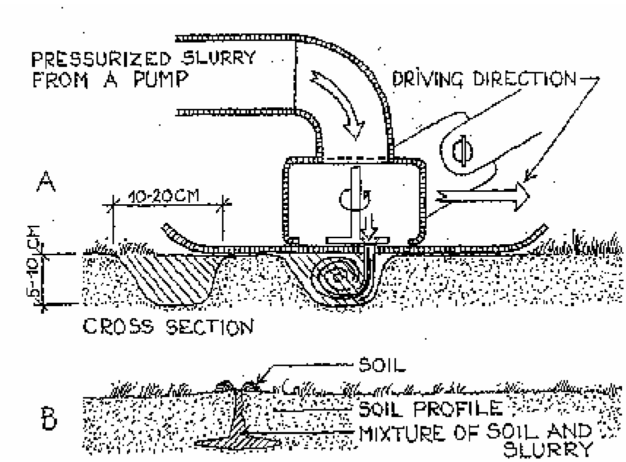

Figure 1: The working principle of the vital part of the pressurised DGI equipment used in the experiment. A: Vertical section of top soil, surface runner, and the slurry jet penetrating the sod. B: Cross section of top soil after injection.

in the tank, so that the emerging seed rates were 0.7, 0.7, and 0.35 g m^{-2}, respectively. The seeds and the concomitant N supply, amounting to 2.0 g mineral N m^{-2}, were injected at two alternative dates in the spring, May 5 or 15.

RESULTS

The sown grass species emerged in satisfactory densities, whilst the emergence of clover was relatively poor, even when accounted for its lower seed rate and relatively high seed weight.

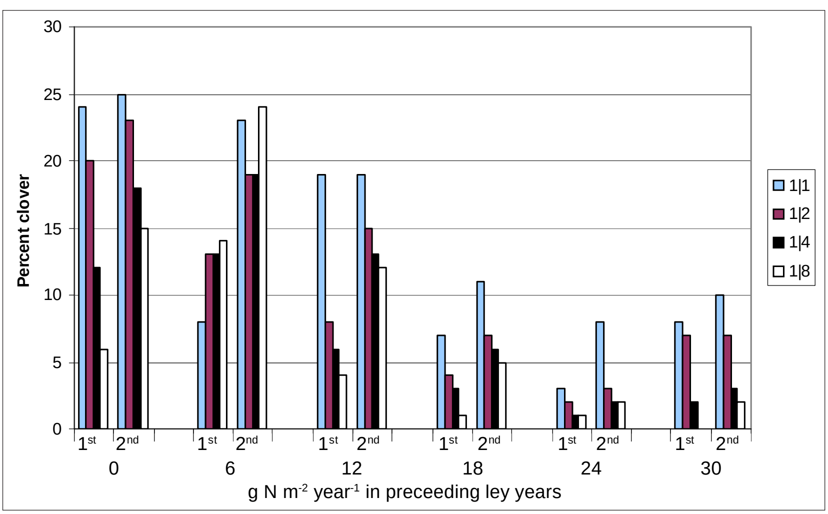

Figure 2: Percent clover in the red clover/meadow fescue stand at 1st and 2nd harvest in the season after the injection, as a function of N-fertilisation during the first two ley years and glyphosate application before reseeding at normal ($^1/_1$) or reduced ($^1/_{2,}$ $^1/_{4,}$ $^1/_8$) rates.

However, seedlings of the two grass species disappeared rapidly in the competition with the old plants, also on the glyphosate treated plots, where the fescue gradually recovered. Among the relatively few clover seedlings, some survived to make out an appreciable part of the total yield in the injection year, and especially during the following year (*Figures 2* and *3*).

DISCUSSION AND CONCLUSION

Reseeding of grass species into established grassland using the DGI method and the procedure described, is totally irrelevant, even for a 'fast establisher' as perennial ryegrass, according to the results. The same seems true even for red clover, since the gain in fodder quality and N-fixation from the (relatively few) new plants introduced (*Figure 3*) probably is much less than the loss in yield during the establishing year. However, considerable improvements of the method are possible. The seed rate was low, and the emergence of clover was poor when compared with that of the grasses. Increasing the seed rate by a factor of at least two, preferably still more, should have significantly improved the establishment of clover irrespective of competition conditions. Early spring sowing is probably a prerequisite for a success when using such a method. Even the first sowing date in this experiment, May 5, was far from the earliest possible. Sowing as soon as the soil moisture makes it convenient, will improve germination conditions and minimise competition for the young seedlings. So we think that refinement of the method in the directions suggested is worth trying and might be of interest, in conventional farming and perhaps especially in ecological management systems.

Figure 3: View of the experimental field the year after injection showing the established red clover before the 2nd harvest

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Relationships between seed yield components in tetraploid red clover (*Trifolium pratense* L.)

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Keywords: *Trifolium pratense* L., breeding, seed yield, tetraploid

ABSTRACT

Red clover varieties and in particular tetraploid varieties are often characterised by a low seed yield. In this experiment we studied the relationship between seed yield characteristics. A highly significant and positive correlation between the number of flower heads and seed yield was found. Using a visual score for the number of flower heads as a breeding criterion could thus be used to increase the seed yield capacity of the varieties. An estimation of the broad sense heritability for seed yield was very high.

INTRODUCTION

The main objectives of red clover breeding (*Trifolium pratense* L.), an important forage crop in temperate regions, are disease resistance, persistence and forage yield. Another important trait, negatively correlated with the last two mentioned and influenced by many components, is seed yield. Red clover varieties are often characterised by an unsatisfactory level of seed yield leading to high production costs. Particularly tetraploid red clover varieties have low seed yields. The nectar secreted at the base of the longer corolla tube is not accessible for bees, a fact that results in a lower degree of visitation and low pollination (McGregor 1976).

In the phenotypical recurrent family selection all plants with insufficient growth and disease resistance are eliminated. Plants are harvested individually and only plants with sufficient seed yield per plant start the next selection cycle. Unfortunately, in this way plants with bad seed yield have intercrossed with the others. It would be preferable to eliminate these plants before pollination. The objective of these trials was to assess associations between seed yield components, and to investigate the heritability of the seed yielding capacity.

MATERIALS AND METHODS

In 2003 we planted 275 spaced plants of tetraploid red clover originating from 11 families. In the nursery field we evaluated the growth and disease resistance. All plants with insufficient growth and disease resistance were eliminated. In the summer of 2004, the 45 remaining tetraploid plants were allowed to intercross and seed was harvested individually. The flower head number per plant was counted, the seed yield per plant was weighed and the thousand seed weight was measured.

For the second trial, out of the 45 remaining plants harvested in 2004, progenies of five high seed yielding plants and five low seed yielding plants were sown. In 2006 thirty plants per progeny of the high seed yielding plants were allowed to intercross in one field and 30 plants per progeny of the low seed yielding plants in another field. The plants were grouped in 6 blocks containing 5 plants of each of the 5 families. The 6 blocks of each polycross were harvested and mean seed weight per family was measured.

RESULTS AND DISCUSSION

There was a high variation in head number per plant and in seed yield per plant (*Table 1*).

Table 1: Seed yield components of 45 tetraploid red clover plants

	Mean	Variation
Head number per plant	314	83 - 718
Seed yield per plant (g)	11.2	1.2 - 36.6
Thousand seed weight (g)	2.98	2.55 - 3.66

The expected high correlation between the seed yield per plant and the seed number per plant (= seed yield/thousand seed weight) was confirmed in this trial. The correlation coefficient between the seed yield and seed number was 0.98.

In most seed production fields, the major component of total seed yield is number of flower heads rather than number of seeds per head or weight per seed (Taylor and Quesenberry 1996). We calculated the correlation coefficients between the head number and the seed number per plant (r = 0.57) and between the head number and the seed yield per plant (r = 0.58). *Figure 1* shows the relationship between the seed yield per plant and the flower head number per plant. There are outliers where we could not predict the seed yield based on the flower head number per plant. On the one hand we observed two plants with more than 600 flower heads with a very low seed yield per plant (1.2 and 5.1 g seeds per plant). On the other hand there were two plants with about 500 flower heads and a remarkably high seed yield per plant (34.9 and 36.6 g seeds per plant). As a result of the elimination of these outliers, the correlation coefficient between flower head number per plant and seed yield per plant increased to 0.82. Herrmann *et al*. (2005) also observed a high correlation between these components.

This high correlation suggest that a visual score of flower head number as a characteristic for breeding red clover with a high seed yielding potential can be used.

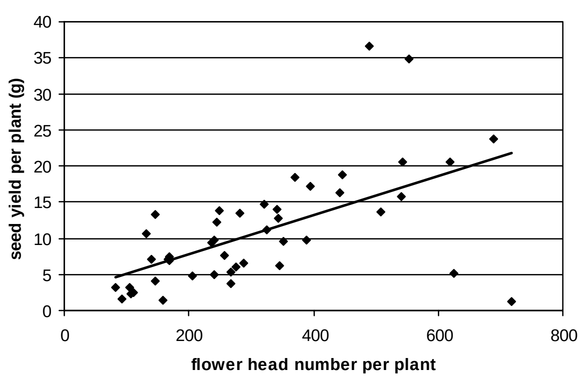

Figure 1: Correlation between seed yield per plant and head number per plant of 45 tetraploid red clover plants

Analysis of variance for the seed yield per offspring family of the high and low seed yielding plants showed a highly significant difference between the two polycrosses. The mean seed yield of the progenies of the high seed yielding plants was double the mean seed yield of the progenies of the low seed yielding families. *Figure 2* shows the seed yield harvested in 2004 on the 10 selected parents and the mean seed yield of the 30 progenies per plant harvested in 2006. The figures are standardized to eliminate year effects. The heritability was estimated as the report between the difference of the mean of the seed yields of the progenies from the high and low seed yielding plants and the difference between the mean of the seed yields of the high and low seed yielding plants. The heritability calculated in this way was very high and amounted to 0.95. This indicates that eliminating all plants with insufficient seed yields for the next cycle of recurrent family selection will improve the seed yield capacity of tetraploid red clover.

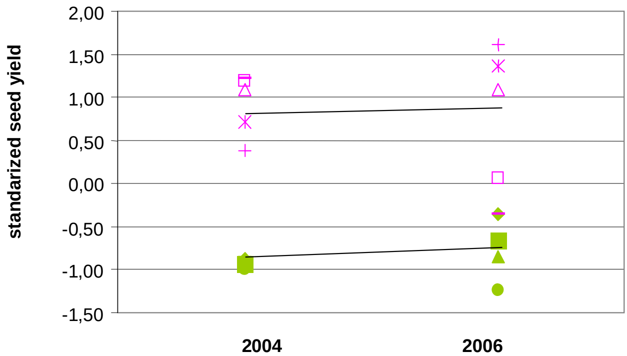

Figure 2: Standardized seed yield of 5 high (□,+,Ä,-, *) and 5 low (■,▲,♦,●,-) seed yielding plants in 2004 and the mean of their progenies in 2006 (the lines connect the means of high and low seed yielding plants in 2004 with the means of their progenies in 2006).

CONCLUSIONS

Plants with a high number of flower heads tend to have a better seed yield per plant. The estimation of the heritability for seed yield was very high. Selection for high flower head number per plant and eliminating plants with a low seed weight in recurrent selection is recommended for increasing seed yielding capacity in tetraploid red clover.

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A comparative study on the forage yield in winter and spring cultivars of pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.)

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Keywords: forage pea, common vetch, biomass production

ABSTRACT

A small-plot trial was carried out for two years in Serbia with a total of twenty forage peas and vetches of diverse origin. The cultivars were sown either in early October (winter cultivars), or in early March (spring cultivars), and were cut at the stages of full flowering and forming of the first pods. The winter cultivars of pea had the greatest plant height (132 cm) and the highest yields of green forage (41.2 t ha^{-1}) and dry matter of forage per plant (10.98 g). The winter cultivars of common vetch had the greatest values for number of stems (3.2 plant^{-1}) and number of internodes (23.9 stem^{-1}), as well as the highest yield of dry matter of forage per area unit (9.6 t ha^{-1}). Spring cultivars of common vetch had the highest portion of dry matter of forage (0.25).

INTRODUCTION

Pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.) are traditional winter and spring forage species in Serbia and throughout the Balkan Peninsula (Mihailovic *et al*. 2005a). It is estimated that forage pea, together with feed pea, is grown on between 25,000 ha and 30,000 ha, while common vetch, together with other vetches, take about 7,000 ha in Serbia today. Both species are utilised as green forage, hay, forage meal, silage and haylage (Mikic *et al*. 2006).

The study was aimed towards determining the potential for yield of forage in winter and spring cultivars of forage pea and common vetch and examining their components of yield of forage.

MATERIALS AND METHODS

A small-plot trial has been carried out during 2005 and 2006 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šancevi on a carbonated chernozem soil. The trial included five winter cultivars of pea, NS-Pionir and NS-Dunav from Serbia, Champagne from France, Mir from Bulgaria and Osjecki Zeleni from Croatia, five spring cultivars of pea, NS-Lim and NS-Junior from Serbia, Nadja and Poneka from Germany and Timo from Sweden, five winter cultivars of common vetch, NS-Sirmium, Neoplanta, L-386, L-387 and Kadmos NS from Serbia, and five spring cultivars of common vetch, Novi Beograd from Serbia, Armantes from Spain, Languedoc and Blanchefleur from France and Morava from Australia. The cultivars were sown in early October, for winter cultivars, and early March, for spring cultivars, with a plot size of 5 m², three replicates and a crop density of 120 viable seeds per m² for winter cultivars of pea, 100 viable seeds per m² for spring cultivars of pea, 180 viable seeds per m² for winter cultivars of common vetch and 150 viable seeds per m² for spring cultivars of common vetch. All twenty cultivars were cut at the stages of full flowering and forming of the first pods (Mihailovic *et al.* 2005b). The results were processed by analysis of variance (ANOVA) with the Least Significant Difference (LSD) test applied.

RESULTS AND DISCUSSION

As an important component of yield of forage (Mihailovic *et al.* 2005c), number of plants before cutting ranged from 73 plants m^{-2} in Mir to 168 plants m^{-2} in L-386 (*Table 1*), without significant differences in number of plants before cutting between the cultivars of each group

Table 1: Components of yield of forage in twenty winter and spring cultivars of forage pea and common vetch during 2005 and 2006 at Rimski Šancevi

Cultivar name	Number of plants (m^{-2})	Plant height (cm)	Number of stems ($plant^{-1}$)	Number of internodes ($stem^{-1}$)	Yield of green forage (g $plant^{-1}$)	Yield of dry matter (g $plant^{-1}$)	Yield of dry matter (t ha^{-1})	Portion of dry matter of forage
Winter forage pea								
NS-Pionir	88	140	2.1	21.3	53.00	11.81	10.6	0.22
Champagne	79	124	1.8	20.7	62.31	12.48	10.5	0.25
Mir	73	108	1.9	16.3	43.33	7.79	5.8	0.18
NS-Dunav	86	147	2.3	22.0	51.00	11.37	9.9	0.22
Osjeèki Zeleni	79	140	1.8	23.7	47.67	11.44	9.6	0.24
Average	81	132	2.0	20.8	51.46	10.98	9.3	0.22
Spring forage pea								
NS-Lim	88	94	2.7	19.3	39.67	9.51	8.9	0.24
NS-Junior	93	106	2.6	19.3	47.33	9.93	9.6	0.21
Nadja	95	127	1.8	19.7	43.33	10.08	10.1	0.25
Poneka	88	101	2.2	17.3	41.00	8.22	7.6	0.20
Timo	83	99	2.6	22.0	44.67	9.96	9.1	0.22
Average	89	105	2.4	19.5	43.20	9.84	9.2	0.23
Winter common vetch								
NS-Sirmium	143	114	3.6	24.0	27.00	6.48	8.1	0.24
Neoplanta	160	122	3.1	23.7	24.00	5.35	9.1	0.22
L-386	168	125	3.1	25.0	30.67	6.44	9.1	0.21
L-387	167	127	3.1	24.7	25.33	6.33	10.7	0.25
Kadmos NS	163	107	3.3	22.3	27.00	7.32	11.2	0.27
Average	160	119	3.2	23.9	26.80	6.38	9.6	0.24
Spring common vetch								
Novi Beograd	173	111	2.1	19.0	19.83	4.96	8.7	0.25
Armantes	150	75	3.1	22.0	17.00	4.76	7.6	0.28
Languedoc	155	84	2.5	17.7	19.22	4.29	6.1	0.22
Blanchefleur	161	70	2.0	17.0	17.73	4.29	7.3	0.24
Morava	157	93	2.0	17.7	24.33	6.22	9.3	0.25
Average	159	86	2.3	18.7	19.62	4.90	7.8	0.25
LSD								
0.05	22	25	1.7	4.5	7.0	2.7	1.8	0.03
0.01	30	37	2.4	5.9	9.3	3.6	3.0	0.05

and with significant differences among cultivars in whole. The winter cultivars of forage pea and common vetch had significantly greater plant height than the spring cultivars, namely 132 cm and 119 cm to 105 cm and 86 cm, being within the limits of the species in general (Angelova and Yancheva 1996). There were no significant differences in number of stems at both levels, while there were significant differences in number of internodes at both levels.

Among the cultivars of the four groups, the highest yields of green forage per area unit were in NS-Pionir (47.3 t ha^{-1}), NS-Junior (45.7 t ha^{-1}), L-386 (43.3 t ha^{-1}) and Morava (36.7 t ha^{-1}), with significant differences between spring cultivars of common vetch and the other three groups. The highest yields of dry matter of forage per area of the four groups were in NS-Pionir and Champagne (10.6 t ha^{-1} and 10.5 t ha^{-1}), Nadja (10.1 t ha^{-1}), Kadmos NS (11.2 t ha^{-1}) and Morava (9.3 t ha^{-1}). The average proportion of dry matter of forage varied from 0.18 in Mir to 0.28 in Armantes, with a significant difference at the level of 0.05 between the winter cultivars of pea (0.22) and the spring cultivars of common vetch (0.25).

CONCLUSIONS

Winter cultivars of forage pea and common vetch represent an important source of both green forage and dry matter, while spring cultivars of both species show a considerable potential for production of high yields of green forage and dry matter in a relatively brief period of time.

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Seed production of subalpine and alpine leguminosae

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ABSTRACT

Species-rich seed mixtures in a mountain environment at middle and high altitude, primarily aiming at site-specific vegetation for nature protection rather than to the production of forage, have gained significance in recent years. In a time of the relegation and destruction of extensively used grassland, the areas suitable for restoration must also be seen as areas of potential ecological balance. A prerequisite for the successful realisation of these aims is the production and availability of seed of suitable site-specific species.

Four different leguminosae (*Anthyllis vulneraria* ssp. *alpestris*, *Trifolium alpinum*, *Trifolium badium* and *Trifolium pratense* ssp. *nivale*), all naturally occurring in the middle and higher zones of the Alps, were investigated during the last decade. The possibilities and conditions of their successful seed production as well as their applicability for agricultural utilisation were assessed. All four species proved to be suitable for restoration and agricultural utilisation of areas in the middle and higher zones of the Austrian Alps. The seed production of *Trifolium alpinum* and *Trifolium badium* is considered too expensive because of a very slow juvenile growth, low competitiveness and seed yields clearly below 100 kg ha^{-1}. The seed production of *Anthyllis vulneraria* ssp. *alpestris* and *Trifolium pratense* ssp. *nivale* is more realistic and can be recommended for site-specific seed mixtures in the sub-alpine and alpine vegetation belt.

Keywords - leguminosae, seed production, site-specific, mountain environment

INTRODUCTION

Due to the climatic and edaphic constraints at high altitudes of the Alpine area, the use of site-specific seed mixtures has gained more and more significance in recent years. Above all in restoration activities connected with large building projects (separation of wood and pasture, revitalisation and improvement of sub-alpine and alpine grassland, ski lifts, ski runs, snow-making facilities, reservoir power stations, roads and tourist infrastructure), this type of restoration has become prominent (Krautzer *et al.* 2006). However, together with the standard demands of rapid surface protection, restoration stability and forage quality, the function of protecting biotopes is to be increasingly considered. In a time of the relegation and destruction of extensively used grassland, the areas suitable for restoration must also be seen as areas of potential ecological balance. If these aims are to be met the production, the availability and the use of seed of suitable site-specific species must be taken care of.

During the last fifteen years, research work was carried out in order to test the possibilities and conditions of the successful use of site-specific species, occurring naturally in the middle and higher zones, in landscape construction. Detailed instructions for the economic seed production and use of four different sub-alpine and alpine leguminosae were assessed in Austria, Italy and Germany.

MATERIAL AND METHODS

Seeds of *Anthyllis vulneraria* ssp. *alpestris* (Alpine Kidney-vetch), *Trifolium alpinum* (Alpine clover), *Trifolium badium* (Brown clover) and *Trifolium pratense* ssp. *nivale* (Snow clover), all occurring naturally in the middle and higher zones of the Alps (Hegi 1975), were collected from natural plant stands in the Alps and important characteristics for their general suitability for agricultural use were assessed (Krautzer 1995). In order to develop and optimise the seed propagation in lowland regions, different trials on small (trial range 3-15 m^2) and large scale (trial range 350-8 000 m^2, see *Table 1*) were established at differing altitude in Austria and Germany (Krautzer *et al.* 2003). Several observations and assessments were carried out in order to get basic knowledge about seed properties, field preparation, maintenance, fertilization, weed and disease control as well as harvesting methods, yield and product quality (Peratoner 2003, Krautzer *et al.* 2004).

Table 1: Description of sites

Site	Country	Altitude	pH $CaCl_2$	Soil type	Soil texture
Hochwurzen	Styria, A	1.830	6.6	leptosol	gravelly loamy sand
Gumpenstein	Styria, A	710	5.2-6.4	cambisol	loamy sand to silty sand
Hebenshausen	Hessen, D	220	6.5-7.1	luvisol	silt loam

RESULTS AND DISCUSSION

Suitability for agricultural use

Investigations on natural plant stands of all four assessed species showed different distribution as well as different demands on soil properties and different tolerance against agricultural utilisation (*Table 2*). In general, all four species can be considered suitable for the use in site-specific seed mixtures at middle and high altitude in mountain environment.

Table 2: Important characteristics and suitability for agricultural use

	vegetation belt			parent rock		moisture	
species	montane	subalpine	alpine	silicious	calcareous	dry	wet
Anthyllis vulneraria ssp. *alpestris*	+	(+)	-	(-)	+	+	-
Trifolium alpinum	-	(+)	+	+	-	(+)	(+)
Trifolium badium	(+)	+	+	+	+	+	+
Trifolium pratense ssp. *nivale*	-	+	+	+	(+)	(+)	+

	tolerance against			nutritional	sward
species	fertilisation	cutting	trampling	value	density
Anthyllis vulneraria ssp. *alpestris*	(+)	(-)	(+)	(-)	-
Trifolium alpinum	+	+	+	+	-
Trifolium badium	(+)	+	+	+	(-)
Trifolium pratense ssp. *nivale*	(+)	+	+	+	(-)

+ = very good, (+) = good, (-) = poor, - = very poor

Cultivation and fertilisation

Alpine leguminosae are generally susceptible to pests and diseases. Soils are preferred that are intermediate to medium heavy, deep, rich in humus, not too acid, and easily warmed. With the exception of *Anthyllis vulneraria* ssp. *alpestris*, arable land that tends to drought should be avoided for seed production.

The optimal seed rate for the species assessed mainly depends on the dimension and „Thousand Seed Weight" (TSW) of the seeds. *Table 3* gives an overall view on seed rate and recommended row spacing.

Effects of different cover crops on Alpine kidney-vetch, brown clover and snow clover were assessed in several trials. The effect on the different species (positive or negative) mainly depended on the type of cover crop. In general, winter cereals were not suitable as cover crop. Spring cereals, especially durum wheat and spring barley, were most promising. However, there is a wide range of suitable alternatives for the use of cover crops. In practice, the choice must depend on the effect on agricultural production and farm management. Seeding in pure stand would also be a successful method, especially for *Trifolium pratense* ssp. *nivale*, that can be seeded up to the end of August, after crop harvest. Such an approach always requires more efforts for successful weed control.

Table 3: Characteristics for cultivation and fertilization

specie	seed rate kg ha^{-1}	row spacing cm	fertilisation N kg ha^{-1}	P_2O_5	K_2O	notes
Anthyllis vulneraria ssp. *alpestris*	7 - 10	20 - 45	30*	70	120	no potassium cloride
Trifolium alpinum	10 - 14	12 - 24 or 45	30*	40	70	nematode-free soils, pH < 5,5 recommended
Trifolium badium	8 - 10	15 - 20	30*	40	70	low growth rate no potassium cloride
Trifolium pratense ssp. *nivale*	6 - 8	20 - 25	30*	70	120	cut in spring recommended no potassium cloride

* at sowing in pure stands

Three assessed leguminosae showed best results on soils with a pH around 6, medium to high contents of nutrients and semi-intensive fertilization. For *Trifolium alpinum*, soils with limestone should be avoided. Inoculation with soil from the site stimulates the growth rate, as experimentally shown (Peratoner 2003).

Leguminosae need adequate amounts of phosphorous and potassium (*Table 3*). If sown in pure stands, 30 kg ha^{-1} nitrogen after sowing speeds up the development of the seedlings.

Compared to cultivated species and varieties, all assessed species showed the common characteristic of an early slow development and less resistance to competition. Measures for plant protection must be undertaken as early as possible to avoid large deficiencies in yield. Thus, populations with low weed infestation are only possible if there is a mechanical weed control (weeding combined with brushing and hoeing between the rows) optimally combined with a chemical control. *Trifolium pratense* ssp. *nivale* showed relatively high competitiveness and can be treated like normal red clover. The risk of crossbreeding with the lowland *Trifolium pratense* ssp. *pratense* can though cause problems. However, it can be used successfully for restoration at high altitudes for three to four generations.

Harvest, yield and seed quality

Under normal conditions, the first harvest takes place during the second growing season. Only *Trifolium pratense* ssp. *nivale* was able to produce ripe seeds during the first growing season, having been sown in pure stand in early April. Seed ripened from the end of July to August, with the exception of *Trifolium badium*, showing a harvesting period from the end of June to the beginning of July (*Table 4*). For *Anthyllis vulneraria* ssp. *alpestris*, the genetic characteristics of the propagated ecotype were decisive for the possibility of a second harvesting year. The plants of some ecotypes wilted after harvest like *Trifolium badium,* but most of the assessed provenances had enough surviving plants for a second harvest. For *Trifolium badium* and *Trifolium alpinum,* low competitiveness and slow plant development were reflected in poor yields. For these species, successful seed production is not yet possible.

According to the rules of the „International Seed Testing Association“ (ISTA 2004), the average seed quality, characterised by thousand seed weight (TSW in g), purity (% of weight) and germination capacity (GC in %) was assessed (*Table 4*). With the exception of *Trifolium alpinum*, results of our trials showed that seed germination is positively correlated with the seed weight. Higher germination rates were found for seed propagated at low altitudes compared to seeds from natural plant stands (Krautzer 1995). In general, the assessed species achieved a seed quality comparable to commercially produced varieties of forage leguminosae.

External quality such as purity and germinating capacity are mostly within the producers sphere of influence. Time and technique of harvesting as well as the quality of the drying equipment can decisively influence the quality of the product.

Table 4: Harvest, yield and seed quality of the investigated species

specie	period of harvest	number of harvesting years	average yield 1st harvest kg ha^{-1}	average yield 2nd harvest kg ha^{-1}	yield kg ha^{-1}	TSW* g	GC** %	purity %
Anthyllis vulneraria ssp. *alpestris*	01.08. - 10.08.	1 - 2	300	260	100 - 250	3,28	94	97
Trifolium alpinum	01.07. - 12.07.	1 - 2	10	20	0 - 40	5	98,5***	98
Trifolium badium	23.06. - 04 07.	1	70	0	21 - 80	0,98	78	98
Trifolium pratense ssp. *nivale*	25.07. - 30.08.	2 - 3	100	120	120 - 380	1,1	86	97

* thousand seed weight ** germination capacity *** with scarification

CONCLUSIONS

Seed production of site-specific leguminosae is difficult and risky. In view of low competitiveness and yields, the seed production of *Trifolium alpinum* and *Trifolium badium* is not reasonable. On the other hand, *Anthyllis vulneraria* ssp. *alpestris* and *Trifolium pratense* ssp. *nivale* can be recommended for seed production.

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Modelling ecological succession using long-term microbial chronosequences

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Keywords: ecological succession, bacterial community, ARISA, correspondence analysis

ABSTRACT

Ecological succession occurs following the formation of new substrates or catastrophic disturbance (Wardle *et al.* 2004). In this study, long-term chronosequences (sites varying in age since surface formation after a catastrophic disturbance) in Alaska, Hawaii and Australia are used to model ecosystem succession of microbial communities. The structure of soil bacterial communities in chronosequence samples was assessed using automated ribosomal intergenic spacer analysis (ARISA); a recent molecular advance in community profiling that is capable of providing a fuller characterisation of microbial community structure than previously possible.

The resulting profiles, which indicated the number and relative abundance of fungal and bacterial species per sample, were compared between succession stages at each chronosequence. A range of multivariate statistical analyses was used to elucidate patterns of the response of soil microbial community development during succession, including multiple ANOVA and canonical correspondence analysis.

INTRODUCTION

Ecosystem succession of microbial communities was compared over three long-term chronosequences in Alaska, Hawaii and Australia. Six sites were identified at each of the three locations, representing six different stages of the chronosequence. Stages were characterised by the variable Time; the youngest surface was allocated to Time level 1, the oldest to Time level 6. The study design consisted of eighteen sites in total. At each site a composite soil sample was collected by pooling about 30 samples collected throughout the site. Humus was collected to the full humus depth. For the Australian sequence, humus was not present, and the top 5 cm of sand substrate was used instead. The structure of bacterial communities in the soil was determined using ARISA, and the effect of Time on these communities was assessed by comparing samples across chronosequences for each site.

METHODS AND RESULTS

Community profiles produced by ARISA indicated the number and relative abundance of bacterial species per sample. Bacterial profiles from each chronosequence were explored using canonical correspondence analysis (CCA) after initial analysis by detrended canonical correspondence analysis (DCCA) revealed that the data exhibited a unimodal, rather than linear, response to Time.

Due to very long composition gradients, the scaling of ordination scores was carried out using Hill's scaling with its focus on inter-sample distances. The resulting ordination joint plots (*Figure 1*) show species scores which approximate the weighted average of each species (in this case, weights are the relative abundances) with respect to all six levels of Time. Species scores are represented by triangles. The distance between the scores approximates the dissimilarity of distribution of relative abundance of those species across the samples, measured by their Chi-squared distance. Triangles in proximity correspond to species often occurring together.

Centroids of the categorical variable Time are illustrated by numbered grey squares corresponding to the six levels of Time. They can be interpreted as groups of samples. Therefore, the distance between the squares approximates the average dissimilarity of species composition between the two sample classes being compared, measured by their Chi-square distance.

The distance between a species score and squares representing Time levels shows the relative occurrence of that species at the individual levels of Time. A species is predicted to occur with the highest relative frequency in levels with corresponding squares close to that species' triangle.

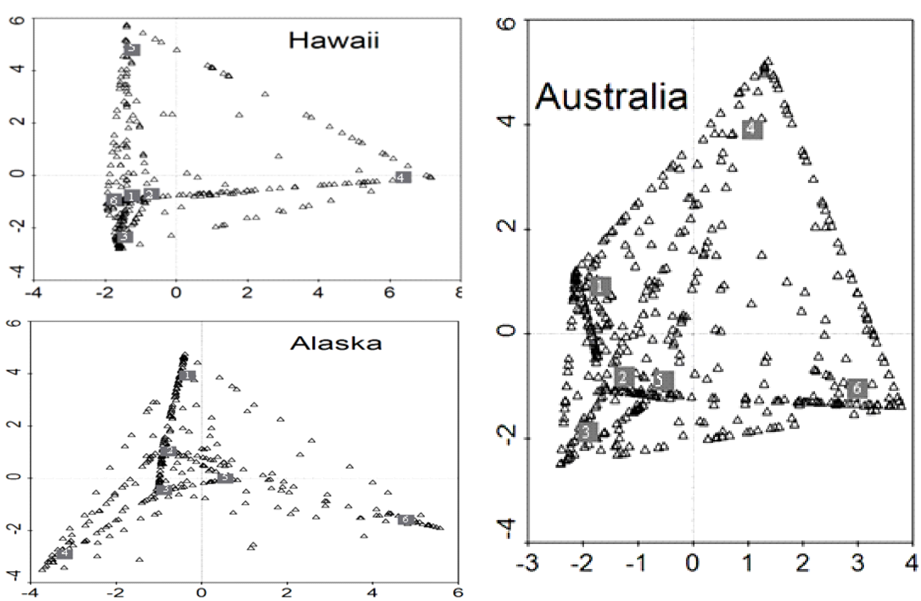

Figure 1: Ordination plots produced by CCA for Hawaii, Alaska and Australia. The diagrams provide a graphical representation of the dissimilarities among species and Time levels. Both axes on each plot show the scale of distances by which the dissimilarities are measured. Species scores are illustrated by triangles. The centroids of each Time level are represented by squares, numbered according to their associated level of Time.

All three ordination diagrams in *Figure 1* show species clustering at the Time category centroids. This indicates large species turnover between different stages of the chronosequences. In particular, Alaska has very spread out centroids denoting few species in common between sites at different chronosequence stages. A species score that lies on a direct line from one Time level centroid to another is present only in those two Time levels. The location of a species triangle on such a line indicates the relative occurrence of that species between the two Time levels.

Bacterial ARISA profiles were also converted into binary form to illustrate the presence and absence of species in each sample. Average numbers of species in common between two samples were calculated. These averages were calculated for samples within sites, between sites in the same location and between sites from different locations. The results are displayed in *Figure 2* below, where numbers in the white boxes indicate the average number of species in common between two samples from different locations. Note that the average number of species in a site in Hawaii, Alaska and Australia was 65, 61 and 75 respectively.

Hawaii observed a higher average number of common species within site than between sites (3.4 > 2.9). This result is not surprising given that we expect samples from the same site to be more homogeneous than samples from different sites. However, in Alaska and Australia the converse was true, indicating a lack of response to the study design (2 < 2.7 in Alaska, 3.2 < 3.9 in Australia). Bizarrely, the average number of species in common between a sample in Alaska and a sample in either Australia (2.9) or Hawaii (2.4) is higher than the average number of species in common between two samples in Alaska (2.0).

Hawaii (6 sites) 3.4 within site 2.9 between sites		
2,4	**Alaska (6 sites)** 2.0 within site 2.7 between sites	
2,7	2,9	**Australia (8 sites)** 3.2 within site 3.9 between sites

Figure 2: Illustration of the average number of species in common between two samples, where the samples were taken within site and between sites at each location (numbers within grey boxes), and also between sites from different locations (white boxes).

CONCLUSIONS

These initial results lead us to conclude that there is very little structure present in the dataset. Patterns of the response of soil microbial community development during succession have yet to be determined. Alternative multivariate methods, including Non-Metric Multidimensional Scaling will be applied to the data for further exploration.

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Effect of white clover companion on the establishment of contrasting varieties of *Lotus corniculatus* in low fertility conditions

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ABSTRACT

Birdsfoot trefoil (*Lotus corniculatus*) is a forage species commonly used in North America, New Zealand and central Europe. However, the current emphasis on low input extensive systems in British agriculture has stimulated interest in developing systems which include species that are able to enhance the performance and the health of grazing animals. Birdsfoot trefoil, which has a lower soil nutrient requirement than traditional pasture forage legumes, contains condensed tannins in the leaves and stems and these compounds not only prevent bloat in ruminants but also protect proteins in the rumen and allow more efficient absorption. Thus, this useful species has been associated with increases in performance and reductions in health problems in a range of grazing animals. An experiment was established at Bronydd Mawr Research centre in 2005 to investigate the performance of nine birdsfoot trefoil cultivars in a low fertility soil when sown with contrasting morphological types of white clover (*Trifolium repens* L.). Early results indicate that establishment of the trefoil was enhanced by the presence of white clover.

Keywords: White clover, Lotus, low fertility, establishment

INTRODUCTION

The current emphasis on quality rather than quantity in agriculture has stimulated interest in developing systems which include species that are able to enhance the performance and the health of grazing animals, particularly in the less favoured areas. Birdsfoot trefoil is a desirable species for inclusion in swards and may play an important role in the development of low fertility, diverse grazing systems. However, previous experiments in the UK have demonstrated the difficulty in maintaining the legume within swards under a range of managements, particularly when grown in combination with white clover. There is therefore a clear need for a greater insight into the factors affecting the persistence of birdsfoot trefoil in swards, particularly under low fertility conditions. In a previous experiment carried out in the glasshouse (Johansen *et al.* 2005) we concluded that birdsfoot trefoil was more persistent when grown with a non invasive clover (white clover with short stolons) compared to more aggressive forms under a lax cutting regime. However, this experiment was conducted under optimum fertility conditions and there was a need to gain information under more realistic field conditions where the legume could provide useful production from a low fertility environment.

MATERIALS AND METHODS

An experiment was established at Bronydd Mawr Upland Research Centre (51°59'N, 03°38'W) in 2005 to test the hypothesis that the presence and morphology of the white clover companion will affect the establishment and stability of birdsfoot trefoil within low fertility systems in an upland environment. The experimental site was on an acid brown earth soil of the Milford series overlying Devonian Red Sandstone (Rudeforth *et al.* 1984) at 372 m above sea level. Soil analysis at the site in 2004 showed P to be 3.9 ppm, whilst K, Na, Mg and Ca contents were 0.26, 0.17, 0.74 and 4.60 meq%, respectively, and the pH to be 5.14. No fertilizer had been applied to the site for 12 years preceding the establishment of the experiment.

Nine selections of Birdsfoot trefoil (germplasm previously identified as showing potential for UK conditions) were sown with two contrasting white clovers and a no clover control in a split-plot design with clover as the main plots and birdsfoot trefoil as the sub plots. Each treatment was replicated 3 times. The birdsfoot trefoil was sown at a seed rate of 5 kg ha^{-1}, the white clover was sown at 1.5 kg ha^{-1} and the companion grass, meadow fescue (*Festuca pratensis*), which was common to all plots, was sown at a seed rate of 15 kg ha^{-1}. The plot size was 3 x 2 m, the experiment was sown on 26th July 2005 and managed under a lax cutting regime.

RESULTS AND DISCUSSION

Visual establishment ground cover scores were taken on a 0 - 9 scale (0 - low, 9 - High) in early June 2006 and are presented in *Figures 1* and *2*. The data were transformed using a square root transformation and the resultant data analysed by analysis of variance. However, the untransformed data is presented for ease of interpretation. There were highly significant differences in establishment scores between birdsfoot trefoil populations ($P < 0.001$) and clover companion ($P < 0.01$) but no significant interaction was detected. In previous experiments white clover has been shown to be highly competitive with birdsfoot trefoil. However, in this study the presence of white clover significantly improved the establishment ground cover scores of birdsfoot trefoil.

The positive association between the establishment of birdsfoot trefoil and the presence of white clover was interesting as white clover has previously been perceived of as being anta-

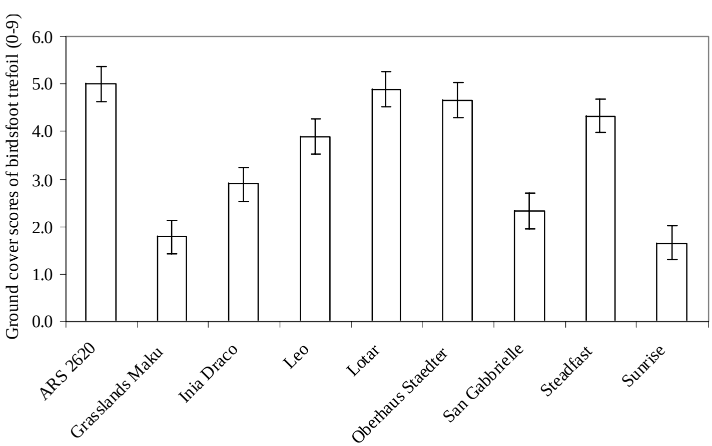

Figure 1: Ground cover scores of nine populations of birdsfoot trefoil (mean of clover treatments). Vertical bars represent s.e.d.

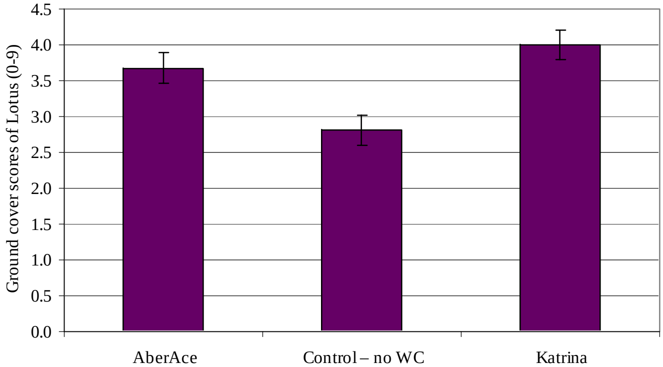

Figure 2: Ground cover scores of birdsfoot trefoil when grown with in three clover treatments (mean of nine birdsfoot trefoil treatments). Vertical bars represent s.e.d.

gonistic towards the development of successful stands of *Lotus corniculatus*. The white clover cultivars differed substantially in morphology. AberAce has extremely high stolon density, small leaf size and a prostrate growth habit whilst Katrina is classified as having a medium to large leaf size and a much more erect growth habit. Despite these extreme variations in white clover morphology little difference could be detected on establishment of birdsfoot trefoil. The establishment effects noted in this study are likely to be a consequence of the low fertility conditions combined with a low seed rate for white clover. The subsequent development of the associations will be monitored as the experiment progresses.

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Abundance of, and colonisation by native arbuscular mycorrhizal fungal populations in four forage species, and the effect of the resulting rhizosphere soils on the development of pathogen-infested barley

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Keywords: AMF, forage species, barley, *Drechlera*, *Bipolaris*

ABSTRACT

Arbuscular mycorrhizal fungal (AMF) spore abundance and root colonisation in field plots cropped with either *Trifolium pratense* L., *T. repens* L., *Lolium perenne* L. or *Dactylis glomerata* L., or a mixture of all four species, was determined. The field plots were fertilised each year with either 0 or 180 kg nitrogen ha^{-1}. The effects of AMF on a succeeding crop was assessed by studying the development of barley plants, grown from seeds naturally infested with *Drechslera* sp. and *Bipolaris sorokiniana,* when grown in soils from these field plots. The results indicate that AMF abundance and colonisation was greater in *L. perenne* than in *D. glomerata*, and greater in *T. pratense* than in *T. repens*. Barley plants grown in the test soils were generally healthier than plants grown in the control soil.

INTRODUCTION

Plant rhizospheres provide dynamic habitats for their microbial inhabitants, where continuous supplies of organic nutrients support their multiplication and activity. The species diversity and composition of these inhabitants is determined by interactions between them and the plant species present. A host plant can influence arbuscular mycorrhizal fungal composition and diversity by regulating carbon allocation to roots, by producing secondary metabolites, or by modifying the soil environment. It can also regulate each phase in the life history of AM fungi (e.g. root colonization and/or sporulation, Bever *et al.* 1996, Hetrick and Bloom 1986, Sanders and Fitter 1992) through the influence of its living roots. In return AMF can confer benefits to plant by stimulating its growth primarily through enhanced P supply and from protection from fungal diseases (Newsham *et al.* 1995).

The objective of this study was to compare AMF colonization on roots and spore abundance in the field soil of legume and non-legume forage crops grown in monoculture or in mixture, and the effect of the resulting rhizosphere soils on infection of the succeeding crop by fungal pathogens.

MATERIALS AND METHODS

The soils used in the study were sampled in the autumn 2005 from the upper 10 cm under stands of four plant species: red clover (*Trifolium pratense* L., cv. Fanny), white clover (*T. repens* L., cv. Ramona), perennial ryegrass (*Lolium perenne* L., cv. Helmer) and cocksfoot (*Dactylis glomerata* L., cv. Dactus) sown 2002 and harvested three times a year thereafter. The plant species were sown in early summer year 2002 at Svalöv (56° 55'N, 13° 07'E) in monocultures, and in a mixture consisting of 25% of each species. The plots received annual applications of nitrogen (0 or 180 kg N ha^{-1} $year^{-1}$). The soils were stored at +4 °C for about 6 months until use.

AMF spore abundance was estimated after extracting the spores from 25 g of each soil according to the procedure of Bharadwaj *et al.* (2007). Equal quantities of soil were dried overnight at 80 °C and weighed to enable the transformation of spore data to spore density g^{-1} dry soil. To estimate AMF colonisation, the roots were collected from the same soil samples and processed with KOH followed by staining in trypan blue according to Bharadwaj *et al.* (2007). Stained root pieces were cut into small pieces and 100 pieces per sample were mounted on an object slide for microscopic observations. AMF colonisation was estimated in % root colonised cm^{-1} and occurrence of vesicles, spores and/or arbuscules was also recorded for each root piece.

Spring barley seeds, cvs Baronesse and Pongo, naturally infested with *Drechslera* sp. and *Bipolaris sorokiniana*, respectively, were selected for the study of effect of host - AMF interactions on infection by fungal pathogens in a succeeding crop. This study was carried out in a glasshouse at 18/15 °C (day/night temperatures). In order to precondition the naturally-occurring AMF spores in a uniform manner in the test soils, seeds of spring wheat (cv. Dacke) were sown in all test soils and the plants allowed to grow for two weeks after which they were removed and discarded. Before sowing the barley seeds for the succeeding crop measurements, they were imbibed for half an hour in sterile tap water and heat treated for 1½ hour at 42 °C in a water bath followed by air-drying overnight at 28-30 °C. This was done in order to reduce the severity of the disease infestation in the seeds that were thereafter allowed to germinate in pots containing a mixture of test soils diluted with a commercially available peat based compost (1:3, 50 seeds per pot, n = 2). The seedlings were grown for four weeks. Plant fertilizer and extra light was supplied if needed. Control pots contained the commercial soil only. Pathogen identity was confirmed by incubating barley coleoptiles on a filter-paper moistened with sugar solution (Svensson 1986). Conidia were observed using a light microscope to distinguish *Drechslera* from *B. sorokiniana*. The number of the diseased plants was recorded, and the roots were sampled for estimation of AMF colonisation according to the above procedure. All data were analysed statistically with one-way ANOVA.

RESULTS

Occurrence of AMF spores and colonised roots varied with plant species (*Table 1*). In general the spore density was higher in soils without N fertiliser with the exception of the mixture (MIX 0).

White clover soil contained the lowest spore numbers while ryegrass soil had the highest. Colonisation of the roots of perennial ryegrass and red clover by AMF was significantly greater than in the other two species and the mixture. We found that the stained barley roots lacked AMF colonisation. At the end of the experimental period, they were colonised by oomycetous fungi and carried structures similar to oospores irrespective of the cultivar and the treatment. With a few exceptions, roots from treatments receiving 180 kg ha^{-1} N contained more oospores than roots from control and unfertilised treatments, and the oomycetous fungal colonisation was significantly greater in cv. Pongo control plants than in control plants of cv. Baronesse.

Table 1: AMF spore abundance, number of vesicles and % root colonisation estimated in soils from monoculture and mixture plots

Treatment*	Spore No. [g soil] $^{-1}$	AMF % root colonisation cm^{-1}		Vesicles % [root cm] $^{-1}$	
CF 0	31	0,5	E	0,3	C
CF 180	14	6,8	DE	4,4	C
PR 0	36	20,1	C	26,9	A
PR 180	25	19,7	C	11,9	B
MIX 0	29	8,2	D	3,0	C
MIX 180	30	6,4	DE	5,5	C
WCL 0	19	2,1	DE	0,5	C
WCL 180	9	2,9	DE	1,2	C
RCL 0	27	52,5	A	21,9	A
RCL 180	15	28,4	B	2,1	C
n	2	100		100	
p	NS	p < 0,0001		p < 0,0001	

*CF = cocksfoot, PR = perennial ryegrass, WCL = white clover, RCL = red clover, MIX = mixture of all four species, 0 and 180 denotes N fertilisation. Means with the same letter are not significantly different (Duncan Multiple range test, p<0,05).

Table 2: Root colonisation estimated in barley (cv. Baronesse) at DC 11 stage infested with *Drechslera* sp. and grown in rhizosphere soils, n = 2

Treatment*	% diseased plants	% Colonisation [root cm]$^{-1}$ with oomycetous fungi	Oospores % [root cm]$^{-1}$
CF 0	4,5	16	31
CF 180	5,7	10,5	34
PR (0)	4,4	7,8	30
PR (180)	4,9	17,7	55
MIX(0)	6,6	16,1	29
MIX(180)	8,1	13,1	22
WCL 0	3,5	9,7	53
WCL 180	11,1	7,2	32
RCL 0	1,9	5,7	34
RCL 180	5,8	13,4	37
Control	9,5	12,3	2
p	NS	NS	NS

DISCUSSION

Forage species had a significant influence on spore abundance and root colonisation. The high activity of AMF on red clover and ryegrass suggests that further work should be done to investigate their possible benefit to succeeding crops, such as barley, including the separation of direct and indirect effects of AMF inoculum on nutrient uptake and plant health. The role of N input on AMF activity, species number and dominance of mycorrhizal species needs to be investigated among other factors such as the occurrence of weeds as alternate hosts, sampling date and soil factors. The fact that the barley roots were not colonised by AMF was unexpected. As oomycerous fungi were found in all treatments including the control, it is likely that the commercial compost harboured the oomycetous populations and that their rapid growth out-competed or delayed infection and/or growth of arbuscules of AMF. Of the two spring barley cultivars used, Pongo was more responsive than Baronesse in terms of colonisation by oomycetous fungi including the oospore production.

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Performance of red clover in binary mixtures with various grass species

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ABSTRACT

During the period 2003-2006 a field trial was carried out under the conditions of light-gray forest soils (planosols) with the purpose of determining the productivity and persistence of the red clover cultivar developed at RIMSA in Troyan in pure stands compared to binary mixtures with 8 grasses under a hay-cutting system. Under the conditions of the Central Balkan Mountains, red clover cv. Troyan in association with tall fescue (cv. Elena) ensured a high and stable hay yield across seasons and years. The best balance between the grass and legume component was maintained in this mixture. During the third growing year, in the mixtures with meadow timothy cv. Troyan and orchardgrass cv. Dabrava a considerable significant increase of productivity was observed, as compared to the pure sward of red clover. The studied grasses did not increase the red clover persistence. In the fourth experimental year, in all mixtures red clover had completely declined and the yield only consisted of the grass component.

INTRODUCTION

The establishment of a productive and persistent mixture considerably depends on the regime of use (Prigge *et al.* 1999) and varietal compatibility of grass and legume component (Sanderson and Elwinger 1999, Pedersen and Brink 1988). According to Connolly (1970) the choice of the grass component influences the seasonal distribution of biomass yield and quality rather than the total annual productivity. The suitable grass component has a critical role in maintenance of the balance under which no one of the species predominates in the mixture (Helgadottir and Kristjansdottir 2004).

The objective of this experiment was to determine the productivity and persistence of mixed swards of our red clover cultivar (cv. Troyan) with eight meadow grasses compared with a pure clover stand.

MATERIAL AND METHODS

The trial was carried out for a 4-year period (2003-2006); location - altitude 384 m, Central Balkan Mountains; soil characteristics - unsaturated planosols, with pH 4.4 in KCl; fertilizing- before sowing with $N_6P_8K_8$ and after the end of vegetation with P_8K_8 every year; trial design-randomized blocks with four replications, trial plot area of 5 m², grass:legume component ratio of 1:1 in binary mixtures. A pure stand of red clover cv. Troyan was used as a control. The studied grass species were represented by cultivars and populations adapted to the experimental conditions. They were: tall fescue (*F. arundinacea*) cv. Elena; red fescue (*F. rubra*), local ecotype, meadow fescue (*F. pratensis*), local ecotype; timothy (*Phl. pratense*) cv. Troyan, smooth bromegrass (*Br. inermis*) cv. Nika, perennial ryegress (*L. perenne*), breeding population; orchardgrass (*D. glomerata*) cv. Dabrava; crested wheatgrass (*A. cristatum*), ecotype. The sowing date was 21 March 2003.

Table 1: Rainfall (mm) from May to September, 2003-2005

	May	June	July	August	September
1967-2002	79 mm	98 mm	77 mm	70 mm	54 mm
			% of long - term average		
2003	226	35	119	7	106
2004	182	136	85	141	161
2005	327	67	329	322	441

In the sowing year the months of June and August were very dry and no cut was made. In the second and third experimental years the rainfall was higher than normal, exceeding average values two to three times in the summer months of 2005 (*Table 1*).

RESULTS AND DISCUSSION

The mixture productivity over seasons and years depended on the rhythm of species growth and development. The maximum production of red clover growth was found during the first harvest year after sowing (2004). Then there were no significant differences in the annual yield of dry matter between the mixtures and the pure clover stand (*Table 2*). The treatment with wheatgrass was inferior in fresh matter yield to the control by 17.4% ($P < 0.05$). In the spring cut of 2004 the mixtures tended to be superior to the control, but the difference was only statistically significant in the treatment with tall fescue cv. Elena. In the summer re-growth of 2004 the mixtures were less productive and in autumn mixture yields were equal to those in the pure stand (*Figure 1*). The mixture with timothy had the most stable yield across seasons, i.e., the clover percentage increased from cut 1 till cut 3.

Table 2: Dry matter yield (t ha^{-1}) of nine treatments and yield relative to red clover (%) in 2004 and 2005, and overall average

	1st harvest year (2004)		2nd harvest year (2005)		Mean	
Treatments	t ha^{-1}	%	t ha^{-1}	%	t ha^{-1} yr^{-1}	%
1. red clover control	19.53	100	9.46	100	14.49	100
2. + tall fescue	21.65	111	10.77	114	16.21	112
3. + red fescue	20.23	104	8.87	94	14.55	100
4. + meadow fescue	20.81	107	10.50	111	15.65	108
5. + timothy	20.14	103	12.32	130	16.23	112
6. + smooth bromegrass	20.33	104	9.66	102	14.99	103
7. + perennial ryegress	19.80	101	7.78	82	13.79	95
8. + orchardgrass	19.71	101	12.38	131	16.05	111
9. + crested wheatgrass	17.85	91	11.99	127	14.92	103
$LSD_{0.05}$	ns		2.83		ns	

In the second harvest year after sowing (2005) the grass species significantly influenced the hay yield in the summer cut, and hence the annual yield. The mixtures with timothy and orchardgrass were superior to the standard in dry matter yield by 30% ($P < 0.05$). In these two treatments the grass species greatly predominated in the spring cut, whereas in the summer cut their best balance was observed (*Figure 2*). In the mixtures with tall fescue there was a good

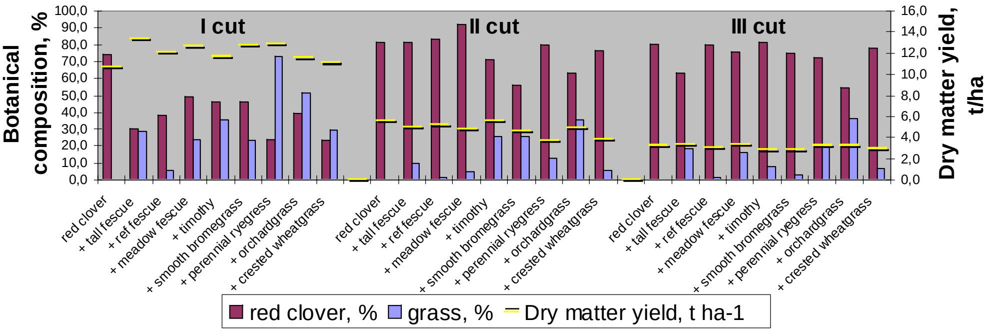

Figure 1: Botanical composition and productivity of red clover and 8 grass-clover mixtures during three cuts in 2004

compatibility under which this balance between the grass and legume component was maintained by years and seasons. This is the treatment with the most stable yield by seasons and years. During the first year after sowing, red clover greatly predominated in the treatments with smooth bromegrass, red and meadow fescue and during the second year after sowing, in those with perennial ryegrass and wheatgrass. After the second winter, perennial ryegrass declined and the sward became heavily infested with weeds. On average for the experimental period, the superiority of the mixtures with tall fescue cv. Elena, meadow timothy cv. Troyan and orchardgrass cv. Dabrava was not statistically significant, but these treatments gave a considerably higher hay yield during 2005, the second harvest year after sowing. The grasses studied did not increase the red clover persistence. In the fourth experimental year, in all mixtures red clover had completely declined and the yield only consisted of grass and weeds.

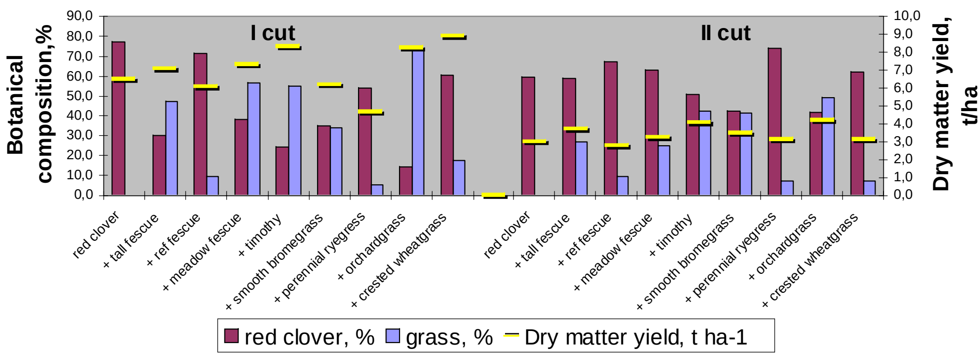

Figure 2: Botanical composition and productivity of red clover and 8 grass-clover mixtures during three cuts in 2005

CONCLUSIONS

Under the conditions of the Central Balkan Mountains the growing of red clover cv. Troyan in association with tall fescue (cv. Elena) ensured a high and stable hay yield by seasons and years. The best balance between the grass and legume component was maintained in this mixture. During the third year (i.e., the second harvest year after the establishment year), in the mixtures with meadow timothy cv. Troyan and orchardgrass cv. Dabrava a considerable significant increase of productivity was observed compared to the pure sward of red clover.

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Effects of genotype and cutting frequency on lucerne yields and quality

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Keywords: cutting frequency, lucerne, populations, quality

ABSTRACT

Lucerne yields and quality can be increased by cutting in the earlier stages of development, and this requires cultivars tolerant of frequent cuts. The objective of this paper was to determine the tolerance of experimental lucerne populations towards intensive cutting in dry farming. A field trial was carried out during 2004-2006. One set of half-sib populations was cut three times in the 2004 and five times in 2005 and 2006 (intensive utilization), while another was cut two times in 2004 and four times in 2005 and 2006. Cutting in an earlier stage resulted in a larger leaf contribution and shorter plants (480 g kg^{-1} and 59 cm height versus 405 g kg^{-1} and 80,5 cm) compared to cutting at full flowering. The related experimental populations from different sources differed in their tolerance to intensive cutting. Intensive cutting resulted not only in higher yields of the experimental populations but in better forage quality as well.

INTRODUCTION

Lucerne yield and quality do depend on the cultivar, but are much more dependant on the stage of crop development. Cutting lucerne in the earlier stages of plant development results in more cuts, higher annual yields and significantly better dry matter quality because of the higher proportion of leaves, which contain more protein and less crude fiber.

Cultivars that are winter-hardy, resistant to drought, diseases and pests and regrow rapidly are better adapted to frequent cutting. Lucerne breeding for better quality can be done by selecting luxuriant plants tolerant of frequent harvesting (Rotili *et al.* 2001). Lucerne cultivars differ in their winter hardiness and regrowth rate, resulting in different yields and persistence under the influence of frequent harvesting (Kallenbach *et al.* 2002, Katic et al. (2003). Veronesi *et al.* (2006) also indicated the possibility of developing cultivars tolerant to frequent cutting.

The objective of this study was to determine yields and regrowth rate in 24 lucerne populations and to assess their dry matter quality in the intensive, five-cut system compared to the standard, four-cut one.

MATERIAL AND METHODS

In 1998 17 genotypes of significantly different origin that had shown good adaptability to growing conditions in Serbia in previous studies were planted in a nursery. The source material consisted of the European cultivars Europe, Orca, Warrote Fany, Vertus, Zuzana, RSI 20, RGI 50, RN 30, and RCIN 60, the Serbian cultivars Tisa, NS Banat ZMS II, and NS Mediana ZMS V, and the genotypes 12 x 5 x 15 and 10 x 10 x 4, developed by crossing plants of the US varieties Vernal, Saranac and Iroqois, obtained by inbreeding (S_7).

Each source was represented by 120 plants and was planted in three replications with 40 plants each. The rows were 0.8 m apart and plant-to-plant spacing was 0.5 m. In the first year and in the first cut of the second year, plant vigor, regrowth rate, plant height and green forage yield were assessed. At the start of regrowth after the second cut, the poor plants were removed from the crop to prevent them from taking part in pollination. From all of the sources, a total of 150 female plants were selected for planting half-sib families. In 2001, they were sown in rows 10 m long and 0.8 m apart in three replications with three rows per replication. To prevent the poorer half-sib families from taking part in pollination, such families were cut before flowering in the second cut of the second year. The seed of the selected half-sib families was harvested separately. During the first and second year, the families were evaluated for luxuriance, regrowth rate, and disease appearance (on the leaves), the best 24 families were selected and their seed was sown in a progeny test in 2004 for evaluation purposes. The progenies were assessed for tolerance to less and more intensive cutting (four and five cuts, respectively). The trial was in the form of two identical sets of half-sib families planted in three replications with 5 m^2 basic plot size. Green forage yield was determined in the field, while dry matter yield was calculated using a 0.5 kg sample to determine dry matter content. Regrowth rate was measured 15 days after cutting.

Data were analyzed by two-factor ANOVA with cut as Factor A and half-sib families as Factor B. Significance of differences among the half-sib families was determined by the LSD test.

RESULTS AND DISCUSSION

The half-sib families differed significantly in their green forage yield, dry matter yield and regrowth rate after cutting ($p > 0.01$) (*Table 1*). Cutting frequency x genotype (half-sib population) interaction was significant ($p > 0.05$) (*Table 1*).

Ten of the half-sib families studied produced higher yields (green forage) with five cuts per year, while one did so with four cuts a year. However, thirteen families had no significant interaction between cutting intensity and genotype (*Table 1*).

The cutting frequency x genotype interaction was significant ($p > 0.01$). Notably, nine half-sib populations gave higher dry matter yields more with more frequent cutting and four with less frequent harvesting. In eleven half-sib populations, there was no significant interaction between harvesting frequency and genotype for dry matter yield.

Regrowth rate after cutting differed significantly among the half-sib populations. Cutting frequency x genotype interaction manifested itself in only seven half-sib populations, and in favor of four cuts per year at that. The rest of the families did not respond to harvesting frequency with respect to regrowth rate (*Table 1*). However, intensive cutting resulted in higher forage and dry matter yields, slower regrowth, shorter plants, and higher contribution of leaves to yield, i.e. better quality (*Table 2*).

Study results indicate that by choosing luxuriant plants with rapid regrowth after cutting it is possible to select half-sib populations tolerant of frequent cutting. Their use in breeding makes it possible to develop high-yielding varieties well able to withstand intensive utilization (Chatterton et al. 1977, Rotili et al. 2001). Modern cultivars used for hay production are better

Table 1: Yields of half-sib genotypes in 2004-2006 as affected by cutting intensity (cutting frequency x half-sib family interaction)

Genotype Source	Half-sib family	Green forage t/ha 5 cuts	Green forage t/ha 4 cuts	DM t/ha 5 cuts	DM t/ha 4 cuts	Regrowth cm 5 cuts	Regrowth cm 4 cuts
1. RSI 20	133 04	61.63	60.53	13.47	13.77	34.1	33.8
2. Europe II/II	134 04	66.57	56.93	14.67	12.60	31.1	31.1
3. Orca bbgj	135 04	65.90	62.13	14.37	14.40	30.3	31.0
4. RN 30	136 04	70.83	62.50	15.54	13.96	34.7	33.5
5. Mediana	137 04	68.70	58.40	14.87	12.95	35.4	34.7
6. Zuzana	138 04	65.90	59.97	14.22	13.70	32.5	31.2
7. Storgozia	139 04	59.80	55.20	12.50	12.44	30.1	32.5
8. Europe II/III	140 04	67.33	61.90	14.50	13.94	31.0	33.0
9. Warrrote	141 04	59.67	62.60	11.49	14.02	29.3	30.4
10. Orca H	142 04	61.97	64.05	13.28	14.43	28.3	29.9
11. 10 x 10x 4	143 04	63.00	53.57	13.35	12.04	28.7	29.6
12. Rgi 50	144 04	65.67	59.20	15.09	13.30	33.4	33.1
13. Fany	169 04	56.73	59.63	12.44	14.06	31.1	31.7
14. Orca 3/02	170 04	62.70	63.73	13.68	14.03	30.0	31.6
15. Vertus	171 04	63.23	65.00	13.90	14.45	30.8	31.4
16. Victoria	172 04	68.80	66.53	15.12	14.01	30.5	32.0
17. Banat o b	173 04	73.00	63.00	15.91	14.37	31.9	32.2
18. Europe I /02	174 04	66.70	66.67	14.38	14.58	30.2	30.8
19. 12 x 5x 15	175 04	67.93	65.07	14.34	14.67	27.8	31.5
20. RCIN 60	176 04	67.43	59.23	14.87	13.44	32.2	33.0
21. Orka 1/02	177 04	64.80	62.97	14.17	14.26	30.6	31.0
22. Tisa	178 04	72.03	63.00	15.47	14.23	32.6	33.9
23. Orca 0/02	179 04	*57.57*	*66.93*	12.86	15.20	29.6	31.6
24. Evropa I/02	180 04	69.37	68.07	15.28	15.49	32.6	33.0
	0.05	4.85		1.09		1.4	
LSD	0.01	6.39		1.43		1.9	

Table 2: Effect of cutting frequency (2004-2006) on yield and quality of lucerne

	Green forage t/ha	DM t/ha	Reg. rate (cm)	Proportion of leaves (g kg^{-1})	Plant height (cm)
5 cuts	65.30	14.16	31.2	480	59.0
4 cuts	61.95	13.93	32.0	405	80.5
0.05	1.716	3.847	0.5	2.3	3.4
LSD 0.01	2.261	5.068	0.7	3.3	4.7

adapted to intensive cutting systems than the old cultivars (Kallenbach *et al.* 2002). Frequent cutting can be used for lucerne utilization in dry farming conditions without the danger of yield losses or reduced stand life (Gossen 1994).

Harvesting lucerne five times a year increases yields and leaf contribution to yield, as indicated by Rotili *et al.* (2001). Lucerne changes morphologically as it matures and from flowering on the proportion of leaves in yield drops from 48 to 40%, while the contribution of stem increases from 52 to 60% (Orloff and Puntam 2004).

The development of lucerne cultivars tolerant of frequent cutting makes it possible to increase the yield and quality of this crop in Serbia under dry farming conditions.

CONCLUSIONS

The selected half-sib lucerne populations from different sources differed significantly in forage and dry matter yield and regrowth rate.

Cutting frequency had significant effect on yield, quality and regrowth rate in the half-sib populations. The half-sib families selected were shown to be tolerant of frequent cutting. The selection of half-sib families for the development of lucerne cultivars tolerant of frequent harvesting and the use of the intensive utilization system could help increase the yields of this crop in dry farming in Serbia.

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Decomposition of plant residues from early succession on abandoned fields of the dehesa ecosystems, Spain

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ABSTRACT

In order to better understand the recycling of elements associated with the turnover of organic matter, litter weight loss due to decomposition was analyzed during early secondary succession on former arable land in Salamanca, Spain. Following the dry matter dynamic and bioelement release during the litter decomposition process, 9 main plots representing different plant diversity treatments were chosen at random. Nylon litter bags containing 10 g of recently fallen litter, from their own plot, previously dried (room temperature) to a constant weight, were placed inside each bag, in the same proportion of the three groups weight of the each plot (legumes, grasses and forbs).

The first sampling was done in August 2003 and thereafter every second month. Organic matter loss of leaves confined into litterbags at the end of the first vegetative cycle was lower in both low and high sow treatments as compared to natural colonization treatment. The dynamics of total C, N and P throughout the study period was similar in the three treatments, although there were seasonal rate differences

Keywords: Biodiversity, secondary succession, litter decomposition, bioelement release, litter bags.

INTRODUCTION

Due to the intensification of agricultural practices and developments, the European Union has introduced set-aside measures for agricultural land. When agricultural land is set-aside permanently, restoration of natural ecosystems may be achieved in which the ecosystem has maximum carrying capacity in terms of biodiversity, biomass and nutrient cycling.

Two types of natural development at set-aside agricultural ecosystems will be studied: one towards forest ecosystems and the second towards grassland ecosystems (Murphy *et al.* 1998). According to Leps *et al.* (2001), the succession to a forest soil includes colonization of soil organisms adapted to a low nutrient community, with fungi as main microbial decomposers while the succession at former agricultural fields may lead to higher species diversity. However, the most important change will be towards a more complex community structure.

Annual litter, its decomposition and simultaneous release of bioelements is one of the fundamental pathway of the flow of energy and matter in Earths ecosystems. This stage involves a reorganization of organic substances and a diversification in the transfer rates of nutrients. Bioelement fluxes vary according to the rate and duration of decomposition processes and entails appreciable differences in the productivity of plant populations (Prescott 1995, Ferrari 1999). The litter decomposition process affects the functional and structural aspects of the Earth's ecosystems (Berg and Staaf 1981, Boeken and Orenstein 2001). In turn, the distribution and recycling of organic matter contribute to the structural matrix of the ecosystem forming the organic matter in the soil (Thomas and Asakawa 1993, Murphy *et al.* 2001, Santa Regina 2001).

Litter decomposition is a result of numerous and complex interactions of biotic and abiotic factors in the soil. If the succession of the different states of decomposition, both physical and biological, is relatively constant, decomposition depends only on the species, the climate and the soil. Many authors have attempted to characterize the kinetics of the biodegradation of plant matter, using the classical technique of litter bags (Berg and Staaf 1981).

Most studies have reported on decomposition of litter artificially composed only of the leaves of a single species. The aim of this study is to compare the decay of litter in different phases of the process and nutrient release on the function of the artificially created meadow communities on previously abandoned arable land.

MATERIAL AND METHODS

Site description

The study area was located at 840 m asl. 15 km to the west of Salamanca, Spain, (40º54'00''N, 5º45'30''W), where a 1 ha experimental plot was chosen at the Muñovela experimental farm (C.S.I.C.). The plot is edafically homogenous, with a dehesa-like woodland. Previously it was heavily grazed although it is now fenced off to prevent the access of domestic animals.

The climate of the zone features rainy winters and hot summers and may be classified as semiarid Mediterranean (C_1 B'_1 S_2 b'_4). Long-term mean rainfall and temperature have mean values of 500 mm y^{-1} and 10.8ºC, respectively. November being the rainiest month (99 mm) and July the driest (17 mm). January is normally the coldest month (0.8ºC) and August the hottest (29.9ºC).

The soil is a Chromic Luvisol (FAO, 1989), developed over red clays and Miocene conglomerates. Soil texture A/B is loam/clay, the slope of the plot is 2%.

Methods

A field experiment on former agricultural land was carried out on abandoned arable land in Spain with sown low (LSD) and high (HSD) diversity treatments, and natural colonisation (NC) (Leps *et al.* 2001). There were five replicate blocks. Mid-succession types of functional groups of plants were experimentally sown in both low and high diversity mixtures. The low and high diversity mixtures consisted of the same amounts of seed (grasses: 2500 seeds m^{-2}, legumes: 500 seeds m^{-2}, and other forbs, also 500 seeds m^{-2}). Fifteen species (five per functional group) were sown as high sown diversity treatment. For the low sown diversity treatment low diversity seed mixtures (two grasses, one legume and one other forb species) were composed at random.

Following the dry matter dynamic and bioelements release during the litter decomposition process, 9 main plots have been chosen at random: 3B (natural colonization), 3C (low diversity) and 3D (high diversity), 12 nylon litterbags 1 mm mesh bags, with a surface area of 4 dm^2 (20 cm x 20 cm) have been placed at random over the holorganic horizon, three replicates (12 x 3 x 3 = 108); 10 g of recently fallen litter, from their own plot, previously dried (room temperature) to a constant weight, were placed inside each bag, in the same proportion of the three groups weight of the each plot (legumes, grasses and forbs). Every second month during one year, 1 bag per plot was collected for analyses.

RESULTS AND DISCUSSION

A similar type of behaviour was observed in the three treatments, but with occasional divergences, or occurred at different rates *(Figure 1)*. Seasonal variations played a major role. At the beginning, during the first 3 months a noteworthy loss of weight was observed. During the ensuing summer period, the process ceased, and a second, slower stage of degradation occurred that affected molecules with stronger bonds. During this phase, soil microorganisms play a more active role, with a deceleration or interruption of decomposition (due to drought and typical Mediterranean high temperatures). Finally, a new acceleration of decomposition was observed in weight loss from the autumn to spring period. This was more pronounced in the natural colonization treatment.

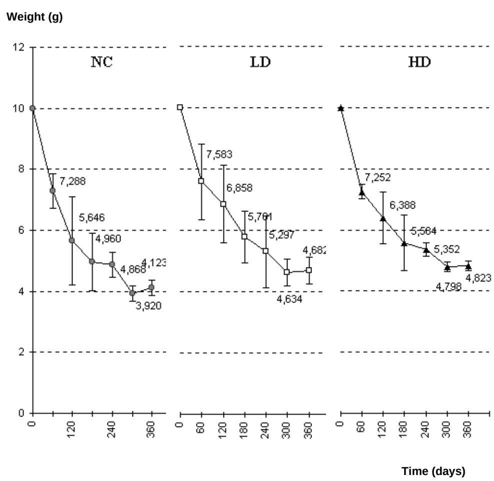

Figure 1: Evolution of dry matter in decomposing biomass of the three considered treatments

Loss of carbon (absolute values) was observed in the three treatments throughout the decomposition period (*Figure 2*). In relative terms, certain temporal increases were noted; these occurred in parallel with the processes of humification and were more pronounced in the HD treatment. During the first 3 or 4 months of the field experiment an elevated loss of C was observed in HD, LD and NC treatments, accompanied by a weight loss. Following the initial 3 or 4 months, strong losses were observed in the three treatments. Stability in this concentration indicates a close parallel between weight loss and the disappearance of organic compounds.

No significant differences were found in the concentration of N of the vegetal species in decomposition in the three treatments. During this process, after the first 6 months, a relative increase in the percentage of N was seen (*Figure 2*). Generally there were periods of continuous mineralization or leaching, along with others in which mineralization was reduced or had practically ceased. The losses of nitrogen are similar in all sites at the end of the decomposition process, but the greatest N loss was seen in the NC treatment after 12 months. Berg and Staaf (1981) reported a certain relationship between the decomposition process and accumulation of nitrogen. Low nitrogen concentrations in soil give rise to greater N increases during the initial stages of decomposition.

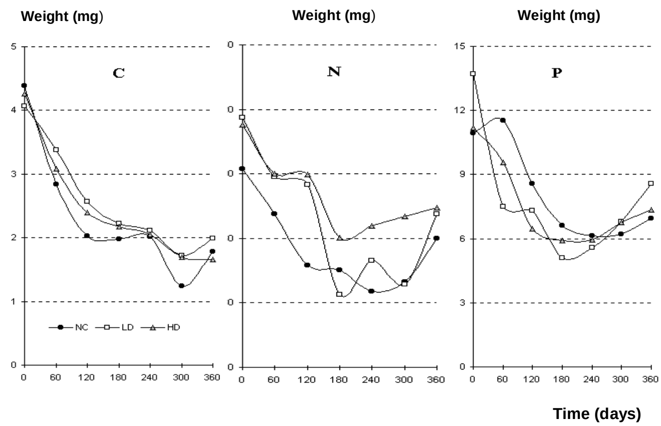

Figure 2: Evolution of C, N and P loss in the decomposition of litter at the three treatments

Initial P loss was very rapid in all treatments during the first 4 months (*Figure 2*). There was an increase in relative concentration from the sixth months after decomposition began. Considering the initial content and the low rate of P in the plant biomass, it is probable that this element could be limiting for biomass production. Immobilization seemed to coincide with the humid autumn months and, on occasion with the end of spring, when microbial activity was at its maximum. This would indicate retention of P in these leaves, which may indicate a limiting factor in this ecosystem.

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Effects of seeding rate of white clover in mixtures with grasses for pastures grazed by sheep

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ABSTRACT

A grazing study was conducted to determine the influence of grass-clover mixture composition on dry matter yield and crude protein content in pasture swards. The experimental pasture was situated on northern part of West Carpathian Plateau, 240 m a.s.l. on a brown soil classified as light loamy sand. Four grass-white clover mixtures were sown with 10, 20, 30 and 40% clover, respectively, with a grass mixture of perennial ryegrass, meadow fescue, cocksfoot and timothy in equal proportions.

The pasture was grazed by sheep 3 times in 2004 and 5 times in 2005 and 2006. The botanical composition of the four mixtures changed over time, depending on the clover content in the seed mixture. The proportion of white clover in the sward increased during the first year, to 30-70% of DM, but declined in the 2nd and 3rd years to 5-30% of DM. The mixture sown with 30% white clover had the highest dry matter and crude protein yield in 2004 and 2005, and the mixture with 40% clover the lowest.

Keywords: pasture, grass, clover, mixture, yield

INTRODUCTION

Pastures respond to nitrogen fertilization. Legumes that are present can transfer nitrogen to the grass. White clover (*Trifolium repens* L.) is recognized for its high forage quality, ability to support high levels of intake by livestock, and capacity to fix nitrogen as the ideal pasture plant (Barnes 1990, Frame 2001, Sanderson 2006). Major reasons for the declined interest in white clover in mountain and upland areas of Poland is that frigid winters and hot, dry periods during the summer are responsible for unpredictable persistence and productivity of white clover in this region (Kasperczyk and Szewczyk 2003). Intentionally sown or as volunteer species, white clover can in some years make up 20% or more of the forage production in many pastures (Barnett 2006). In order to maintain the legume in the stand, the aim of this study was to investigate differences in performance (persistence, yield and crop quality) between four white clover seeding rates in grass-legumes mixtures.

MATERIALS AND METHODS

The grazing study was established in April 2004 in southern Poland. The experimental pasture was situated on the northern part of the West Carpathian Plateau, 240 m.a.s.l. on brown soil (cambisol) classified as light loamy sand with followed chemical properties: pH in KCl = 4.45; pH in H_2O = 5.02; available P content 32 mg kg^{-1}; available K content 40 mg kg^{-1}; Mg content 28 mg kg^{-1}. Total nitrogen and organic matter content in the soil were relatively 0.112 and 1.65% of soil DM. Other site characteristics (means of the actual data in the three years) were as follows: annual rainfall was 597 mm and annual temperature was 6.9 °C .

The experimental area was ploughed and reseeded with grass-clover mixtures. Grasses in mixture were represented equally by follow species: *Lolium perenne* L., *Festuca pratensis* Huds., *Dactylis glomerata* L. and *Phleum pratense* L. The size of each of the 16 plots was 12 m². They were located on the newly sown fenced area and grazed as only pasture in certain day with the sheep. The number of animals were estimated according to currently available fresh mass. The following grass-clover mixtures in four replicates were used in randomized blocks:

M-1 - *Trifolium repens* 10%, Grasses 90%

M-2 - *Trifolium repens* 20%, Grasses 80%

M-3 - *Trifolium repens* 30%, Grasses 70%

M-4 - *Trifolium repens* 40%, Grasses 60%

As pre-sowing fertilization (April, 2004) 60 kg N, 30 kg P and 60 kg of K ha^{-1} were applied. In subsequent years similar PK fertilization was applied, but divided into two doses given in spring and after 2^{nd} grazing. Nitrogen fertilization was reduced to 30 kg N ha^{-1} and applied for first regrowths only. The pasture was grazed by sheep 3 times during first year of utilization. In 2005 and 2006 grazing was performed 5 times and animals were turned in at an average sward height 10-15 cm. After grazing the residual sward yield was in range 5 to 30% of the original DM yield. Botanical composition of the swards was estimated before each grazing period by separating of the fresh herbage samples into species, and data were expressed as % of DM. Samples for dry matter estimation and chemical analysis were taken before each grazing. Dry matter content was obtained at 105 °C and crude protein (CP) concentration was measured by the Kjeldahl method.

RESULTS AND DISCUSSION

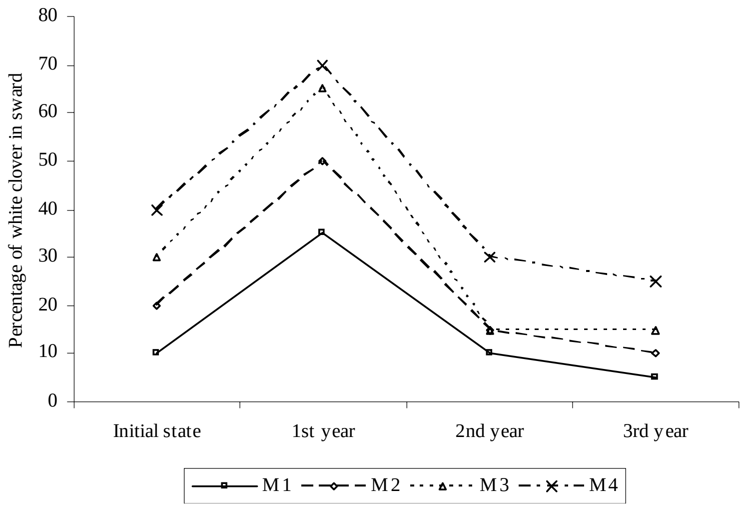
The white clover contribution to the sward yield increased during the establishment year, with mixtures with the highest clover sowing rates showing the highest clover proportions. In the year after sowing the proportions of clover had distinctly decreased in all swards, and in the next year there was a minor further decline. Changes of clover proportion were regular and rapid (*Figure 1*).

Grasses had a slow establishment, but after the first year competitive companion grasses in mixtures seemed to influence proportion of white clover in the sward (Diana *et al*. 2006). In this experiment, cocksfoot was the most successful competitor among the grass species that were sown. Excluding the sowing year, an increasing rate of this species in DM yield was considerable (*Figure 1*).

During the sowing year (2004) and the years there after, significant differences in dry matter (DM) and crude protein productivity among mixtures were observed (*Table 1*). The highest total production was obtained by mixtures with a seeding rate of 30% white clover. Except for mixture M2, the productivity of mixtures increased with time.

CONCLUSIONS

Due to rapid decrease of legumes in swards grazed by sheep, the proportion of white clover in mixtures affected dry matter yield and crude protein concentration less than expected.



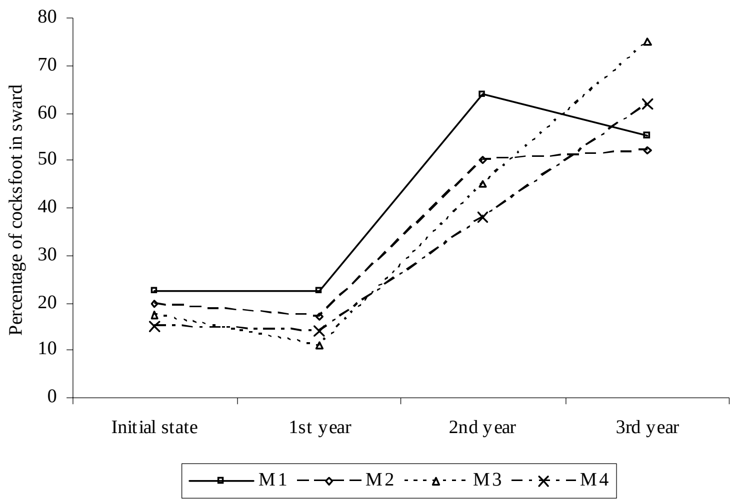


Figure 1: Percentage (% of DM) of white clover and cocksfoot in pasture 4 swards (average of all regrowths in certain year)

Table 1: Dry matter and crude protein concentrations (annual mean values) of four grass-white clover mixtures during three years.

	Dry matter (t ha^{-1})			Crude protein (kg ha^{-1})		
Mixture	2004	2005	2006	2004	2005	2006
M1	3.57	5.94	7.40	835	1391	1731
M2	3.45	5.74	5.34	832	1386	1288
M3	3.83	6.39	7.90	860	1434	1773
M4	2.95	4.92	6.50	763	1271	1680
$LSD_{0.05}$	0.62	1.06	0.97	72	120	193

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Dry matter yield of sainfoin swards under a frequent cutting regime

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ABSTRACT

A field trial was carried out at the Institute of Forage Crops, Pleven, Bulgaria during the period 2003-2005. The trial was laid out under non-irrigated conditions on slightly leached chernozem soil. Sainfoin cv. „Jubilejna“ in pure stand and in binary mixtures with perennial ryegrass (cv. „Meretti“) and cocksfoot (cv. „Dabrava“) were tested under a frequent cutting regime. The legume:grass ratio was 1:1 in the mixed stands on the basis of sowing rate. Two, five and six cuts were obtained in 2003, 2004 and 2005, respectively. It was found that the highest dry matter yield was obtained from the mixture of sainfoin and ryegrass in the second year, and from the pure sainfoin stand in the third year. The mixture of ryegrass and sainfoin (on average for second and third year) had a higher dry matter yield in the first cut and a lower yield in the third and fifth cut, as compared to the pure sainfoin stand. The mixture of sainfoin and cocksfoot had a significantly higher yield in the fourth cut.

Keywords: sainfoin, perennial ryegrass, cocksfoot, dry matter, mixture

INTRODUCTION

Sainfoin is a perennial legume which is useful for grazing, hay-making and silage conservation (Borreani *et al.* 2003, Vuckovic 2004). It is a suitable component of mixtures under nonirrigated conditions. In agronomic terms, improved compatibility in sainfoin-grass mixtures results in higher dry matter productivity (Chakarov 1998), due to the more effective utilisation of resources brought about by complementary seasonal growth patterns and better persistence. The objective of this study was to determine the dry matter yield of sainfoin in binary mixtures with cocksfoot and perennial ryegrass under a frequent cutting regime.

MATERIALS AND METHODS

During the period 2003-2005 a field trial was carried out without irrigation by the long plot method with six variants, four replications and a plot size of 10 m^2. The soil subtype is slightly leached chernozem. Sainfoin cv. „Jubilejna“ was studied in a pure stand, as well as in binary mixtures with perennial ryegrass (cv. „Meretti“) and with cocksfoot (cv. „Dabrava“). Before basic cultivation 300 kg ha^{-1} P_2O_5 and 150 kg ha^{-1} K_2O were applied as reserve fertilizer, depending on soil supply. Nitrogen (50 kg ha^{-1}) was applied at the time of sowing. Sainfoin was sown at a rate of 120 kg ha^{-1} nonpodded seed, and 25 kg ha^{-1} for perennial ryegrass and cocksfoot. The legume:grass ratio was 1:1 in the mixed stands on the basis of sowing rate. Chemical weed control was not conducted. The swards were managed under a cutting regime (mowing at 20-25 cm height). Two, five and six cuts were obtained in 2003, 2004 and 2005, respectively. Dry matter yield was determined and the data was submitted to analyses of variance and mean, which were compared to those obtained from pure sainfoin stands using LSD.

RESULTS AND DISCUSSION

Rainfall distribution was irregular during the period of active species growth in the first two years (*Figure 1*).

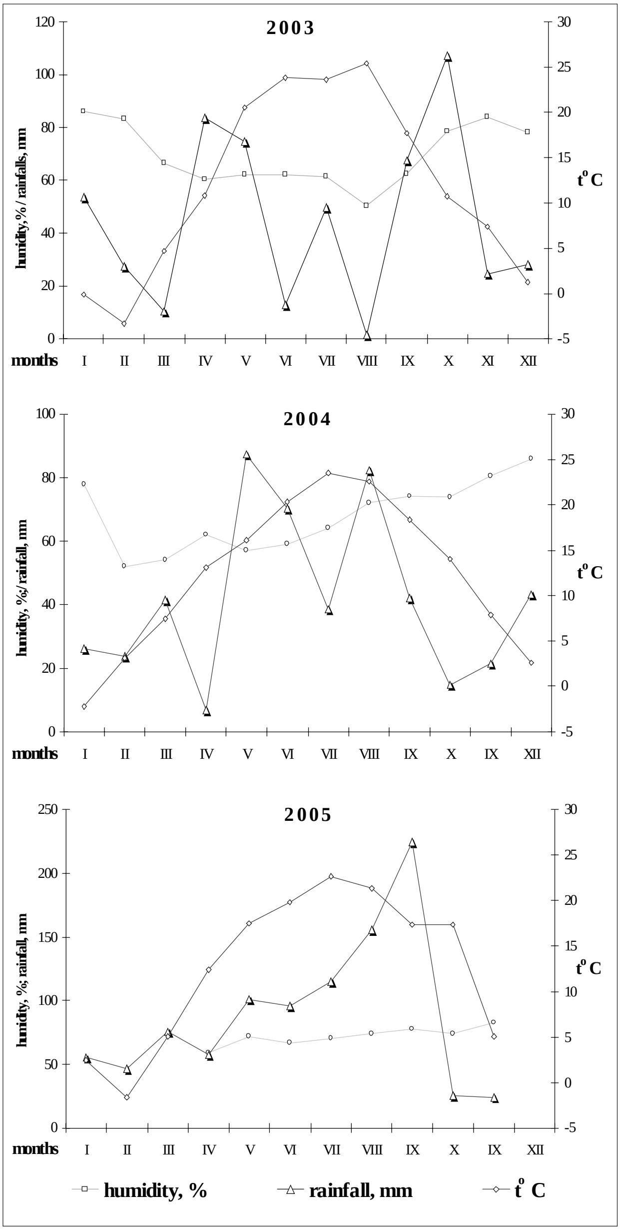

Figure 1: Meteorological conditions during the period of study

In the first year after sowing there was no rainfall during the first two ten-day periods of May and the average diurnal temperature was high (20.5 °C). June was characterized by the smallest rainfall amount (13 mm) and the highest average day temperature (23.9 °C). These unfavourable meteorological conditions resulted in low sward productivity in 2003. In spite of the greater rainfall amount in May and June of 2004, as compared to the same months of 2003, the early spring drought in April (6.9 mm) reflected on productivity unfavourably, though to a smaller degree. In 2005 the rainfall increased and created favourable conditions for plant growth.

There were no significant differences in dry matter yield between pure sainfoin and its mixture with grasses in the year of stand establishment. The highest dry matter yields were obtained from the mixture of sainfoin and ryegrass in the second year and from the pure sainfoin in the third year. These results can be explained by the contrasting species dynamics in the mixed swards. Ryegrass, as a species with a faster developmental rate and good compatibility with sainfoin, contributed to the dry matter yield and to a greater proportion of sainfoin in the sward. Cocksfoot, due to its slower developmental rate and greater interspecies competition, contributed to a lower mixture yield in the second year, as well as on average for the period. The highest dry matter yield was obtained from the pure sainfoin in the third year and on average for the period, but this result was due to the large weed infestation of the sward.

Table 1: Dry matter annual yield (kg ha^{-1}) of the swards

Treatments	2003	2004	Years 2005	average	% sainfoin
Ryegrass	1410	4567	6038	4005	51.4
Cocksfoot	1318	4509	7273	4367	56.1
Ryegrass + Cocksfoot	1405	5582	6394	4460	57.3
LSD 5%	215	2008	1589	1684	
Sainfoin + Ryegrass	2930	9324	9999	7418	95.2
Sainfoin + Cocksfoot	2992	8560	10092	7215	92.6
Sainfoin	3084	8630	11657	7790	100.0
LSD 5%	258	344	590	406	

Dry matter yield by cuts on average for second and third year depended on the biological characteristics of the species, their interrelations and meteorological conditions during regrowth. In first cut the dry matter yield from the mixture with ryegrass was higher than the yield obtained from the pure sainfoin and the mixture with cocksfoot (*Table 2*).

In third cut the yield from the pure sainfoin was superior to that from the mixtures, and the mixture with cocksfoot had a higher yield than the mixture with ryegrass. In fourth cut the pure sainfoin had a lower yield than the mixtures, while in fifth cut the opposite occurred.

CONCLUSIONS

The highest dry matter yield was obtained from the mixture of sainfoin and ryegrass in the second year, and from the pure sainfoin stand in the third year. The mixture of ryegrass and sainfoin (on average for second and third year) had a higher dry matter yield in the first cut

Table 2: Dry matter yield (kg ha^{-1}) of the swards (average for 2004-2005)

Treatments	I	II	III	Cuts IV	V	VI	Total	% to pure sainfoin
Ryegrass	2002	1760	664	228	339	308	5301	52.3
Cocksfoot	1688	1401	1304	508	670	320	5891	58.1
Ryegrass + Cocksfoot	1811	1833	1073	374	564	332	5987	59.0
Sainfoin + Ryegrass	4486	2763	996	394	666	356	9661	95.2
Sainfoin + Cocksfoot	3702	3000	1260	384	616	364	9326	91.9
Sainfoin	3854	3141	1635	289	856	369	10144	100.0
LSD 5%	433	422	254	83	88	126	456	

and a lower one in third and fifth cut, as compared to the pure sainfoin stand. The mixture of sainfoin and cocksfoot had a significantly higher yield in the fourth cut.

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Botanical composition and crude protein content of sainfoin swards under a frequent cutting regime

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ABSTRACT

A field trial under non-irrigated conditions on slightly leached chernozem soil was carried out at the Institute of Forage Crops, Pleven. Sainfoin cultivar „Jubilejna" in pure stand and in binary mixtures with perennial ryegrass and cocksfoot were tested under a frequent cutting regime. It was found that sainfoin predominated in the swards, except for third and fifth cut, on average for the second and third year. Its proportion was greater in the mixture with ryegrass than with cocksfoot. Weed infestation in the pure sainfoin stand was heavier than that in the mixtures. Crude protein content in the mixture of sainfoin with ryegrass was higher than that in mixture with cocksfoot, due to its greater portion in the sward. There were no differences in crude protein yields between the pure sainfoin stand and mixtures with grasses.

Keywords: sainfoin, perennial ryegrass, cocksfoot, crude protein, mixture

INTRODUCTION

Sainfoin is a suitable component of perennial herbaceous mixtures under nonirrigated conditions (Chakarov 1998). It cohabits very well with other perennial grasses and legume species and weed infestation is twice as low than that in pure stands (Chakarov and Dimitrova 2003). The objective of this study was to determine the botanical composition of the sward and the crude protein yield from sainfoin in binary mixtures with cocksfoot and perennial ryegrass under a frequent defoliation regime, with the aim to mimick performance under grazing.

MATERIALS AND METHODS

During the period 2003-2005 a field trial was carried out with six treatments in four replications and a plot size of 10 m^2 (1.3 x 8.0 m) on a slightly leached chernozem soil without irrigation. Sainfoin variety 'Yubileyna', perennial ryegrass variety 'Meretti' and cocksfoot variety 'Dabrava' were studied in pure stands, as well as in binary grass-sainfoin mixtures. Before basic cultivation, 300 kg ha^{-1} P_2O_5 and 150 kg ha^{-1} K_2O were applied, depending on soil supply. Nitrogen (50 kg ha^{-1}) was applied at the time of sowing (first decade of April). Chemical weed control was not conducted. The sowing rate of sainfoin was 120 kg ha^{-1} non-podded seed, and 25 kg ha^{-1} for perennial ryegrass and cocksfoot. The legume:grass ratio was 1:1 in the mixed stands on the basis of sowing rates. The swards were used under a cutting regime (mowing at 20-25 cm height). Five and six cuts were obtained in 2004 and 2005, respectively. A botanical analysis of harvested herbage was conducted following each cut. Crude protein content (CP) in dry matter was determined by the Kjeldahl method, and calcium content complexometrically. Data of dry matter and crude protein yield was submitted to analyses of variance and mean, which were compared to those obtained from pure sainfoin stands using LSD.

RESULTS AND DISCUSSION

The average botanical composition of the swards after the establishment year (2004 and 2005) is presented in *Figure 1*. Under a hay-cutting regime of utilization, sainfoin and grasses form reproductive stems mainly after the first cut and represent 65% of annual yield, in the next cuts they form only aftergrass (Chakarov 1998, Katova 2005).

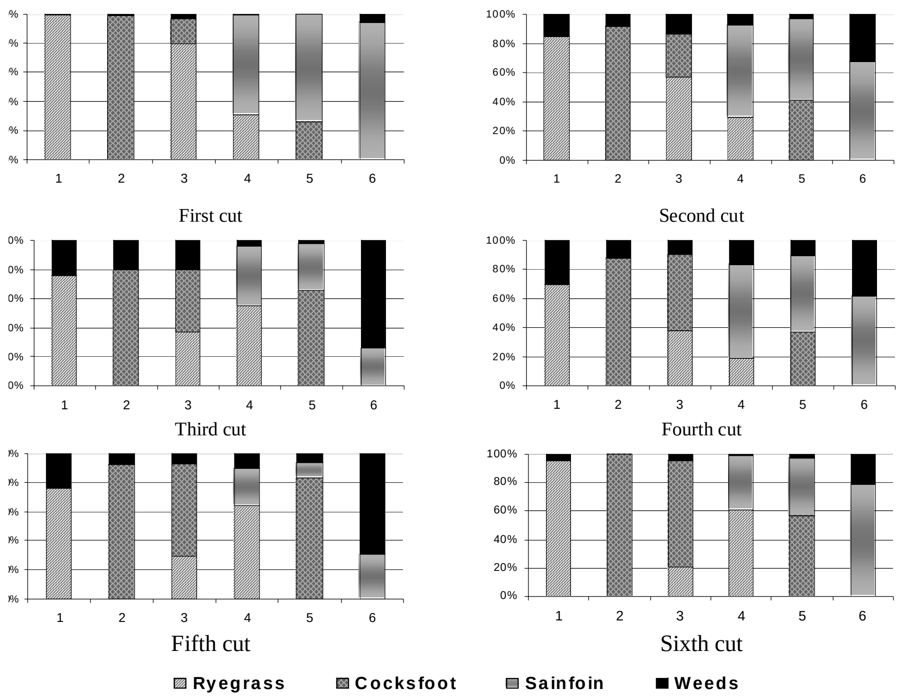

Figure 1: Botanical composition of six swards with perennial ryegrass (R), cocksfoot (C) and sainfoin (S) (1-6: R, C, RC, SR, SC, S) during six cuts (average for 2004-2005)

There were significant differences in the yields between pure sainfoin and sainfoin mixtures with ryegrass. Weeds (*Setaria viridis*, *Echinochloa crus galli*, *Amaranthus restroflexus*, *Erigeron canadensis*) formed a considerable proportion of pure sainfoin sward biomass in the second and fourth cut, and predominated in third and fifth cut. In general, the weed infestation of grass swards was lower, except for ryegrass in fourth and fifth cut, and the weed proportions were lowest in the mixtures.

Biological characteristics of the pasture species in the different cuts, formed under respective meteorological conditions, determined the dynamics in the botanical composition of the sward. That was reflected in the nutrient composition of the forage. A greater proportion of sainfoin in the swards resulted in an increase of CP and calcium concentrations (*Table 1*).

Changes in botanical composition of the swards between the cuts (dynamics in the proportion of sainfoin, grasses, weeds, *Figure 1*) were reflected in different CP concentrations, e.g. the CP decline in the sainfoin swards during the third and fifth cut coincided with high weed

Table 1: Concentrations of crude protein, calcium and phosphorus (g kg^{-1} DM) in six sward types during six cuts (average of 2004 and 2005)

Stands	CP	Ca	P	CP	Ca	P
		First cut			Second cut	
Ryegrass	91.3	3.16	2.03	99.8	3.82	2.30
Cocksfoot	89.7	3.51	2.12	98.8	3.75	2.32
Ryegrass+ Cocksfoot	104.2	3.43	2.18	101.5	5.31	3.14
Sainfoin + Ryegrass	169.4	6.19	2.98	178.1	9.40	2.59
Sainfoin + Cocksfoot	150.1	6.26	2.91	152.4	7.51	2.76
Sainfoin	166.2	7.14	3.40	120.8	6.72	2.78
		Third cut			Fourth cut	
Ryegrass	127.0	6.19	2.54	95.1	7.50	2.20
Cocksfoot	124.8	5.55	3.08	98.5	8.36	1.90
Ryegrass + Cocksfoot	126.1	4.12	2.44	101.8	6.58	2.32
Sainfoin + Ryegrass	132.9	6.46	2.70	123.0	14.07	1.48
Sainfoin + Cocksfoot	130.9	6.78	3.80	115.1	14.22	1.68
Sainfoin	98.4	5.40	2.83	109.1	10.79	1.41
		Fifth cut			Sixth cut	
Ryegrass	127.6	6.05	3.22	114.4	4.14	2.66
Cocksfoot	129.7	6.06	3.37	110.3	4.26	2.46
Ryegrass + Cocksfoot	126.2	5.55	2.75	112.8	4.30	2.47
Sainfoin + Ryegrass	144.3	7.57	2.82	147.5	6.93	2.91
Sainfoin + Cocksfoot	131.7	5.44	2.64	140.4	5.94	2.86
Sainfoin	91.4	6.39	2.02	151.5	8.54	2.79

proportions during these cuts. The lowest crude protein yield (*Table 2*) was recorded for the grasses because of their low DM yields and low CP concentrations (*Table 1*). Sainfoin inclusion in the sward considerably increased the yield of both dry matter and CP. The greater sainfoin proportion in the mixture with ryegrass slightly increased the dry matter yield, but considerably enhanced the crude protein yield, as compared to the mixture with cocksfoot. There were no significant differences in the yields between pure sainfoin and its mixture with grasses.

Table 2: Annual crude protein yield (kg ha^{-1}), average for 2004-2005

Stands	Cuts I	II	III	IV	V	VI	Annual yield total	DM
Ryegrass	183	176	84	22	43	35	543	5301
Cocksfoot	151	138	163	50	87	35	625	5891
Ryegrass + Cocksfoot	189	186	135	38	71	37	657	5987
Sainfoin + Ryegrass	760	492	132	48	96	53	1581	9661
Sainfoin + Cocksfoot	556	457	165	44	81	51	1354	9326
Sainfoin	641	379	161	32	78	56	1347	10144
Average	413	305	140	39	76	45	1018	
$LSD_{0.5\%}$ ±	120.5	108.5	55	12.9	14.6	13.1	242.3	1680

CONCLUSIONS

Sainfoin predominated in the swards, except for third and fifth cut, during the second and third year. Its proportion was greater in the mixture with ryegrass than with cocksfoot. Weed infestation in the pure sainfoin stand was heavier than that in the mixtures.

Crude protein content in the mixture of sainfoin with ryegrass was higher, as compared to that in mixture with cocksfoot, due to its greater proportion in the sward. There were no differences in the crude protein yields between the pure sainfoin stand and sainfoin mixtures with grasses.

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Effects of genotype, microbial fertilizer and seeding rate on forage yields of red clover (*Trifolium pratense* L.)

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ABSTRACT

Along with alfalfa, red clover (*Trifolium pratense* L.) is the most important forage legume. In addition to being a source of high quality animal feed, red clover is a highly suitable preceding crop for almost any field crop, because it leaves behind a soil rich in nitrogen, which is especially important for optimizing mineral nutrition through reduced mineral fertilizer use. The objective of our study was to investigate the effects of genotype (Kolubara, K-17, Viola), microbial fertilizer (two microbial strains) and seeding rate (three seeding rates: 12, 15, 18 kg ha^{-1}) on red clover forage yields. The study was carried out at the Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad. In both study years, the forage yield depended mostly on the genotype.

Keywords: *Trifolium pratense* L., forage yield, genotype, microbial fertilizer, seeding rate

INTRODUCTION

Along with alfalfa, red clover (*Trifolium pratense* L.) is the most important forage legume. Although Serbia has favorable agroecological conditions for red clover growing, especially in the hilly and mountainous areas, hay yields obtained in the country are still low (3-4 t ha^{-1}), despite the fact that the domestic cultivars of this crop have a considerably higher genetic potential (9-15 t ha^{-1} hay). The low yields and a shortage of variety seed have led to a decline in the area sown with this forage legume in Serbia. However, the advancement of agricultural production and increased livestock production in the country demand a higher contribution of red clover to high quality feed production, which can be done by the introduction of necessary cultural practices into large-scale production and by planting new, higher-yielding varieties of improved quality.

MATERIALS AND METHODS

The objective of our study was to investigate the effects of genotype (Kolubara, K-17, Viola), microbial fertilizer (two microbial strains) and seeding rate (three seeding rates: 12, 15, 18 kg ha^{-1}) on red clover forage yields, of both green forage and dry matter yield. The study was carried out in 2004 and 2005 on a chernozem soils at the Rimski Šancevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad. The basic plot size was 10 m² and row-to-row distance 20 cm. Statistical data processing was done by three-factor ANOVA and the results were tested by the LSD test.

Meteorological conditions

The locality of Novi Sad has a moderate continental climate, with the long-term (1948-1993) average rainfall during the growing season of 338 mm and the mean temperature for the same period of 17.8°C.

Red clover has specific requirements with regard to climatic conditions. The optimum annual rainfall for successful growth of red clover is about 800 mm. As the long-term average annual rainfall in the locality of Novi Sad is about 590 mm, supplementary irrigation has to be performed in dry years. The total annual sums of precipitation at the Rimski Šancevi site in 2004 and 2005 were 814 and 738 mm, respectively, which favored the obtainment of high forage yields in red clover. With regard to its thermal requirements, red clover is a cool-climate plant.

RESULTS AND DISCUSSION

The domestic cultivars Kolubara and K-17 produced significantly higher total yields of green forage and, especially, dry matter during the 2004-2005 period (*Table 1*, *Table 2*), indicating the importance of the local materials these cultivars had been developed from. Many authors (Simon 1998, Boller *et al.* 2003, Gaue and Ingwersen 2003, Herrmann *et al.* 2003, Boller *et al.* 2004, Vasiljevic *et al.* 2005) point out the advantage local red clover ecotypes have over materials from other regions because of the fact acclimatized ecotypes produce higher forage yields than populations and varieties that are less adapted.

Table 1: Total green forage yield of red clover during 2001-2002

		Seeding rate (C)			Average	Average
Genotype (A)	Microbial fertilizer (B)	12 kg ha^{1}	15 kg ha^{1}	18 kg ha^{1}	(AB)	(A)
Kolubara	1. microb. strain1	122.1	122.6	132.7	128.1	127.8*
	2. microb. strain2	129.4	126.0	123.1	126.2	
	3. control	131.1	132.9	123.5	129.1	
	Average (AC)	127.5	129.5	126.4		
K-17	1. microb. strain1	115.8	123.6	115.9	118.4	124.9
	2. microb. strain2	129.6	123.4	133.3	128.8*	
	3. control	126.8	126.6	129.3	127.6*	
	Average (AC)	124.1	124.5	126.2		
Viola	1. microb. strain1	126.7	126.9	123.2	125.6	123.1
	2. microb. strain2	119.8	127.4	119.7	122.3	
	3. control	120.3	126.0	118.3	121.5	
	Average (AC)	122.3	126.8	120.4	Average (B)	
Average (BC)	1. microb. strain1	121.6	126.7	123.9	124.0	
	2. microb. strain2	126.3	125.6	125.4	125.7	
	3. control	126.1	128.5	123.7	126.1	
Average (C)		124.6	126.9	124.3		

	%	A	B	C	AB	AC	BC	ABC
LSD	5	3.9	4.2	4.4	7.9	8.4	8.4	20.5
	1	5.3	5.6	5.9	11.4	12.1	12.1	37.7

The *Rhizobium leguminosarum* bivar. *trifoli* strains used had negative effect on dry matter yield, with the control treatment having a significantly higher yield (*Table 2*). The negative interaction between the *Rhizobium* strains and genotype was particularly pronounced in the domestic cultivar K-17 (*Table 1*, *Table 2*). In Shejbal *et al.* 2001, the negative influence of the microbial fertilizer Rizobin on dry matter yield in the tetraploid red clover variety Vulkan was due to the incongruence (incompatibility) between the strain that was used and the domesticated, active strain of *Rhizobium leguminosarum* bivar. *trifoli*. Burton 1985 reported that the process of infection may be aborted at any stage or the nodule may develop into either an effective or ineffective nodule.

The highest forage yield was obtained with the seeding rate of 15 kg ha^{-1} (*Table 1*, *Table 2*), but the difference relative to the other rates was not statistically significant, which leads to the conclusion that the optimal seeding rate for red clover establishment at the Rimski Šancevi site is 12-15 kg ha^{-1}.

Seeding rates vary depending on geographic location (climatic conditions), soil type and crop utilization method. In the eastern parts of the U.S., red clover for combined use (forage/seed) is planted using 10-15 kg ha^{-1} (Rincker and Ramptom 1985). In the Canadian province of Alberta, according to Fairey (1988), the seeding rate for red clover growing in continuous cropping was 7.5 do 8 kg ha^{-1}, which is similar to the optimal seeding rate for conditions in Poland (8-10 kg ha^{-1}) (Bawolski and Scibior 1985).

Table 2: Total dry matter yield of red clover during 2001-2002

		Seeding rate (C)			Average	Average
Genotype (A)	Microbial fertilizer (B)	12 kg ha^{1}	15 kg ha^{1}	18 kg ha^{1}	(AB)	(A)
Kolubara	1. microb. strain1	26.8	27.1	27.9	27.3	
	2. microb. strain2	27.2	26.4	26.8	26.8	27.2**
	3. control	26.8	28.3	27.1	27.4	
	Average (AC)	26.9	27.3	27.3		
K-17	1. microb. strain1	27.4	28.3	26.8	27.5	
	2. microb. strain2	26.1	26.6	28.7	27.1	27.9**
	3. control	30.4	28.5	28.3	29.1*	
	Average (AC)	27.9	27.8	27.9		
Viola	1. microb. strain1	24.2	24.6	25.1	24.6	
	2. microb. strain2	25.1	26.8	23.9	25.3	25.2
	3. control	24.3	27.4	25.1	25.6	
	Average (AC)	24.5	26.3	24.7	Average (B)	
Average (BC)	1. microb. strain1	26.1	26.6	26.6	26.5	
	2. microb. strain2	26.1	26.6	26.5	26.4	
	3. control	27.2	28.1	26.8	27.4*	
Average (C)		26.5	27.1	26.6		

	%	A	B	C	AB	AC	BC	ABC
LSD	5	0.7	1.0	1.0	1.9	2.0	2.0	4.9
	1	0.9	1.3	1.4	2.7	2.9	2.9	8.9

CONCLUSIONS

Over the two-year study period (2004-2005), the total yields of green forage and dry matter depended the most on genotype. The domestic cultivars Kolubara and K-17 produced significantly higher forage yields than Viola, the most commonly grown foreign cultivar in the country.

Statistically significant negative effects on dry matter yield were found for both of the Rhizobium leguminosarum bivar. Trifoli strains used. Especially pronounced was the negative interaction between strain 1 and the variety K-17.

The optimal seeding rate for red clover for combined use (forage/seed) at Rimski Sancevi was 12-15 kg ha^{-1}.

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