

EUCARPIA

European Association for Research on Plant Breeding

FODDER CROPS SECTION

BREEDING FOR QUALITY

**Proceedings of the 19th Fodder Crops Section Meeting held in Brugge,
Belgium, 5-8 October 1994**

Editors: Dirk REHEUL and An GHESQUIERE

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Organising Committee

Rijksstation voor Plantenveredeling
Burgemeester Van Gansberghelaan 109
9820 Merelbeke

Preface

The 19th meeting of the Fodder Crops Section of Eucarpia was held in Belgium, from the 5th to the 8th of October, 1994, at the kind invitation of the Belgian Government, Rijksstation voor Plantenveredeling. This was a most welcome return of the section to Belgium, since our previous meeting held at Ghent some thirteen years ago. This time our meeting was held in the pleasant ambience of the historic town of Brugge. The earlier meeting, had as its topic for discussion, "Breeding for High Yielding Forages combined with High Seed Yield" which, at a time when production was still the primary demand, was most appropriate. The theme of this meeting, "Breeding for Quality", was again most timely in view of the changing demands now facing forage breeders. Current requirements are for the development of a more sustainable agriculture and for environmentally acceptable grassland management systems. Coupled with this, there is an ever increasing demand for the wider use of land for non-agricultural purposes. In this context, quality is an overriding criteria in its influence on maintaining animal production without recourse to expensive inputs. Further aspects of the changing role of breeding was recognised in the organization of the meeting by the inclusion into the programme, of a session devoted to amenity species. Undoubtedly, breeding work on these species will become a regular feature of the Section meetings.

The meeting, which was opened with an address by Mr. De Baerdemaeker, the first secretary to the cabinet of the Belgian Minister of Agriculture, was attended by 92 participants from 18 countries with a good representation from eastern Europe. It consisted of a series of paper presentations, posters and workshop sessions, each of which engendered their own lively discussions. How to determine quality in a product which, in general, has to be processed by the animal, will always generate problems especially when trying to translate any measures into meaningful selection criteria.

As well as these formal proceedings the Conference had a visit to the RvP Station at Merelbeke to see some of the breeding and allied research work in progress. The extent of the breeding programmes, together with the technical backup for the determination of quality parameters of potential selections, was an impressive demonstration of the manner in which effective cultivar development is conducted. In addition to these formal sessions, opportunity was taken to visit the exhibition, of one of the most famous painters of Brugge, Hans Memling. A guided tour of the city gave members a chance to see something of the historic architecture of the old town.

The section most gratefully acknowledges the support for this meeting provided by the Belgian Government and Rijksstation voor Plantenveredeling.

M.D.Hayward.
President, Eucarpia Fodder Crops Section.

October, 1994

Organisation of the meeting

SESSION 1 : The importance of quality parameters

PAPERS

Jan INGRAM (UK). The practical importance of quality tests made in herbage variety evaluation

Johan DE BOEVER (B). The importance of the digestibility of preserved grass for the dairy cow

Lucien CARLIER (B). A report on the topic "Forage quality, feeding value and animal performance", presented and discussed during the General Meeting of the European Grassland Federation, Wageningen, June 1994.

SESSION 2 : The measurement of quality parameters

PAPERS

Pierre DARDENNE (B). Forage analysis by NIRS

Robert VAN LOO (NL), D. REHEUL (B), J.W. CONE (NL). Genetic variation in perennial ryegrass for gas production during in vitro rumen fermentation

Annemarie DE SMET (B), D. DE BRABANDER (B), J. DE BOEVER (B). The evaluation of physical structure of fresh and preserved grass by measuring chewing activity and related characteristics

POSTERS

Petter MARUM (N), A.H. AASTVEIT (N). Near-infrared reflectance spectroscopy - local calibrations

Dirk REHEUL (B), A. GHESQUIERE (B), L. CARLIER (B). Analysing digestibility in ryegrasses

SESSION 3 : Amenity grasses

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Ulf FEUERSTEIN (D) , N. ROULUND (DK). Quality in turfgrass breeding : influence of red thread

Charles SNIJDERS (NL), G.D. WINKELHORST (NL). Selection for resistance to red thread disease (*Laetisaria fuciformis*) in *Lolium perenne* and *Festuca rubra* by artificial inoculation

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Slawomir PRONCZUK (PL), M. PRONCZUK (PL). Looking for shade tolerance in lawn grasses

Mario FALCINELLI (I), F. VERONESI (I), B. LUCARONI (I). Evaluation of different amenity grasses in central Italy

SESSION 4 : Germplasm screening and genetic variation for quality characteristics

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Evelin WILLNER (D). Evaluation of alfalfa genetic resources

Efisio PIANO (I), N. BERARDO (I), L. PECETTI (I), P. VALENTINI (I), M. ODOARDI (I). Some observations on the qualitative characteristics of grazing-type lucerne

Giuditta DE SANTIS (I), E. CHIARAVALLE (I), D. PALERMO (I), P. MARTINIELLO (I). Variation in crude protein and fibre content : their relationship with dry matter and its components in alfalfa cultivars, in mediterranean environment

Miriam ODOARDI (I), G. SANTELLI (I), R. CREMONA (I), N. BERARDO (I), L. STRINGI (I). Screening *Atriplex halimus* and *Medicago arborea* populations for saponin content

Luca RICCIONI (I), E. PIANO (I). Occurrence of endophytic fungi in natural populations of tall fescue from Sardinia (Italy)

Catherine RAVEL (F), C. CHARBONNEL (F), G. CHARMET (F). A survey of *Acremonium* endophytes in wild populations of perennial ryegrass collected in France

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Ulrich POSSELT (D) : Selection parameters of quality traits in perennial ryegrass

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Angelica GRUSEA (R). Variation and inheritance of quality of *Dactylis glomerata* varieties obtained by different breeding methods

POSTERS

Jose Alberto OLIVEIRA (E), P. CASTRO (E). Variability and selection of genotypes of perennial ryegrass for nutritive value using NIRS

Robert VAN LOO (NL), O. DOLSTRA (NL), H.J.P. MARVIN (NL), A.L.F. DE VOS (NL). Genotype x N-supply interaction in perennial ryegrass for nitrogen use efficiency related to forage quality

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Wojciech JOKS (PL), Z. ZWIERZYKOWSKI (PL), E. JOKS (PL), T. NOWAK (PL). Agronomic value of festulolium (*Festuca pratensis* x *Lolium multiflorum*) strains

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SESSION 1 : The importance of quality parameters

THE PRACTICAL IMPORTANCE OF THE QUALITY TESTS MADE IN HERBAGE VARIETY EVALUATION

Jan INGRAM

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Summary

The paper examines the practical value of some of the characters that are or might be measured in herbage trials. It concludes that digestibility is important because it has such a large effect on animal production but that the measurement of other characters which affect intake might also be useful.

Introduction

Leys sown in UK agriculture concentrate heavily on ryegrass, with some timothy and white clover added. Past breeding has concentrated on increasing the longevity of swards with spectacular success in the early heading maturity groups. More recently, new varieties have produced a steady improvement in yield of dry matter at a rate of about a half percent per annum (Jordan, 1988). There have been suggestions that for some crops, for example maize and wheat, one of the consequences of genetic selection for yield has been a reduction in quality. This appeared to be beginning to happen in the case of grass for both digestibility and protein content. Indeed there are good physiological arguments why selection for high yield should tend to reduce digestibility, as the maximum growth rate is achieved by stem production which is relatively indigestible (Figure 1).

With the overproduction of ruminant livestock products in the EEC, and the consequent transfer of emphasis from increasing output to improving the economy of production, the need to increase the proportion of ruminant diets provided by cheap fodder has become more important. Hence the emphasis has shifted away from varieties with improved yield towards improving quality. Herbage quality differences can affect livestock production in three ways :

- a. the content of utilizable component is altered,
- b. the intake is altered,
- c. management factors may be altered and particularly those relating to silage making.

Many of the measurements we can make affect more than one of these aspects either directly or indirectly. In considering various measures of quality we have to consider :

- a. How large a change might be achieved by plant breeding and the consequence of these changes to practical farming ?
- b. Do improvements bring consequential disadvantages with them ?

Dry Matter Content

For silage there seems to be a considerable difference in opinion as to the value of higher dry matter silage in improving animal output - a problem which this paper does not aim to address ! It appears that a high dry matter content achieved by a prolonged period of wilting in the field can lose more feed value in the drying than it gains from its improved dry matter. However, varieties might improve silage dry matter content through a high initial plant dry matter content, or through an improved rate of drying after cutting. The latter characteristic appears to be rather difficult to assess although there are a number of published works which indicate that tall fescue dries more rapidly than other species. This should be of use to grass driers, although my experience has been that most of them use tall fescue for its other advantages, rather than for its rapid drying rate. For silage crops, the dry matter content rises as the crop matures and any assessment of varieties would have to take maturity into account which makes its assessment more difficult.

For many years, opinion seems to have been that the dry matter content of fodder crops has little effect on the intake of fresh material. A number of recent papers have appeared which suggest that there may be a quite large increase in intake with improving dry matter content in perennial ryegrass. Osoro and Cebrian (1989) suggested this only occurred when the digestibility was greater than 70 % but the effect was then about 5 % intake per % DM. A similar effect had been noticed by Sonneveld (1965) for both ryegrass and timothy. Intake of Italian ryegrass was almost doubled as dry matter content rose from 10.2 % to 20.5 % in South Africa (Meissner *et al.*, 1992), but only increased by 40 % in New Zealand as dry matter content increased between 10 % and 18 % (John and Ulyatt, 1987). The fact that the benefit of higher dry matter content only becomes apparent when digestibilities are high, might account for some of the apparently conflicting results for silage.

The dry matter contents of a frequent cut management are more relevant to grazing. A preliminary investigation suggests that there is a considerable range between existing ryegrass varieties. An experiment containing about 42 lines showed varietal differences of 3-4 % which were consistent through a season and across 4 sites (Figure 2). Furthermore there does not seem to be a strong relationship between dry matter content and digestibility. As the dry matter determination is part of the yield measurement, no extra work is needed. Further consideration of the differences between varieties in dry matter content might be useful.

Protein Content

Protein content in grasses seems to be highly and inversely correlated with dry matter yield. To be effective breeders must breed for protein yield otherwise selection for high protein exerts a selection for low yield. The protein content of grass is rarely thought to be limiting. Protein contents as low as 6 % can be encountered in the first conservation cut of high yielding crops. Farmers seem to attach little importance to protein content in the crop preferring to supplement with high protein concentrates which also improve the protein quality.

Soluble Carbohydrate Content

Water soluble carbohydrate is completely digestible and therefore might be expected to be associated with good digestibility, but results suggest that the correlation is not good (Dent and Aldrich, 1966). As water soluble carbohydrate rises with increasing maturity (ap Griffiths, 1963), there is a danger that selecting soluble carbohydrate material will lead to the selection of less digestible material. Carbohydrate content has been identified as one of the main determinants of silage fermentation quality. Wilkinson *et al.* (1981) suggested that the minimum level for silage should be 3 % of the wet matter. Ryegrass will usually meet this level but other species of grass and legumes have a lower content so that rye-grass/clover mixtures may be below optimal levels. However, silage additives can mitigate the adverse affects of low soluble carbohydrate content. Soluble carbohydrate content has occasionally been linked with animal preference (Jones and Roberts, 1991 ; Bland and Dent, 1964).

Mineral Content

Supplementation with minerals has sometimes been shown to improve animal performance. Two varieties of ryegrass have been introduced in the UK recently with improved contents of magnesium. In NIAB trials the improvement was about one third. Moseley and Baker (1991) showed that the high magnesium line dramatically reduced the incidence of hypomagnesaemia and increased intake and lamb liveweight gain by 10 %. Breeding for an additional character such as high magnesium content, reduces the effort that can be applied to improving the conventional characters, so that high mineral content has to be seen by farmers as a positive benefit offsetting the otherwise slightly poorer agronomic performance. The yield of the better of these two varieties is between 5 and 10 % below the best 'conventional' varieties, but even then farmers seem to prefer to provide extra minerals rather than lose the grass yield. In addition there is no guarantee that a high magnesium variety will completely cure hypomagnesaemia in extreme situations. It seems, therefore, that the chances of a high mineral content variety being successful, probably depends on a demonstration that the improved animal performance achieved by the increased mineral content in the grass, cannot be achieved by supplementation of the diet with minerals in some other way.

Digestibility

Tables for the theoretical effect of improvements in metabolizable energy of grass diets on livestock output have been published (Holmes, 1989). Metabolizable energy is normally determined by some form of digestibility determination. Thus the tables show that changes in digestibility from 62 to 68 could result in changes in live weight gain of 33 %.

Comparisons of silages made from varieties of differing digestibilities have been made by a number of workers using cattle who have shown almost linear responses to digestibility between 60 and 70 % DOMD ; amongst them, Steen (1992) LWG 0.06 kg/head/D value unit (Figure 3), Ryan (1981) LWG 0.025 kg/head/D value unit and Connolly (1977) LWG 0.03 kg/head/D value unit. Experiments can be found where improved digestibility has not produced the expected animal output improvement. There are indications that the lack of response in some experiments maybe partly due to the need to provide ad lib forage if intake improvements are to be effective, and partly due to the differences in the measure of quality

being confounded with other factors. Perhaps Oloroso and Cebrian (1989) identified one of these situations when they published a paper suggesting that although digestibility drives intake up to 70D, it is dry matter content which is more important at digestibilities higher than 70D.

Experiments in New Zealand, where swards were conditioned to produce different digestibilities, showed that improved digestibilities in grazed pastures did improve milk fat output (Holmes, 1987). The importance to grazing animals of differences in digestibility between varieties has been further emphasised by two experiments, one using dairy cows (Gately, 1984) and the other with sheep (Davies *et al.*, 1989, 1991 and 1992). In addition there have been on-farm experiments conducted in Wales which have shown 15 % animal production advantages to a more digestible variety (Walters, 1984). It is often suggested that the relative digestibility of varieties changes with time, and particularly should this be so at around heading time. NIAB results (Figure 4) have shown that while relativities do change from cut to cut, nevertheless there are varieties which are consistently good over all cuts and others that are consistently poor and this seems to be confirmed by some of the papers mentioned above (Figure 5).

Improved digestibility improves animal output by two routes. It increases the energy that can be utilised per kilogram of feed eaten by about 1.5 % per 1D-value unit improvement in digestibility. It also increases the amount of fodder consumed. Which is the more important may depend on the feeding regime of the stock, but some authors have gone on record as suggesting that the intake effect is the more important. It is well known that intake seems to be driven by the speed of passage of the feed through the animal, which in turn is a function of the speed of breakdown of the feed. Work in Australia has suggested that the breakdown is caused partly by enzymatic action causing longitudinal breaks and partly by mastication causing lateral breakage (Wilson *et al.*, 1989). In this case, one might expect intake to be partially but incompletely correlated with digestibility. The introduction of a wet maceration test which mimicked chewing might lead to a better assessment of the value of herbage to stock, and various workers are engaged in developing one.

Leaf Shear

Leaf shear has been measured by a number of workers and differences detected between genotypes which in one case amounted to almost 100 %. This difference has been related to differences in speed of eating or bite size (Moseley and Thorhallsdottir, 1991 ; Mackinnon *et al.*, 1988). It has been shown that these improvements improve the rate of intake. It seems likely, but I know of no evidence, that leaf shear and rate of breakdown under wet maceration will be correlated. As yet, no varieties have been produced in the UK which claim reduced shear strength so their value in farm practice is unknown.

Sward Architecture

A considerable amount of work has been done to define the limitations to intake caused by the mass of herbage on offer. However, at a given mass this material can be available as a short dense crop or as a taller thinner crop. A tall thin crop therefore has a lower bulk density.

Unfortunately, changes in height and bulk density are often confounded with other changes. This has limited many experiments to assessing the effect of bulk density and sward height on either bite size or the rate of intake, with the implication that a high rate of intake will lead to a high intake per day. It has been found that increases in rate of biting and grazing time do not completely compensate for reductions in bite size. There is clear evidence that rate of intake or bite weight, is more influenced by sward height than sward density. Allden and Whitaker (1970) suggested that doubling the sward height at a constant mass could double the rate of intake, whereas Laca *et al.* (1992) suggested that the increase in bite weight was about 50 %. A similar result was reported by Black and Kenny (1984) working on ryegrass in Australia.

However clear cut evidence that this advantage to tall swards is reflected in animal performance is more difficult to find. Illius (1987) has presented results which suggest that increasing sward height at a constant weight by 135 % increased LWG by about 10 %. If the effect of sward architecture is real, the intake of Italian ryegrass should be better than perennial when all other characters are equal. However tall stands tend to be associated with stems which have lower digestibility and slower comminution. This is probably why Jackson (1974 and 1975) observed an improved intake to Italian ryegrass over perennial ryegrass in cattle, but liveweight gains were the same for the two species.

I am not aware that the amount of genetic variability for height at a constant (high !) yield has been fully defined. Certainly casual observation suggests that there is considerable variation in plant habit. Measurements of DUS plots show that the height to width ratio of perennial ryegrass varieties can vary from 1.1 to 0.6. If there was a corresponding difference in swards then it might well be possible to select for better intake via more upright varieties (Figure 6).

Wheeler and Corbett (1989) has published a survey of research workers which asked them to place quality criteria "in order of importance in selecting forages of high nutritive value". They placed digestibility as the most important single quality character followed by ease of comminution. Sward architecture was placed last behind high crude protein content. However, the respondents were largely in the USA and the Antipodes and were considering animal live weight gain or wool production and not intensive milk production where intake may be a more important factor.

Preference and Taste

NIAB and many other workers have shown that animals show marked preferences for some species and varieties of grass. In ryegrass, animals consistently prefer tetraploid varieties. Within a ploidy, differences between varieties have been much smaller. They often seem to be associated with differences in digestibility, but there are exceptions which may be connected with stemminess. Stemmy varieties do not seem to be well grazed even if they have digestible stems. Experimental work indicates that, for some species, taste may also influence animal preference. How much preference influences no choice intake is debatable. Australian work which showed sheep to have a distinct distaste for white clover (Kenny and Black, 1984), but to eat it readily in a no choice situation suggests the answer is not much, unless it happens to be linked to one of the other more important measures such as digestibility or ease of comminution.

Nevertheless, preference is a measure which carries considerable weight with farmers. This is partly because farmers are constantly transferring animals from field to field, and an unacceptable field leads to a short term drop in milk production which may not be completely recovered. This is perceived as a need to have varieties with good preference scores, although experimental work would suggest the problem might be solved by having all fields at the same level of preference even if that level is relatively low.

Conclusion

Digestibility seems frequently to be highly correlated with animal performance when differences between varieties within a species are considered. However it has been shown that there are big differences in intake between species at the same digestibility. This suggests that there are other characters which could be improved to increase intake. In conserved grass this might be by a wet maceration test but for grazed herbage consideration of sward architecture might also be important.

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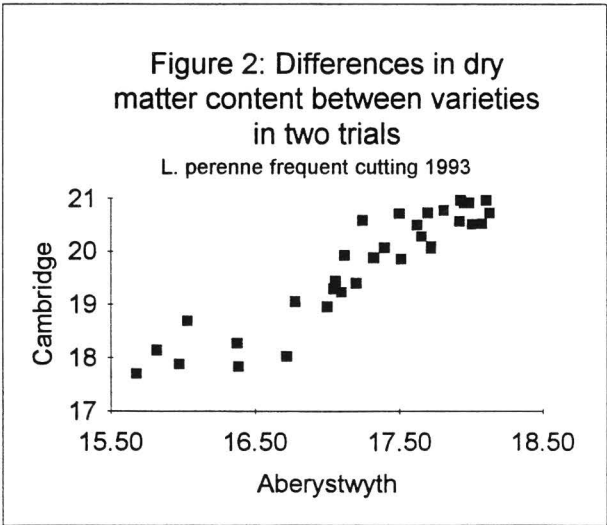
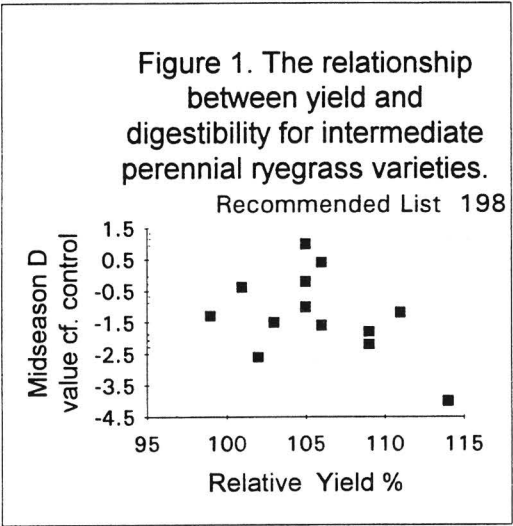


Figure 3: Effect of silage digestibility on cattle production (after Steen, 1992)

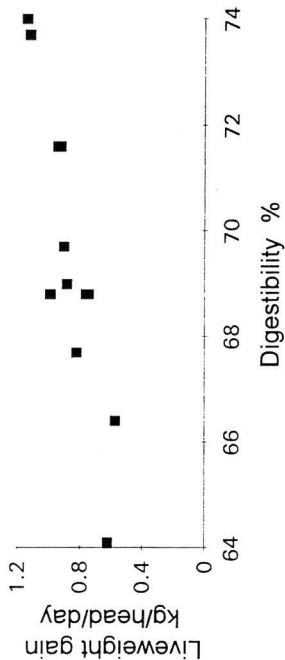


Figure 4: Changes of digestibility in perennial ryegrass varieties within a season (2 sites)

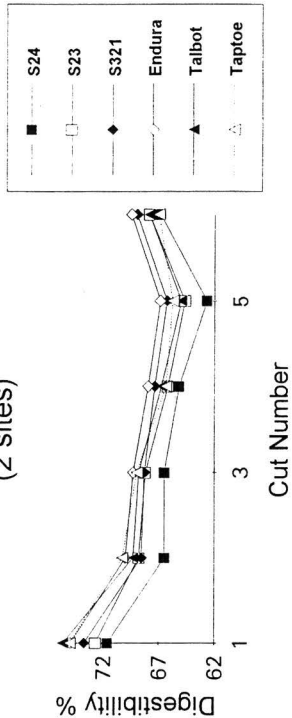


Figure 5: Changes in digestibility for two varieties under grazing (after Gately 1984)

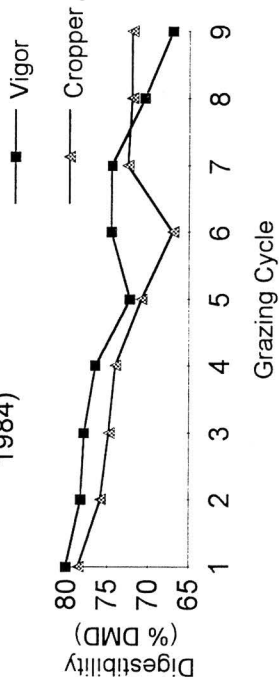
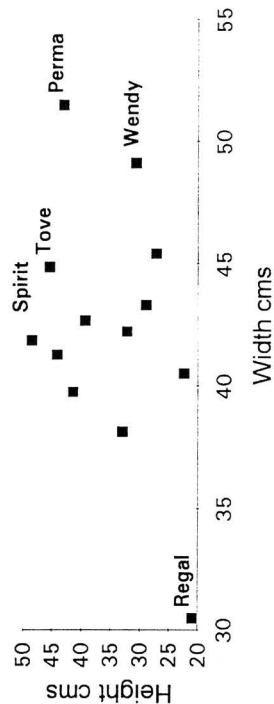


Figure 6: Differences in habit in spaced plants of perennial ryegrass



THE IMPORTANCE OF THE DIGESTIBILITY OF PRESERVED GRASS FOR THE DAIRY COW¹

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Summary

The study was based on in vivo experiments with 73 grass silages and 32 grass hays, varying in digestibility from 55 to 83 %. Rumen fluid and enzymatic methods accurately predict in vivo digestibility. For NIRS-calibrations and comparative purposes however, in vitro results should be corrected with standard samples. The organic matter digestibility of the preserved grasses was mainly determined by the digestibility of the cell-walls through the degree of lignification. It was calculated that a percent-unit increase in digestibility increased metabolizable energy and net energy for lactation with 0.16 and 0.11 MJ/kg OM, respectively and increased digestible protein in the intestine with 1.2 and 2.2 g/kg OM for grass silages and hays, respectively. A percentage increase of digestibility increased voluntary intake by dairy cows with on average 0.18 kg DM/day for prewilted grass silages and hays, but had no effect for wet silages. The corresponding response in potential roughage milk production based on energy and protein intake amounted to 0.6 kg for grass hays, 0.5 kg for prewilted silages and 0.4 kg for wet silages.

Introduction

Grass is mainly grown to provide feed for ruminants. However, it is only recently, particularly since the introduction of the milk quota system, that grass breeders consider feeding quality as a potential selection criterion. Up to now only in the UK, digestibility is actually mentioned in the Recommended List. The feeding value is defined in terms of animal production potential as the result of intake of digestible nutrients and the efficiency of utilization, in the assumption that toxic factors are absent and no essential element is in short supply. From the three components in this definition, digestibility is the most appropriate parameter to measure because of methodological reasons. Digestibility further exerts an important influence on the other two components, intake and efficiency of utilization. It is self-evident that determining digestibility with animals is not suited for breeding purposes. During the last three decades less time-consuming (three to five days) and less cumbersome in vitro simulation techniques have been developed, making use of either rumen fluid or commercial enzymes. More recently, the measurement of digestibility became extremely attractive with the entrance of the very fast ($\pm 1'$) near infrared reflectance spectroscopy (NIRS) technique. This study discusses the importance of digestibility as feed quality parameter for grass, based on data from preserved grasses, varying in digestibility mainly because of the growth stage at

¹ Communication n° 906 of the institute.

harvest. First, the prediction of in vivo digestibility by in vitro methods and NIRS will be considered. For breeders it can be interesting to know which plant parts or components have most impact on global digestibility. Therefore, the effect of chemical composition and partial digestion coefficients on digestibility will be examined. Finally, possible consequences of an increase in digestibility for the energy and protein value and the voluntary intake by dairy cows will be discussed.

Material and methods

The data originated from 73 grass silages and 32 grass hays with known in vivo digestibility. From 37 grass silages and 16 grass hays also the voluntary intake by dairy cows was determined. Grass crops from either *Lolium perenne* (n = 24 for silages, n = 11 for hays), *Lolium multiflorum* (n = 24 for silages, n = 3 for hays) or mixed pastures (n = 25 for silages, n = 18 for hays) were harvested at the institute (sandy-loam soil ; 700 mm precipitation/year) during the last 25 years. Of the silages, 33 were first-cut (or cut before 30 June), 25 second-cut (before 31 August) and 15 third-cut (after 31 August) ; 20, 10 and 2 hays were respectively first-, second- and third-cut. Ensiling occurred either directly (n = 45) or after prewilting (n = 28).

Chemical analyses were carried out on oven-dried ($\pm 60^{\circ}\text{C}$) samples, ground to pass a 1-mm screen. Crude protein (CP : N x 6.25) was determined with a Kjel-foss apparatus. For determination of oil, samples before 1985 were extracted with diethyl ether (DE) and with petroleum ether (PE) afterwards for a minimum of 6 hours. The diethyl ether values of the grass silages were corrected with the formula : $\text{PE} (\%) = 1/(0.35 - 0.0165 \text{ DE} (\%))$ (n = 34 ; $R^2 = 31\%$) (De Boever, unpublished). Crude fibre (CF) was analyzed with a Fibertec apparatus (Tecator). Ash content was obtained after incineration at 600°C . Cell-wall analysis occurred with a Tecator apparatus by sequentially adding neutral detergent, acid detergent and 72 % (wt/wt) H_2SO_4 (Van Soest *et al.*, 1991). Neutral detergent fibre (NDF) represents total cell-walls. Hemicellulose was calculated from the difference of NDF-acid detergent fibre (ADF) and cellulose from ADF-lignin. Water soluble carbohydrates (WSC) were obtained following the anthron method (MAFF, 1986).

Digestibility was determined with 5 adult wethers or 5 non lactating cows, fed near maintenance level with the herbage as sole feedstuff. After an adaptation period of 2 to 3 weeks, feces were totally collected during 10 days. Rumen fluid digestibility was determined following the classical two-stage technique of Tilley and Terry (1963). Cellulase digestibility was obtained according to the procedure of De Boever *et al.* (1986).

Metabolizable (ME) and net energy for lactation (NEL) were both derived from chemical composition and in vivo digestion coefficients by means of formulae proposed by Van Es (1978). The protein value was calculated as digestible protein in the intestine (DPI) according to the Dutch system (CVB, 1992).

The voluntary intake was determined with Belgian White-Red (dual purpose breed) and Holstein-Friesian lactating cows. All trials fell in the period from 8 weeks after parturition until 8 weeks before the next one. Concentrates were individually offered according to the energy and protein requirements. Most grass silages were chopped at a theoretical length of ± 24 mm. All data were corrected for a body weight of 600 kg and a daily yield of 15 kg FCM.

Results and discussion

The chemical and cell-wall composition, the digestibility, the protein and energy value of the grass silages and hays are given in Table 1. To avoid the disturbing effect of soil contamination, contents are expressed on organic matter (OM) basis. From this table a large variation appears in all parameters for both grass silages and hays. In vivo digestibility of the preserved grassland products ranged from 55 to 83 %.

a. Prediction of in vivo digestibility by in vitro techniques

From Figure 1 a strong relationship appears between in vitro and in vivo digestibility, particularly for grass hays. With the cellulase method almost the same accuracy can be obtained as with rumen fluid so that for reasons of convenience and better reproducibility (De Boever *et al.*, 1986) the former is the method of choice. Rumen fluid digestibility is on average 4.5 and 1.7 %-units lower than in vivo digestibility for grass silages and hays, respectively (Table 1). From Fig. 1 also appears that in vitro values are consistently lower over the whole range tending to decrease at higher digestibility. Some loss of microbial activity in vitro may explain this systematic underestimation. Moreover, the static in vitro conditions do not ideally simulate the in vivo situation e.g. with regard to the substrate/rumen fluid ratio and the retention time of the feed in the rumen. Cellulase digestibility too, is on average lower than in vivo digestibility : 1.3 %-units for grass silages and 1.8 %-units for grass hays (Table 1). However, cellulase values are lower for herbage with low to moderate digestibility and higher for those of high digestibility, the crossing being at ± 75 % in vivo digestibility. The constant amount of cellulases added is apparently insufficient for fibre rich feeds but is in excess for feeds of better quality. On the contrary, when using rumen fluid, living microorganisms are capable to adapt to the nature of the substrate. This phenomenon was also found with concentrates and raw materials (De Boever *et al.*, 1994). Consequently, variation in cellulase values is greater than with rumen fluid or in vivo (Table 1). Because many enzymatic methods or versions are used, comparison of raw cellulase digestibility data from different lists can introduce much confusion. This remark also applies for NIRS-calibrations based on cellulase values. One can avoid that through correction of the cellulase values to a reference level by analyzing standard samples with known in vivo digestibility in each run. It further greatly improves repeatability and reproducibility of the results.

b. Nutritive factors determining digestibility.

In Table 2 the relationships between chemical composition, cell-wall components or partial digestion coefficients and OM-digestibility are given. The determination of chemical composition according to the Weende scheme gives a bad picture of the nature of the carbohydrates : crude fibre mainly represents cellulose, whereas the other cell-wall components, hemicellulose and lignin, arrive in the NfE-fraction (Van Soest, 1985). The better analysis scheme of Van Soest reveals that the organic matter of preserved grasses contains 45 to 80 % cell-walls, composed for about half of cellulose, somewhat less than half of hemicellulose and a few percents of lignin (Table 1). The rest of the OM, considered as completely available for the animal, consists of nitrogenous substances, sugars, lipids and for silages of fermentation products. Therefore, it is not surprising that OM-digestibility was mainly determined by the partial digestion coefficients of NfE and CF. The digestibility of the cell-walls on its turn was media-

ted through the extent of lignification. Cellulose content also had a pronounced negative effect on digestibility for grass hays but not for silages. Hemicellulose had a slightly negative influence on digestibility. Crude protein, water soluble carbohydrates and fat were slightly positive or not related to digestibility.

c. Possible consequences of an increase in digestibility.

c.1 Energy value

Table 3 represents the linear relationship between *in vivo* digestibility and three energy value expressions. There was no relationship between digestibility and gross energy content. The higher mean GE content on OM-basis for grass silages than for grass hays is due to the higher fat and protein content of the former ; however, when expressed on DM-basis, grass silages contained on average less energy because of the considerable soil contamination. Digestibility had a strong positive and linear effect on the ME - and NEL value, particularly for grass hays. The greater contribution of fat to the energy value of grass silages may explain the lower determination coefficient of the relationship. For an increase in digestibility of 1 percent, ME and NEL of herbage products increased with about 0.16 and 0.11 MJ/kg OM, respectively. This relationship is logical in fact as energy values were calculated from multiple linear regression equations based on digestible nutrients. Thus, although the greatest energy loss, i.e. through feces, is measured, losses in urine, methane and heat are estimated. Givens *et al.* (1989) found for spring-grown herbages that the proportional loss of gross energy in urine, varying from 2.5 to 11.4 %, strongly increased with crude protein content. Methane losses, ranging from 5.8 to 8.2 % of GE for fresh grasses harvested over the year, appeared not related to digestibility or chemical composition (Givens *et al.*, 1993). For a given type of animal production, the efficiency of ME utilization is assumed to vary directly with ME concentration of the feed. However, calorimetric data for sheep or cattle grown or fattened exclusively on roughages showed a wide variation in the relationship between ME and energy retention (Greenhalgh and Wainman, 1980). On forage diets the energy costs of physical comminution (eating and rumination), fermentation and absorption would account only some 40-45 % of the heat increment of the feed, the remainder being associated with the liver and other tissues outside the gut. Moreover, the decline of efficiency of ME utilization with more fibrous feeds would be mainly due to the nature of the substrates made available by digestion and the metabolic reactions for which they are used (Webster *et al.*, 1975). Current empirical energy evaluation systems not only ignore the nature of the end-products formed but also energy-protein relationships. The studies of Ørskov *et al.* (1979) with sheep, maintained entirely on intra-ruminal infusions suggest that variation in the proportions of individual volatile fatty acids (within the normal range) has little influence on the utilization of ME. On the other hand, a shift of organic matter digestion towards the small intestine may increase the uptake of amino acids and improve the utilization of the energy-yielding substrate by providing extra gluconeogenic precursors required for fat synthesis (Ribeiro *et al.*, 1981). Apart from the site of digestion, the form in which energy is retained may be another cause for the variation in the efficiency of ME utilization. So, Rattray and Joyce (1976) calculated from the results of comparative slaughter trials with lambs fed mainly on forages that fat synthesis appeared far more efficient than protein synthesis (0.80 vs 0.20).

c2. Protein value

Since 1992 the protein value of a feed is expressed as the amount of true protein absorbable from the small intestine. The digestible protein in the intestine (DPI) originates from feed protein escaping rumen degradation (DPI_F) and microbial protein synthesized in the rumen (DPI_M), corrected for a metabolic fecal protein fraction (DPI_{MF}). From Table 1 it appears that despite a higher CP content (17.3 vs. 14.3 %/OM), DPI content of grass silages is almost half that of grass hays (4.8 vs. 9.1 %/OM). The reason may be sought in the extensive proteolysis in the silo and the fact that most of the fermentation products do not contribute to the energy supply of the microorganisms (Van Vuuren, 1993). Table 3 gives the effect of digestibility on the protein value and its composing fractions. Per unit increase of OM-digestibility the DPI-value increases with 1.2 and 2.2 g/kg OM for grass silages and hays, respectively. This effect is mainly due to the strong increase of the microbial protein fraction as more fermentable organic matter comes available. Digestibility has a slightly negative influence on the rumen-bypass protein fraction for grass silages, whereas no effect for grass hays. Loss of endogenous protein in feces logically decreases with higher digestibility. The rumen protein balance (RPB), which is a measure of the potential nitrogen output in the environment, appeared not affected by digestibility.

c3. Voluntary intake

The effect of the digestibility of preserved grass on voluntary intake and potential roughage milk production is given in Table 4. Because intake of wet and prewilted silages is different, results were separately discussed. A percentage increase of OM-digestibility increased voluntary intake with on average 0.18 kg DM for prewilted grass silages and hays; no significant effect was observed for direct ensiled grasses. Taking the higher energy content into account, potential roughage milk production (PRMP) would increase with about 0.6 kg milk per unit increase in digestibility. In practice, ad libitum access to a 1 % more digestible grassland product would thus economize 0.3 kg concentrates per animal per day. The PRMP based on digestible protein intake increased at the same rate for grass hays, but somewhat slower for prewilted grass silages (0.5 kg/day). For wet silages, PRMP increased with about 0.4 kg/day, but only as a consequence of the higher energy and protein value of more digestible grass. In a review of experiments with well-preserved silages, Thomas (1980) calculated that silage-DM intake increased on average by 0.15 kg and actual milk yield by 0.29 kg per unit increase in D-value (digestible organic matter in the dry matter). From experiments with 15 grass silages, Castle (1975) obtained a response in milk yield of 0.23 kg per unit increase in D-value. Regression of our data for prewilted grass silages with the D-value resulted in responses for DM-intake of 0.15 kg and for PRMP from energy of 0.75 kg. Thus, our results for DM-intake are similar to those of Thomas (1980), but those for milk yield were considerably higher than observed by Castle (1975) and Thomas (1980). The difference may partially be explained by the different way of concentrate supplementation. In our trials the amount of concentrates was calculated from the difference between the animal requirements and the PRMP, while in the cited experiments concentrates were allotted at a constant rate per kg actual milk production. The concentrate level was higher than in our experiments and this difference increased with better digestible silages as the quality of the forage was not considered. It is known that the substitution rate increases as concentrate level (Steen and Gordon, 1980) and digestibility of the basal ration increases (Blaxter and Wilson, 1963). Another possible cause may be that the actual efficiency of ME utilization with grass silage is lower than calculated. This effect may arise from a nutritional imbalance because of the poor

utilization of silage protein (Thomas and Thomas, 1985). Concerning milk composition, Thomas (1984) found that providing a better digestible grass silage at a constant concentrate level resulted in a reduction of fat content but an increase in protein content.

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Table 1 : Chemical and cell-wall composition, in vivo and in vitro digestibility, protein and energy value of 73 grass silages and 32 grass hays.

	Grass silages		Grass hays	
	mean \pm SD	range	mean \pm SD	range
DM (%)	28.0 \pm 12.1	14.7 - 67.1	84.3 \pm 2.6	79.0 - 89.6
Ash (%/DM)	19.2 \pm 9.3	9.9 - 49.6	9.6 \pm 2.4	5.6 - 16.2
<i>Chemical composition (%/OM)</i>				
CP	17.4 \pm 3.5	11.2 - 26.6	14.3 \pm 4.5	8.0 - 26.1
CF	32.0 \pm 3.9	20.8 - 45.4	32.1 \pm 3.8	23.8 - 38.7
Fat	5.3 \pm 0.9	3.9 - 7.9	2.9 \pm 0.9	1.6 - 4.8
NfE	45.3 \pm 3.6	38.4 - 53.7	50.7 \pm 4.6	42.5 - 58.3
<i>Cell-wall composition (%/OM)</i>				
NDF	61.3 \pm 5.8	44.2 - 80.3	69.6 \pm 6.4	57.3 - 80.7
ADF	35.6 \pm 3.8	24.5 - 47.2	36.9 \pm 5.0	27.0 - 46.1
Lignin	3.2 \pm 0.8	2.1 - 5.4	3.4 \pm 1.1	1.4 - 6.1
Hemicellulose	25.7 \pm 3.3	17.9 - 33.1	32.7 \pm 2.4	28.5 - 37.5
Cellulose	32.4 \pm 3.6	22.4 - 43.6	33.6 \pm 4.1	25.3 - 40.6
WSC(%/OM)	1.3 \pm 1.3	0.3 - 5.9	5.6 \pm 2.6	1.5 - 10.1
<i>In vivo digestibility (%)</i>				
CP	66.2 \pm 6.3	38.7 - 76.4	62.7 \pm 9.0	48.8 - 80.4
CF	80.7 \pm 6.1	63.4 - 90.0	74.5 \pm 7.1	58.8 - 87.3
Fat	73.5 \pm 5.7	53.6 - 85.5	51.5 \pm 8.8	34.1 - 65.9
NfE	69.2 \pm 7.5	42.7 - 83.6	68.6 \pm 7.8	52.5 - 83.2
OM	73.0 \pm 5.4	55.2 - 82.6	69.4 \pm 6.6	54.6 - 81.1
<i>In vitro OM digestibility (%)</i>				
Rumen fluid	68.5 \pm 5.6	52.9 - 79.0	67.7 \pm 6.5	50.9 - 79.6
Cellulase	71.7 \pm 7.1	53.9 - 87.3	67.6 \pm 8.9	47.7 - 85.2
DPI (%/OM)	4.8 \pm 1.7	0.0 - 7.8	9.1 \pm 2.1	5.9 - 15.0
GE (MJ/kg OM)	20.93 \pm 0.55	19.88 - 22.44	19.84 \pm 0.37	19.26 - 20.78
ME (MJ/kg OM)	11.91 \pm 0.91	8.85 - 13.80	10.48 \pm 1.11	8.12 - 12.39
NEL (MJ/kg OM)	6.97 \pm 0.63	4.87 - 8.22	6.04 \pm 0.76	4.45 - 7.40

Table 2 : Linear relationship between chemical composition, cell-wall components or partial digestion coefficients and in vivo OM-digestibility

	Grass silages n = 73			Grass hays n = 32		
	a + bX	R ²	P ¹	a + bX	R ²	P
CP	NS			60.2 + 0.64X	19.0	*
CF	NS			111.2 - 1.30X	57.0	***
Fat	62.5 + 1.98X	11.6	**	57.2 + 4.14X	29.8	**
NfE	NS			NS		
NDF	92.6 - 0.32X	11.7	**	133.4 - 0.92X	73.8	***
ADF	NS			112.2 - 1.16X	72.5	***
Lignin	89.4 - 5.12X	53.0	***	88.0 - 5.58X	81.7	***
Hemicellulose	93.8 - 0.81X	24.1	***	117.8 - 1.49X	26.4	**
Cellulose	NS			113.6 - 1.32X	62.9	***
WSC	NS			61.3 + 1.43X	30.0	**
CPdig.	41.3 + 0.48X	31.5	***	35.3 + 0.54X	54.9	***
CFdig.	9.2 + 0.79X	79.1	***	7.0 + 0.84X	82.8	***
Fatdig.	NS			53.4 + 0.31X	17.3	*
NfEdig.	27.0 + 0.66X	86.2	***	13.6 + 0.81X	92.4	***

¹ P < 0.05: * ; P < 0.01: ** ; P < 0.001: ***

Table 3 : Effect of in vivo digestibility on energy and protein value

	Grass silages n = 73			Grass hays n = 32		
	a + bX	R ²	P ¹	a + bX	R ²	P
<i>Energy value (MJ/kg OM)</i>						
GE	NS			NS		
ME	1.36 + 0.145X	74.2	***	-1.06 + 0.166X	96.9	***
NEL	-0.55 + 0.103X	78.3	***	-1.90 + 0.114X	97.2	***
<i>Protein value (g/kg OM)</i>						
DPI _F ²	59.9 - 0.43X	9.9	**	NS		
DPI _M ²	-10.5 + 0.88X	67.9	***	1.5 + 0.80X	87.0	***
DPI _{MF} ²	89.4 - 0.76X	9.8	**	59.3 - 0.65X	84.0	***
DPI ²	-38.3 + 1.18X	14.7	***	-64.0 + 2.23X	49.7	***
RPB ³	NS			NS		

¹ P < 0.05: * ; P < 0.01: ** ; P < 0.001: ***

² DPI = digestible protein in the intestine, respectively from feed (F) and microbial (M) origin and for metabolic fecal (MF) requirements

³ Rumen protein balance

Table 4 : Effect of in vivo digestibility on voluntary intake and potential roughage milk production (daily basis).

	wet silages n = 14			prewilted silages n = 23			hays n = 16		
	a + bX	R ²	P ¹	a + bX	R ²	P	a + bX	R ²	P
<i>Voluntary intake</i>									
DM (kg)	NS			0.5 + 0.17X	65.9	***	-2.3 + 0.19X	51.6	**
NEL (MJ)	-21.3 + 1.17X	49.8	**	-71.0 + 2.01X	81.9	***	74.4 + 1.94X	76.0	***
DPI (g)	-939 + 18.4X	45.1	**	-1152 + 25.4X	74.0	***	-1463 + 31.7X	74.3	***
<i>Potential roughage milk production (kg)</i>									
energy	-18.6 + 0.39X	49.8	**	-34.9 + 0.66 X	81.9	***	-32.7 + 0.59X	71.0	***
protein	-20.2 + 0.35X	45.1	**	-24.3 + 0.49X	74.0	***	-30.3 + 0.61X	74.3	***

¹ P < 0.05: * ; P < 0.01: ** ; P < 0.001: ***

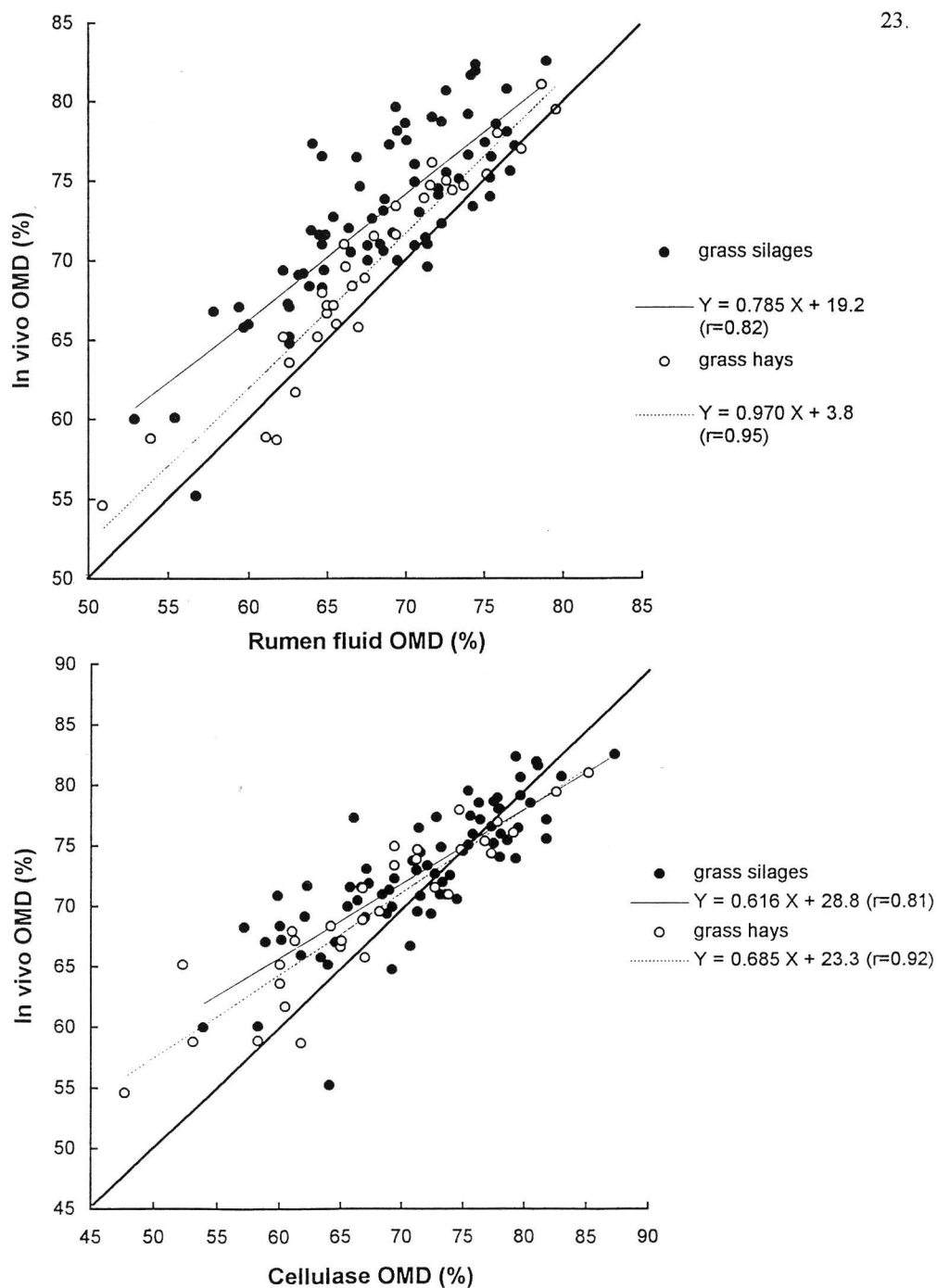


Figure 1 : Prediction of in vivo digestibility by rumen fluid and cellulase digestibility for grass silages (n=73) and hays (n=32)

BREEDING, FORAGE QUALITY, FEEDING VALUE AND ANIMAL PERFORMANCE

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During the last General Meeting of the European Grassland Federation in Wageningen concerning "Grassland and Society", one of the items was "Forage production and utilization". Mr. Beever (1994) gave a remarkable introduction about "Forage quality, feeding value and animal performance" and Mr. Van Vuuren (1994) coordinated the workshop Digestibility and intake.

In this introduction Mr. Beever (1994), as commonly is accepted, stated that forages fail as adequate feeds for high producing ruminants. With dairy cows, high energy and -protein feeds of non-forage origin are fed, as milk yields commensurate with the animals genetic merit can not be achieved from forage only diets. As demand increases for animal products of defined protein and fat contents, forage content of the diet is unlikely to increase. If however we could recognize where the current forages fail as adequate feeds for high producing ruminants it may be possible to reverse that trend by breeding forages with improved characteristics which more closely reflect the animals requirements for specific nutrients.

He also stated that the criteria by which forage breeding programmes should be directed towards improving animal performance have not been adequately established, in part due to the obsession to judge the nutritive value of forage in terms of the overall digestibility of nutritional components such as organic matter, with no regard for the composition of individual nutrients. Otherwise plant and animal scientists, involved in the production and utilisation of forage, have not communicated adequately. Breeders however mostly don't receive adequate answers on their questions for the most relevant breeding topics.

As the rumen is the principal site of forage digestion carbohydrates are involved in two processes. The first, and often rate limiting step is the degradation of the polymer to simple sugars. With fructosans from grasses this process is very rapid but the rate of hemicellulose and cellulose degradation is influenced by the degree of lignification and in particular the cross linkages between lignin and hemicellulose. The second stage of carbohydrate utilisation involves microbial metabolism on the released monosaccharides. Monosaccharides in excess of microbial requirement may be fermented and whilst the VFA produced will be available to the host animal, the associated production of methane and fermentation heat will represent a further loss of energy to the animal.

Acetate and butyrate production predominate on high fibre diets, associated with a net production of hydrogen, which is converted to methane, leading to an overall loss of carbohydrate energy not accounted for as VFA. Production of propionate increases on diets containing significant amounts of readily fermentable carbohydrate (soluble sugars, starches and pectins) but this reaction requires hydrogen and thus methane is not produced.

In this respect it is interesting to refer to a poster presented by Groot and his colleagues from Wageningen concerning "Digestibility of cell walls of ageing grass leaves as estimated from in vitro and gas production techniques". The cumulative gas production technique might prove a useful technique for assessing the degradation of roughages and the resulting formation of fermentation products.

During this meeting Van Loo will discuss this afternoon the results of a cooperative research programme between DLO-CPRO and CLO-RvP about "Genetic variation in perennial ryegrass for gas production during in vitro rumen fermentation".

Studies with perennial ryegrass and white clover fed to grazing cattle indicate that the ruminal losses of dietary N are considerable. Most data for fresh forages, however, suggest that the efficiency of microbial protein synthesis is satisfactory, and rather it is the total yield of microbial biomass that may be compromised in relation to available energy and N supply as a consequence of microbial degradation in the rumen.

Tannin containing forages like some legumes may improve the small intestinal absorption of amino acids. This seems to be related to the formation of a tannin protein complex within the rumen, which either protects some of the dietary protein from degradation or retards the rate of protein degradation in the rumen such that energy and protein availability for microbial growth are in closer synchrony. High tannin concentrations however may result in a depressed feed intake.

In the case of making silage, a large proportion, and often all, of the readily - available carbohydrate will be fermented within the silo to yield lactic and acetic acid, which cause the pH of the silage to fall. At the same time, plant proteases which express optimal activity at different pH values will degrade a substantial proportion of the readily available protein to consecutive amino acids. This represents a serious loss of protein per se, whilst some of the N containing end products (putrescine cadaverine) may have pronounced pharmacological effects upon the animal, possibly resulting in depressed feed intake.

Undoubtedly, one of the major problems of grazed and conserved grasses and legumes is the reduced efficiency of protein utilisation. This can be largely attributed to impaired rumen function as influenced by the nature of the diets fed, and unbalanced supplies of carbohydrate and "protein" to support microbial metabolism, as found on many forages.

It is of crucial importance in any system of animal production based on forages to optimise rumen function, whereby microbial metabolism is not compromised and the transfer of ingested nutrients to absorbed nutrients is optimised. This is essential if maximum forage intake is to be achieved, and only when the requirements of the rumen have been fully met, should consideration be given to the provision of nutrients which are likely to by-pass rumen fermentation. Future forage breeding programmes must establish this important distinction as one of its major criteria. Breeding for yield and OM digestibility alone is no longer adequate. Mr. Beever ended his contribution expressing the hope that forage breeding programmes will concentrate their efforts in relation to improved nutritive value of forages ; that means improved carbohydrate availability and more controlled protein degradation.

In the workshop concerning "Digestibility and intake" coordinated by Van Vuuren (1994) the main question, as I already suggested, was "what selection criteria plant breeders should use in order to obtain a forage composition which is ideal from nutritional point of view ?" In the first part of that workshop the impact of measuring digestibility was discussed. The energy

systems presently used are not accurate enough to guarantee that a small difference in measured digestibility and consequently a higher calculated net energy content result in an improved animal performance. Thus it was concluded that digestibility should no longer be a selection criterion for grass at the high levels we have now reached in Western Europe.

Furthermore from some research work it was concluded that even intake decreased if digestibility of the OM depassed 75 %.

In the second part, the discussion focused on the composition of grass based diets for milk and growth performances.

As was already told, going through the introduction of Beever (1994), a large proportion of the protein may be lost during rumen fermentation, mainly caused by a deficiency in available carbohydrates. To increase the carbohydrate : protein ratio in the diet, either grass composition should be changed or grass should be supplemented with feeds high in available carbohydrates (like fodder beets and maize). On the other hand the protein of grass has to be lowered and must be more available. Selection for an increased N-use efficiency of grass may prevent a large reduction in the nutritive quality and more research has to be done about the influence of tannin upon the protein degradation.

It was concluded that ideal grass contains 50 to 60 % available carbohydrates and 12 % protein of 100 % availability. With respect to carbohydrate composition the possibility to breed starch-containing grasses may be considered. Biotechnology will have a helpful task in this respect.

As a general conclusion : the task of a grass breeder is heavy : his grass must on the one hand look like maize (carbohydrates) and on the other hand look like clover (protein availability). Is this still serious ?

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SESSION 2 : The measurement of quality parameters

FORAGE ANALYSIS BY NIRS

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Near Infrared Spectroscopy enables rapid and non-destructive analysis of organic constituents. Near Infrared Spectroscopy covers spectral aspects, but also associated techniques such as the preparation of samples and data analysis. Most organic substances absorb energy in near infrared (780 - 2500 nm). We can give as example the main constituents of the plant and animal world : protein, fatty matter, carbohydrates, fibres, etc...Each of the constituents has a precise and unique signature in near infrared, depending on deformations of chemical links O-H, C-H and N-H.

Practically, the different constituents are found together and a sample's spectrum is the sum of all the basic absorptions to which are added all the interferences in relation with the physical aspect of the sample. The instrument thus needs to be calibrated, before routine analyses. The first step of this calibration consists in measuring with the instrument numerous (50 to several hundreds) samples which are representative of a given product, for which the different parameters have been measured by reference methods. The second step of the calibration is to optimize a multilinear model which links the optical values to the reference analytical values.

Once the calibration finished, a prediction model is used to accomplish big series of analyses. For 15 years, the Station the Haute Belgique has developed and develops standards to measure the intrinsic and nutritional quality of feed and forage in particular, quality which is linked to the chemical composition and to its digestive use. The direct determination of the digestibility coefficient is one of the most spectacular results of infrared technology. The digestibility of feed organic matter, for which the reference method for animals is very expensive, can be determined with a precision which is superior to that obtained with other recommended laboratory techniques.

Table 1 gives the results of calibrations actually carried out for the analysis of dried and milled forage (pasture grass and hay). One measure of the absorption spectrum enables in a few seconds to determine 18 parameters, by applying specific equations to this spectrum. Data-bases also exist for grass silage, alfalfa, ray-grass and maize (whole plant, stems and leaves, cobs and maize silage). These data-bases have been set-up with forage dried and milled following standardized methods.

The recent development of new types of measuring cells has enabled to consider the analysis of fresh material, i.e. with no preparation, directly from the field, the store or the silo into the spectrometer. For this, the Station de Haute Belgique has a mobile laboratory equipped to set-up the data-bases. This mobile laboratory is an invaluable tool to collect and analyse directly on the field: farms, experimental plots, storage sites, industrial plants, ... Table 2 summarizes present results of standardizations of grass measured fresh. Setting-up these standardizations requires considerable time to select representative samples, to determine reference values and to statistically analyse results.

The comparison of results between "dried and milled forage" and "fresh forage" shows that one reaches similar precision. The first analyses thus let presume that in a near future, the fastidious operations of drying and milling will no longer be necessary. A direct analysis on

fresh material will enable to increase again the number of samples analysed and to define better the production, at plant husbandry trial level and to evaluate farmers stocks. Data-bases with fresh material are being set-up for other products: alfalfa, maize, grass silage. For this last category, the first trials show that parameters such as pH, ammonium nitrogen, volatile fatty acids will also be determinable by infrared.

Near Infrared Spectroscopy seems to be the only technique which is rapid enough to allow a screening of thousands of samples. For this reason, it is a very useful tool for breeders.

Table 1 : Statistical results of forage standardizations. Dried and milled samples.

Variable	N	Moy	ETR	R2
1 : Dry matter	506	94.35	0.54	0.88
2 : Protein	793	14.69	0.79	0.98
3 : Cellulose	823	26.48	1.14	0.95
4 : Soluble sugars	359	10.79	1.40	0.96
5 : Total ashes	696	9.58	0.78	0.92
6 : In vivo digestibility	337	67.60	1.97	0.92
7 : In vitro digestibility	602	76.74	1.76	0.96
8 : Soluble nitrogen	280	5.04	0.60	0.91
9 : NDF	328	48.46	1.36	0.96
10 : ADF	319	28.18	1.04	0.95
11 : ADL	327	5.03	0.58	0.75
12 : Ca	515	7.39	0.77	0.90
13 : Na	439	0.69	0.17	0.63
14 : K	524	26.86	2.01	0.87
15 : Mg	522	2.10	0.17	0.90
16 : P	522	4.04	0.32	0.85
17 : Insoluble ashes	384	2.62	0.35	0.95
18 : Ingestibility	220	64.46	4.83	0.89

N : number of samples

Moy : average

ETR: residual standard deviation

R2 : determination coefficient.

Table 2 : Statistical results of fresh forage

Variable	N	Moy	ETR	R2
1 : Dry matter	431	20.71	0.75	0.98
2 : Protein	498	11.21	0.82	0.96
3 : Cellulose	505	25.88	1.11	0.92
5 : Total ashes	508	8.72	0.67	0.69
7 : In vitro digestibility	500	75.97	1.73	0.95

GENETIC VARIATION IN PERENNIAL RYEGRASS FOR GAS PRODUCTION DURING *IN VITRO* RUMEN FERMENTATION

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Summary

In this study, genetic variation for fermentation kinetics was investigated in perennial ryegrass with a time related gas production technique using buffered rumen fluid. Consistent genetic variation in total gas production was found. Location by genotype interaction for gas production was significant, but was less important than the main genotypic differences. The gas production during the first four hours of fermentation was positively correlated with the soluble, non-protein fraction of organic matter. Gas production during the first 24 hours was highly positively related to organic matter degradability, but only when differences in crude protein content were taken into account. However, not all genetic variation in gas production could be explained by organic matter digestibility, cell wall content or crude protein.

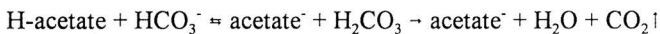
Introduction

As a consequence of environmental policies regarding intensive dairy farming, farmers have to pay more attention to the nutritive value of their forage grass. Improved grass varieties with a higher nutritive value will contribute substantially to economic profit as well as to the reduction of surpluses of mineral nutrients on the dairy farm (Van Loo & Vellinga, 1994).

However, grass breeding for a higher nutritive value is seriously hampered by the lack of an accurate and cost-effective method for the evaluation of nutritive value. Especially, the evaluation of the energy value of feed is problematic. Most methods for the evaluation of the energy value of feeds are based on the disappearance (degradability) of organic matter during *in vitro* or *in sacco* incubations in rumen fluid or cellulase preparations, which methods have some disadvantages. Firstly, the fact that organic matter has disintegrated and has been solubilized does not necessarily mean that the material has been fermented. Secondly, these methods usually only give values after 48 h incubations, so only information on the extent of degradation is obtained and no information on the kinetics of degradation. With the already relatively high degradability as measured after 48 h incubations of the forage grass species grown in North West Europe, improvements of nutritive value also need to include improvements of the rate of degradation and fermentation. Moreover, to improve nitrogen utilisation of dairy cattle, special attention should be given to the asynchronous degradation of soluble sugars, cell wall and protein fraction during rumen fermentation of grass (Beever & Reynolds,

1994). Particularly the degradation of cell walls lags behind the protein degradation. Improvement of the synchronisation of carbohydrate and protein degradation can be achieved by increasing the rate of cell wall carbohydrate fermentation.

Genetic variation for kinetics of feed fermentation may be studied by measuring the formation of the valuable end products of rumen fermentation: volatile fatty acids and microbial biomass. However, at the moment this is not be feasible on a scale required for selection in plant breeding, because multiple incubations are needed. Measurement of gas production during rumen fermentation offers an alternative method for studying rumen fermentation kinetics in single incubations. During fermentation, gasses (e.g. CO₂, CH₄, H₂, NH₃) are released from the rumen fluid. Part of the gas is directly produced with the fermentation of carbohydrates and proteins to volatile fatty acids (VFAs). Furthermore, because the rumen fluid medium is saturated with CO₂, also CO₂ is released indirectly from the rumen fluid as the VFAs release H⁺ in the medium (example for CH₃-COOH, H-acetate) :



As an endpoint measurement for the evaluation of nutritive value, the gas production test has been proposed and used by Menke *et al.* (1979). Beuvinck (1993), Cone *et al.* (1994) and Theodorou *et al.* (in press) have shown that with automatic measurement of the gas production fermentation kinetics may be studied in single incubations. This opens the possibility of studying fermentation kinetics by measurement of gas production for larger scale applications, for example in plant breeding for a higher nutritive value. However, the extent of genetic variation for gas production characteristics has not yet been determined. In the present study some data are presented on the genetic variation in perennial ryegrass (*Lolium perenne* L.) for gas production during *in vitro* rumen fermentation. Gas production kinetics were related to organic matter degradability after 48 h *in vitro* rumen fluid incubation, cell wall and crude protein content.

Materials and methods

The perennial ryegrass material studied consisted of a set of 10 cloned genotypes, 5 inbred families and 5 populations grown at Merelbeke, Belgium and Wageningen, The Netherlands. The material is listed in Table 1. The cloned genotypes were chosen from breeding material of RVP and covered a wide range in digestibility coefficient determined by the pepsine cellulase method (Jones and Hayward, 1975). Inbred families 1 and 3 had a common S3-parent derived from Splendor. Inbred families 7 and 11 also had a common S3-parent derived from Splendor, but not the same parent as inbred families 1 and 3. Inbred family 18 was derived from Lenta III and was not related to the other four inbred families. The populations were either cultivars or collected material. In the first week of July 1993, the material was planted in 1 m rows consisting of 10 plants. For the assessment of quality characteristics, a three-week regrowth was harvested on September 27 in Merelbeke and on September 29 in Wageningen. The harvested material was dried at 70°C for 24 h and ground with a hammer mill with a 1 mm sieve. Crude protein (CP) was determined as 6.25* total N. Total N was determined after Kjeldahl digestion using an autoanalyzer. Cell wall content (NDF) was determined according to Goering and Van Soest (1970). Gas production measurements were carried out using the automated equipment at ID-DLO (Cone *et al.*, 1994) and using a rumen fluid medium

described by Beuvink (1993). This equipment consisted of 12 incubation bottles of which the volume of gas produced was measured continuously. Of each of the 40 samples, the gas production was measured in three separate incubations with rumen fluid from sheep collected on different dates. In each series, one blank incubation to correct for gas produced from the rumen fluid medium itself and one incubation with a standard sample were included. Because only 12 incubations could be carried out at the same time, not all 40 samples could be run in the same series. In total 12 series were carried out. To allow for possible differences between the series due to differences in the collected rumen fluid, an incomplete, balanced block design was used. *In vitro* degradability of organic matter (OMD, % of organic matter) was determined after 48 h incubations in rumen fluid. After incubation, the insoluble residues of organic matter were determined after boiling for 0.5 h in a neutral detergents (Goering and Van Soest, 1970). Each sample was incubated twice.

Results and discussion

On average, differences between the two locations in gas production were not important and only significant after 24 h. Within each of the three groups of material (clones, inbred families and populations) considerable variation was found in the amount of gas produced during *in vitro* rumen fluid incubations (Table 1). For gas production, the relative range of the objects, i.e. the range as a percentage of the mean, decreased with time. Variation in gas production among the different clones was of about the same range as among the populations. Among the inbred families from Splendor, differences in gas production between families with the same common parent were smaller than differences between families with different S3-parents. This indicates that even within cultivars genetic variation exists for gas production characteristics. Although differences in gas production between the perennial ryegrass objects were large and very significant, also fairly large genotype by locations interaction was found, but this interaction was always less important than the main genotypic effects.

Organic matter degradability ranged from 72.7 % (clone 15) to 82.9 % (clone 6), averaged over the two locations (Table 2). In contrast to gas production, organic matter degradability at the Dutch location was on average 11 % higher than at the Belgian location. Also for organic matter degradability the genotype*locations interaction was significant. Cell wall content ranged from 52.7 % for Condesa to 61.0 % for Lp491-1-76, averaged over the two locations. Cell wall content was higher in Belgium than in the Netherlands. Because of the high cell wall degradability, effects of cell wall content on organic matter degradability was small. Based on genotypic means, variance in organic matter degradability could be explained for 98 % by differences in cell wall degradability. Based on genotypic means, a correlation analysis on gas production data, organic matter degradability, cell wall content and crude protein was carried out. Cell wall degradability was not included in this analysis because it was so highly correlated with organic matter degradability. Gas production during the first four hours was highly positively related to the soluble, non-protein content of the grass. The soluble, non protein content (SNP) was calculated as $100 - \text{cell wall content} - \text{crude protein content} - \text{ash content}$ (for this calculation cell wall content was expressed per dry matter). This fraction mainly consists of water soluble carbohydrates. Gas production during the first four hours (V_4 , ml per g organic matter) could be predicted by : $V_4 = 31 + 1.7 \cdot \text{SNP}$, $\text{se} = 4.7$, $R^2_{\text{adj}} = 50.8\%$; se = standard error after fitting. Still considerable variation in V_4 existed that could not be related to any of measured quality characteristics. SNP was not related to the gas production after the

first four hours of fermentation which is logical since the soluble carbohydrates are fermented within the first hours. Gas production during the first 24 hours (V24) was highly positively correlated with organic matter degradability (OMD), but only when differences in crude protein content (CP) were taken into account. The regression formula for V24 was : $V24 = -34.2 + 3.7 \cdot OMD - 3.9 \cdot CP$, $se = 4.8$, $R^2_{adj} = 75.0\%$. A high crude protein content negatively affected V24. This was not only found for the genotypic correlation, but also for the phenotypic correlation. The difference in crude protein content between the locations explained why despite a large difference in organic matter degradability, the locations did not differ very much in gas production. Cell wall content did not correlate with V24.

Conclusions

1. Genetic variation for gas production rate during rumen fermentation has been demonstrated in perennial ryegrass.
2. About 60 % of genetic variation in gas production during the first four hours of rumen fermentation could be accounted for by the content of the soluble, non-protein fraction. The fact that not all variation in gas production could be accounted for by the content of cell wall or crude protein shows that the rate of fermentation during the first hours of incubation as measured with the gas production method cannot be predicted from the amount of soluble carbohydrates only.
3. About 75 % of the genetic variation in gas production during the first 24 h of rumen fermentation could be accounted for by organic matter degradability and crude protein content. Also here, the fact that not all variation in gas production could be accounted for by these two characteristics, shows that some variation in the rate of carbohydrate fermentation cannot be detected when only the solubilisation is studied.
4. Gas production after the first four hours of fermentation was not related to cell wall content, but only to degradability of cell walls or organic matter and to crude protein.

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Table 1 : Time related gas production during *in vitro* rumen fermentation (in ml gas produced per g organic matter). Inbred families denoted with the same superscript have a common S3-parent. Locations : B = Belgium ; N = Netherlands. Significance : * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Within each group of material the objects have been sorted according to their average organic matter degradability. Range % is the range of all genetic objects as a percentage of the mean.

Objects (genotypes, inbred families and populations)		Time							
		4 h		8 h		24 h		48 h	
		B	N	B	N	B	N	B	N
Clone 20		69	75	109	126	182	202	233	232
Clone 7		66	76	105	132	187	207	239	241
Clone 16		76	52	117	97	193	173	242	210
Clone 15		70	78	108	132	188	218	237	255
Clone 12		62	47	102	103	179	184	223	214
Clone 11		80	101	122	160	195	235	241	259
Clone 14		72	73	111	123	188	205	243	240
Clone 1		68	56	113	109	194	191	240	232
Clone 6		65	73	114	131	204	218	250	254
Clone 19		73	68	120	124	203	198	248	230
Inbred family 11 ^b		76	76	105	132	187	207	239	241
Inbred family 7 ^b		70	74	110	127	187	203	235	233
Inbred family 18		75	61	117	114	192	183	239	212
Inbred family 3 ^a		77	78	118	130	195	209	247	244
Inbred family 1 ^a		77	90	124	146	206	226	253	261
Population Lp9984.81		70	74	110	127	187	203	235	233
Population Lp491-1-76		65	57	106	106	186	191	239	230
Population Lp Condesa		88	89	138	139	214	212	259	248
Population Lp Parcour		74	73	122	127	201	207	246	243
Population Lp 979381		73	58	119	110	198	196	242	235
Average		72	70	115	123	193	202	241	237
Range %		37	77	31	51	19	31	15	22
Significance	Location	ns		ns		*		ns	
	Objects	***		***		***		***	
	Location*Objects	**		**		**		**	
LSD Objects ($p = 0.05$)		4.4		5.7		8.8		9.8	

Table 2 : Organic matter degradability (OMD, % of organic matter), cell wall content (NDF, % of organic matter), cell wall degradability (CWD, %) and crude protein content (CP, % of dry matter). Within each group of material the objects have been sorted according to their average organic matter degradability. - = not assessed. Further details : see Table 1.

Objects (genotypes, inbred families and populations)	OMD		NDF		CWD		CP	
	B	N	B	N	B	N	B	N
Clone 20	75.2	91.3	57.6	47.3	56.9	81.6	16.7	27.6
Clone 7	76.3	92.3	58.8	43.6	59.7	82.3	15.5	25.7
Clone 16	78.5	90.7	55.7	52.5	61.4	82.2	16.2	29.8
Clone 15	78.7	92.4	56.9	47.2	62.5	83.8	15.6	24.7
Clone 12	79.4	92.4	55.3	46.7	62.7	83.8	20.5	29.8
Clone 11	79.3	93.1	53.2	39.0	61.1	82.2	17.9	-
Clone 14	79.7	93.0	56.5	50.8	64.0	86.2	16.3	-
Clone 1	81.2	92.3	57.1	53.7	67.0	85.6	18.3	26.1
Clone 6	83.2	92.5	57.8	53.6	71.0	85.9	17.2	24.2
Clone 19	84.2	92.5	56.0	48.2	71.8	84.5	19.2	27.8
Inbred family 11 ^b	80.0	90.1	56.6	54.9	64.7	81.9	15.8	27.7
Inbred family 7 ^b	79.7	91.0	57.6	47.9	64.7	81.1	16.3	24.9
Inbred family 18	80.8	92.6	57.0	45.1	66.3	83.6	19.8	27.3
Inbred family 3 ^a	81.7	92.3	55.1	48.5	66.8	84.1	16.8	26.0
Inbred family 1 ^a	85.8	93.1	54.3	47.1	73.8	85.4	18.3	24.6
Population Lp9984.81	77.8	86.3	58.6	53.0	62.2	74.2	17.3	25.4
Population Lp491-1-76	75.7	92.3	61.0	56.7	60.1	86.5	18.1	29.0
Population Lp Condesa	83.5	87.8	52.7	53.8	68.7	77.2	16.7	-
Population Lp Parcour	80.6	91.0	57.1	50.7	66.1	82.2	17.7	26.1
Population Lp 979381	80.4	91.6	54.5	51.6	64.0	83.7	19.8	28.3
Average	80.1	91.5	56.5	49.6	64.8	82.9	17.5	26.8
RANGE %	13.2	7.4	14.6	35.8	26.0	14.8	28.5	21.1
Significance								
Location	***		***		***		***	
Objects	**		**		**		*	
Location*Objects	**		**				**	
LSD Objects (p=0.05)	2.1		4.1		3.7		2.5	

THE EVALUATION OF PHYSICAL STRUCTURE OF FRESH AND PRESERVED GRASS BY MEASURING CHEWING ACTIVITY AND RELATED PARAMETERS¹

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Summary

The physical structure value of 26 grass silages, 1 hay and 2 batches of fresh grass was evaluated by measuring chewing activity with 8 lactating cows. For that purpose, the eating (EI), ruminating (RI) and total chewing index (CI) were determined, representing the duration of eating, ruminating and total chewing per kg dry matter intake. Grass silages differed in growth stage at harvest, chopping length and preservation method. Mean \pm standard deviation of eating, ruminating and total chewing time amounted to 25.3 ± 4.1 , 47.1 ± 6.8 and 72.3 ± 9.7 min/kg DM respectively. The variation in chewing indexes was primarily caused by differences in growth stage. Preservation method mainly influenced EI but had almost no influence on RI. Chopping particularly decreased RI. Concerning the chewing activity with fresh grass, there was a tendency towards a longer eating and a shorter ruminating time. To predict the chewing indexes with more simple tools, regression equations were derived with chemical, physical and biological feed characteristics. Crude fibre content was the most important determinant. Density, particle size, pH and milling resistance contributed to explain the variation of the chewing indexes.

Introduction

With regard to ration optimization of dairy cattle, the supply of sufficient physical structure has to be subscribed. This is based on the fact that structural material stimulates chewing activity and hence saliva production (Santini *et al.*, 1983). Saliva buffers rumen fluid, thereby maintaining optimal conditions for cellulolytic activity. The structural material itself is degraded gradually, so that very low pH values and high peaks of fermentation products are avoided. Another beneficial effect is the formation of a floating fibre layer in the rumen, stimulating ruminal contractions. A lack of physical structure is often associated with abnormal rumen physiology and other disorders such as depressed feed intake, reduced digestion, lowered milk fat content, acidosis, parakeratosis,...

Although the importance of the fibrousness of diets for ruminants is recognized for a long time, it is only recently that a method of measuring quantitatively the property of fibrousness of feedstuffs is proposed. Many systems suggested a minimum amount of a chemical determined fibre component, such as crude fibre, neutral detergent fibre or acid detergent fibre (NRC, 1978, Mertens, 1985, Woodford *et al.*, 1986, Sutton *et al.*, 1989, Weiss *et al.*, 1989). These recommendations, however, do not consider source or physical form of the fibre. Balch (1971)

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proposed chewing time as a measure for the physical properties of a diet. Sudweeks *et al.* (1981) extended this concept and developed a roughage value index system.

Until now, in Belgium, structural values of feedstuffs are empirically derived. Since 8 years, research is carried out at our institute to develop a roughage evaluation system for dairy cattle. The roughage values of forages and wet by-products are derived by measuring the chewing activity of lactating cows. As measurement of the chewing indexes involves laborious work, the need exists for simple laboratory methods to assess the physical properties of feedstuffs. Chemical, physical and biological parameters are determined to derive best prediction equations for the chewing indexes.

In this study, the chewing indexes of preserved grass, hay and fresh grass will be discussed, as well as the factors influencing these structural indexes. Attention will also be paid to the relation between the chewing indexes and the predictive parameters.

Material and methods

Feedstuffs

Physical structure was determined of 26 grass silages, 1 batch of hay and 2 batches of fresh grass. All grass crops originated from permanent pasture of a 50:50 mixture of diploid and tetraploid perennial ryegrass. Grass silages differed in growth stage at harvest, from early vegetative to full ears. 19 Grass silages were harvested with a precision chopper at a theoretical chopping length of 24 mm (coarse grass). Long material was harvested with a self-loading wagon, provided with 16 knives (6 batches). 1 Parcel was partially cut at a theoretical length of 3.5 mm (short grass).

To investigate the influence of the preservation method, grass was either directly ensiled, or after wilting. When directly ensiled, either no preservative or formic acid or an additive, containing lactic acid bacteria and cellulase, was added.

When grass had to be fed freshly, it was mown and harvested with a small pick-up truck. Batches of fresh grass and hay both consisted of long material.

Chewing activity

Chewing activity measurements were carried out with 8 multiparous moderately producing Holstein cows. Cows were equipped with a leather head-halter. At the height of the lower jaw, a silicone tube was attached, that compressed by chewing. The change in air pressure in the tube was transduced in an electrical signal that was registered on a recorder. After a preliminary period of 10 to 17 days for adaptation to the feed, an experimental period of 4 days followed, during which chewing activity was continuously measured.

The roughages were given *ad libitum*. To assure a normal rumen function, the roughage was supplemented with 3 kg of a protein-balanced concentrate. Roughages and concentrates were offered in 2 about equal meals. At the end of the experimental period, feed removals were recorded.

Mean eating and ruminating time and mean chewing time, as sum of both, were expressed per kg DM intake and were called respectively the eating (EI), ruminating (RI) and total chewing index (CI). These indexes were adjusted for differences in metabolic weight ($BW^{0.75}$) and standardized for a cow of 600 kg.

Laboratory analysis

For the prediction of the chewing indexes, chemical, physical and biological characteristics of the feedstuffs were determined.

Dry matter was determined by drying a sample in a ventilated oven at 60-70°C, followed by oven-drying at 103-105°C during 3h. For the grass silages, DM content was corrected for losses of volatile substances, according to the equation of Dulphy and Demarquilly (1981). Crude protein (CP) was determined with an automatic Kjelfoss-apparatus. The extraction of crude fat (EE) was carried out in a soxlet-apparatus with petroleum ether during 6h. Ash (AS) content was determined after calcination in a furnace at 600°C. Crude fibre (CF) was analysed by Fibertec. Neutral detergent fibre (NDF) was determined according to Van Soest and Wine (1967). For acid detergent fibre (ADF) and lignin, the methods of Van Soest (1963) were applied. NDF and ADF were expressed on ash-free basis. Cellulose was calculated as the difference between ADF and lignin and hemicellulose as the difference between NDF and ADF. Sugars were determined according to the method of Luff-Schoorl (Anonymous, 1971).

Particle size (PS) of the grass samples was determined in an upward air flow at the Institute of Engineering Research at Bedford (UK). Particle size is expressed as the median length in millimeters. Density (De) was determined in a cylindrical recipient which contained about 10 kg fresh feed and was covered with a weight of 95 kg. After 30 minutes under pressure, density was measured and expressed in kg/liter. Milling resistance (MR) was determined as the energy needed to grind 200 g dry material through a Brabender mill, which was connected to a precision impulse counter. MR was expressed in watt-hours per g.

The in vitro digestibility of the organic matter (VDOM) was determined according to the method of Tilley and Terry (1963). The cellulase digestibility of the organic matter (CDOM) was determined following the procedure developed by De Boever *et al.* (1986).

On the grass silage samples, fermentation characteristics were determined. Therefore, 100 g fresh silage was diluted with water up to 1 l. After 1 night soaking, pH, volatile fatty acids (C₂-C₄), alcohols, lactic acid and NH₃ were determined on the filtrate.

Results and discussion

Mean and range in chemical composition of the grass silages, grass hay and fresh grass are presented in table 1. Mean DM content of the grass silages amounted to 333 g/kg and ranged from 158 to 568 g/kg. The difference in growth stages at harvest was reflected in the large range of the CF content, varying from 214 to 304 g/kg DM. For the hay, CF content amounted to 298 g/kg DM. The fresh grass batches showed a mean CF content of 213 g/kg DM.

Table 2 represents the mean chewing indexes of the preserved and fresh grass batches. The standard deviation as a measure of the variation between the batches is mentioned too. For grass silages, mean eating, ruminating and total chewing times of respectively 25.3, 47.1 and 72.3 min/kg DM were observed. EI, RI and CI of hay amounted to respectively 39.6, 52.8 and 92.3 min/kg DM. On fresh grass, cows ate 33.1 min/kg DM and ruminated 33.8 min/kg DM.

Variation between different grass silages amounted to 4.1, 6.8 and 9.7 min/kg DM, respectively for EI, RI and CI.

Differences in chewing indexes of preserved grass were mainly caused by differences in growth stage. The relation between the CF content (g/kg DM), as indicator of the growth stage, and the chewing indexes is represented in the following equations :

	$s_{y,x}$	R^2
EI = $-3.0 + 0.10$ CF	2.1	0.68
RI = $-11.4 + 0.23$ CF	2.2	0.90
CI = $-14.7 + 0.33$ CF	3.6	0.88

$s_{y,x}$: residual standard deviation (min/kg DM)

To avoid influence of other factors, these equations were made up for 11 wilted grass silages, with a theoretical chopping length of 24 mm. The relation with RI and CI indicates that physical structure of grass silages is clearly affected by growth stage. During ageing, grass composition shifts towards more cell-walls and less cell-contents. Moreover, lignification degree of cell-walls increases, which asks more chewing for comminution.

The effect of preservation method is presented in table 3. The influence of direct ensiling versus wilting was compared in 4 experiments. The addition of a preservative was investigated in 3 experiments. Wilting reduced the chewing indexes of grass silages, especially the eating index. The lower palatability and the higher water content of the direct ensiled silages could be responsible for the higher chewing indexes. Addition of a preservative decreased the chewing indexes only marginally.

Table 4 represents the effect of chopping length. Chopping the grass at a theoretical length of 24 mm decreased EI slightly. RI was significantly reduced by chopping. When fine and coarse chopped grass was compared, no effect of finer chopping was observed. In contradiction to these experiments, most studies mention an increase in the eating rate with a smaller particle length (Castle *et al.*, 1979, Deswysen, 1980, Castle *et al.*, 1981, Dulphy *et al.*, 1984). The probability of particles to be ruminated is determined by size and density. After a passage through the rumen, feed particles return to the reticulum, from where dense particles pass into the omasum, while larger and lighter particles are ruminated.

To dispose of more simple tools to evaluate the structural value of grassland products, relations are sought between the chewing indexes and chemical, physical and biological parameters.

In table 5 only the significant relationships between the chewing indexes and the single parameters are presented. None of the parameters explained much of the variation of the eating indexes. This can be ascribed to the voluntary controll of the eating behaviour. With ad libitum access to feed, the beginning and ending of a meal is dictated by the will of the animals and is consequently rather variable. Eating rate depends more on animal linked factors than on the determined chemical, physical or biological feed characteristics. Variation in ruminating activity is for 83 % explained by CF content, which indicates the great effect of growth stage on RI. The other measures of cell-wall content have much less influence on RI, except NDF, which accounts for 70 % of the variability. *In vitro* and cellulase digestibility are negatively correlated with RI. This can be ascribed to the compensatory effect of the rumen function and chewing activity. Grass silages of good quality are digested to a greater extent in the rumen and hence need less comminution by rumination. As total chewing time consists for $\pm 2/3$ of ruminating activity, the variation in CI is mainly explained by the same parameters as RI.

For the prediction of the physical structure of grass silages, multiple regression analysis was carried out. Best prediction equations with 1, 2 or 3 parameters and their accuracy are presented in table 6. RI could be predicted most accurately. For all equations, CF content is

the most explaining parameter. Density and particle size help to explain variation of EI. For RI, pH and milling resistance had some significant contribution. To predict CI, density in addition to CF gives some more explanation of its variability.

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Table 1 : Mean and range in chemical composition of the grass silages, grass hay and fresh grass

	Grass silages (n = 26)		Grass hay (n = 1)	Fresh grass (n = 2)
	mean	range		mean
DM (g/kg)	333	158-568	837	193
CF (g/kg DM)	258	214-304	298	213
CP (g/kg DM)	156	119-199	142	193
AS (g/kg DM)	120	95-161	100	99
NDF (g/kg DM)	468	395-548	546	413
ADF (g/kg DM)	300	250-352	351	245
Lignin (g/kg DM)	39	26-52	51	23
Sugars (g/kg DM)	45	6-125	n.d. ¹	143

1 : n.d. = not determined

Table 2 : Mean chewing indexes of the preserved and fresh grass with variation between batches

	Grass silages (n = 26)	Grass hay (n = 1)	Fresh grass (n = 2)
EI (min/kg DM)			
mean	25.3		
s _x	4.1	39.6	33.1
RI (min/kg DM)			
mean	47.1		1.0
s _x	6.8	52.8	33.8
CI (min/kg DM)			
mean	72.3		3.9
s _x	9.7	92.3	66.9

s_x : standard deviation between grass batches (min/kg DM)

Table 3 : Influence of preservation method on chewing indexes of grass silages

		Direct ensiled	Direct ensiled + preservative	Wilted
n¹ EI (min/kg DM) \pm s_x² RI (min/kg DM) \pm s_x CI (min/kg DM) \pm s_x	4	30.9 \pm 3.1 49.5 \pm 9.8 80.4 \pm 12.4		23.7* \pm 2.9 46.3 \pm 7.6 69.9* \pm 10.2
n¹ EI (min/kg DM) \pm s_x RI (min/kg DM) \pm s_x CI (min/kg DM) \pm s_x	3	29.6 ^a \pm 1.9 45.3 ^a \pm 6.5 74.8 ^a \pm 7.0	27.4 ^a \pm 2.7 43.6 ^a \pm 3.8 71.0 ^a \pm 4.4	22.9 ^a \pm 3.0 43.9 ^a \pm 7.3 66.7 ^a \pm 9.8

1 : n = number of experiments

2 : s_x = standard deviation between grass silages (min/kg DM)

a = values on the same row with the same superscript are not significantly different (P<0.05)

Table 4 : Influence of chopping length on chewing indexes of grass silages

		3.5 mm	24 mm	Long
n¹ EI (min/kg DM) \pm s_x² RI (min/kg DM) \pm s_x CI (min/kg DM) \pm s_x	5		22.9 \pm 3.0 45.4 \pm 4.0 68.3 \pm 6.9	24.7 \pm 2.6 49.6** \pm 5.2 74.3* \pm 7.2
n¹ EI (min/kg DM) RI (min/kg DM) CI (min/kg DM)	1	21.9 ^a 39.9 ^a 61.8 ^a	21.0 ^a 41.5 ^{ab} 62.4 ^a	21.7 ^a 43.4 ^b 65.0 ^a

1 : n = number of experiments

2 : s_x = standard deviation between grass silages (min/kg DM)

* = significantly different (P<0.05) ; ** = significantly different (P<0.01)

a, b = values on the same row with a different superscript are significantly different (P<0.05)

Table 5 : Linear regression equations of grass silages

	EI (min/kg DM)				RI (min/kg DM)				CI (min/kg DM)			
	a ¹	b ¹	s _{y.x} ²	R ²	a	b	s _{y.x}	R ²	a	b	s _{y.x}	R ²
Chemical parameters												
DM (%)	31.9	-0.20	3.5	0.28		ns			-13.8	ns		
Cr (%/DM)	1.7	0.91	3.4	0.33	-15.4	2.42	2.9	0.83				
CP (°)	41.3	-1.03	3.3	0.36	74.4	-1.75	5.5	0.37	115.8	-2.78	7.4	0.45
NDF (°)		ns ²			-13.3	1.29	3.8	0.70	-4.6	1.65	6.7	0.55
ADF (°)	6.9	0.61	3.8	0.16	-2.8	1.67	5.2	0.43	4.2	2.28	7.9	0.38
ADL (°)		ns			32.8	3.69	6.4	0.16		ns		
cellulose (°)	-1.2	1.02	3.4	0.31	-9.7	2.20	4.8	0.51	-10.9	3.22	6.9	0.53
hemicell. (°)		ns			23.2	1.42	5.3	0.41	43.8	1.70	8.5	0.28
sugars (°)	27.3	-0.44	3.8	0.15		ns				ns		
pH		ns			24.3	5.15	6.3	0.17		ns		
NH ₃ (NH ₃ -N/N)	21.6	0.30	3.6	0.27	41.0	0.51	5.9	0.26	62.6	0.81	8.2	0.33
ac.ac. ³ (g/kg)	21.9	0.24	3.5	0.29		ns				ns		
pro.ac. ³ (g/kg)	24.4	1.43	3.5	0.29		ns			70.5	3.09	8.8	0.23
but.ac. ³ (g/kg)	24.2	0.20	3.7	0.20	45.5	0.30	6.3	0.16	69.7	0.50	8.8	0.22
Physical parameters												
De ⁴ (kg/l)	21.2	7.13	3.8	0.16		ns				ns		
PS ⁴ (mm)		ns			43.9	0.07	6.3	0.15		ns		
MR ⁴ (wh/g)	21.6	1.45	3.7	0.25	39.7	2.93	5.6	0.36	61.3	4.38	7.9	0.39
Biological parameters												
VOMD ⁵ (%)	59.6	-0.46	3.7	0.19	159.7	-1.49	3.4	0.75	219.4	-1.95	6.3	0.61
COMD ⁵ (%)	44.4	-0.25	3.8	0.16	111.8	-0.83	4.0	0.66	156.4	-1.08	6.9	0.53

1 : a = intercept of regression ; b = slope of regression ; 2 : s_{y.x} = residual standard deviation (min/kg DM) ; ns = not significant (P < 0.05)
3 : ac.ac = acetic acid ; pro.ac. = propionic acid ; but.ac = butyric acid ; 4 : De : density ; PS : particle size ; MR : milling resistance
5 : VOMD = in vitro organic matter digestibility ; COMD = cellulase organic matter digestibility

Table 6 : Multiple regression equations and accuracy to predict chewing indexes of grass silages

	$s_{y,x}$	R^2
Eating index (min/kg DM)		
$-16.57^{**} + 1.33 \text{ CF}^{***} + 12.81 \text{ De}^{***}$	2.1	0.74
$-25.32^{***} + 0.82 \text{ NDF}^{***} + 18.68 \text{ De}^{***} + 0.03 \text{ PS}^*$	2.0	0.78
$-16.31^{**} + 1.23 \text{ CF}^{***} + 14.59 \text{ De}^{***} + 0.03 \text{ PS}^*$	1.8	0.80
Ruminating index (min/kg DM)		
$-15.43^* + 2.42 \text{ CF}^{***}$	2.9	0.83
$-21.56^{***} + 2.30 \text{ CF}^{***} + 2.13 \text{ pH}^*$	2.7	0.84
$-19.02^{***} + 2.09 \text{ CF}^{***} + 0.86 \text{ MR}^* + 2.27 \text{ pH}^*$	2.4	0.88
Total chewing index (min/kg DM)		
$-13.83 + 3.34 \text{ CF}^{***}$	4.9	0.76
$-32.16^{***} + 3.76 \text{ CF}^{***} + 12.82 \text{ De}^{***}$	4.2	0.82

$s_{y,x}$: residual standard deviation (min/kg DM)

NEAR-INFRARED REFLECTANCE SPECTROSCOPY- LOCAL CALIBRATIONS

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Introduction

Near-infrared reflectance spectroscopy (NIR) is a rapid and accurate method for determining the quality of forages. The accuracy depends on the quality of the calibration used. In forage quality analyses it is common to have large calibration sets where all samples are used in a "universal" calibration. These calibration sets contain large variation in species, harvest year, cuts, locations etc. The purpose of these studies was to look at different approaches to use all this information in order to improve prediction of forage quality by NIR.

Forages are a heterogeneous group, representing different species, growing conditions, and harvest times. This consideration results in very large differences between different samples for forage quality and between different reflectance spectra. One would intuitively expect to be able to improve the calibration to estimate a given sample by only using samples in the calibration that are similar to the unknown. On the other hand, if one is using too few samples in the calibration, inaccurate results may occur due to low repeatability of the reference method (Aastveit & Marum, 1993).

Principles of local calibration

The basic principles in local calibrations are 1) the n samples in the calibration file with the most similar reflectance spectra to the sample to be predicted are selected. 2) A new calibration equation is developed on the selected samples. 3) and finally the predicted value is obtained by applying the local calibration equation on the predicted sample. This procedure is repeated for each new sample to be predicted.

First study

In a first study we looked at four different methods to utilise a calibration set of about 400 samples. A) a universal calibration, B) a separate calibration for each species, and C) and D) are two different methods of local calibrations. In method C the original principal components were used in the calibration. In method D, new principal components were calculated on the selected samples. This method is more time consuming than method C. In both method C and D, the 30, 50, and 100 closest samples based on the Mahalanobis distance from the sample to predicted were selected. The calibration equations were developed by principal component regression (PCR). We tested systematically 4 to 12 principal components for the different traits. The program to do the calibrations were written by the means of the SAS program.

Multiplicative Scatter Correction (MSC) was used to eliminate or reduce the difference in light scatter between samples before calibration. Calibration equations were made both with and without scatter correction. Standard error of prediction (SEP) was calculated for the different calibration strategies.

We found positive effects from scatter correction for most of the quality traits. Relatively large gains in SEP were found when the calibration sets were used in one of the alternative methods, relative to "universal calibration". On average, the local regression calibration methods were superior to the others.

The effect of scatter correction was only slight when local calibration methods with at least 50 samples in the local calibration file were used. The effect was positive for some traits and negative for others. There were only small differences between calibration strategy C and D on non-scatter-corrected spectra. On scatter-corrected spectra, method D was substantially better than method C. Scatter correction had negative effect with strategy C. Overall, method D was a better strategy than method C. We got the best results with 50 to 100 selected samples for the local calibration. The gain in SEP using method D with 100 samples in the local calibration, were for protein 9 %, NDF 22 %, ADF 21 %, crude fibre 22 %, and IVDMD 10 %.

Second study

In a second study we used two calibration files with 558 samples each. One with diverse forage grass (G), the other containing both forage grass and legumes, and grass- legume mixtures (GL). The calibrations were done with the ISI software v 1.17 using the "Teach automatic sequence" function and BAT files in DOS.

We made a local calibration for all samples in this calibration files, using from 25 to 557 samples. The samples were not scatter corrected. We used the Modified Partial Least Squares regression method (MPLS) for calibration with 10 factors and first derivative math treatments.

The number of samples in a local calibration with the lowest SEP varied in G from 100 for crude fibre to 350 for protein. In GL the number of samples varied from 75 for ADL to 200 for NDF. Lowest SEP in G were for protein 0.75, NDF 1.79, ADF 0.94, ADL 0.37, and for crude fibre 0.80. In GL the SEP were for protein 0.94, NDF 2.09, ADF 1.18, ADL 0.55, and for crude fibre 1.00. The gains in precision in G were for protein 4 %, NDF 5 %, ADF 6 %, ADL 7 %, and for crude fibre 11 %. In the more diverse GL file the gains were 6 %, NDF 13 %, ADF 12 %, ADL 11 % and for crude fibre 13 %. The SEP were higher in the more diverse grass-legume (GL) file than in the pure grass (G) file. The optimum number of samples in the local calibration were generally fewer in the more diverse GL file, and the gain in precision were larger than in G.

Conclusion

In these studies we had from 400 to 558 samples in our calibration files. This number is probably far too few to provide spectra representative of the whole body of chemical and physical forage samples properties. We would therefore expect that with a much larger calibration file, one would improve the advantage of local calibrations. With the speed of

today's computers the local calibration method is an interesting and useful method for practical use.

References

- Aastveit, A.H. and P. Marum. 1993. Near-Infrared Reflectance Spectroscopy : Different strategies for Local Calibrations in Analysis of Forage Quality. *Applied Spectroscopy* 47 : 463-469.

ANALYSING DIGESTIBILITY IN RYEGRASSES

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Introduction

Measuring digestibility by wet chemistry methods is labour intensive. New methods as the NIRS analysis are much faster but preparing the sample continues to take a lot of time. Analysing each forage cut gives the best idea of the digestibility of the crop. Predicting the digestibility on an annual basis based on the results of a single cut or the combination of cuts saves a lot of labour or offers the opportunity to analyse more genotypes with the same efforts.

Material and methods

We have studied over 400 different varieties, synthetics or families during 3 growing seasons. These entries were tested in 14 different yield trials properly designed with 3 to 4 replicates. The trials were mown 4 to mostly 5 times a year, the first cut being taken in the second half of April and further cuts at a Dry Matter Yield (DMY) between 2.5 and 4 tons/ha according to the weather and the crop maturity. In each trial all the cuts were analysed in at least one production year. In perennial ryegrass the sowing year was never considered but it was in Italian ryegrass in the case of a spring sowing.

A sample of 250-300 g fresh weight was taken per plot. Samples were dried at 75°C. The dried samples of the replicates were mixed and ground over a Wiley Brabender Mill with a sieve mesh of 1 mm. The digestibility coefficient was calculated according to Jones and Hayward (1975). One can look in different ways at the digestibility. Either one looks at the analytical digestibility coefficient (DC) (*a*) or at the Digestible Organic Matter Yield (DOMY) (*b*). In case (*a*) the **DC on an annual basis (DCA)** is the arithmetic mean of the DC's of each single cut, in case (*b*) the DCA' is a weighted figure and equals

$$\sum_{\text{cuts}} (\text{DC} \cdot \text{OMY}) / \sum_{\text{cuts}} \text{OMY}$$

Results

Tables 1 and 2 give the correlation coefficients between a single cut or a combination of cuts and the DCA. Table 3 shows the correlation between the DCA (arithmetic mean) and the annual DMY.

Conclusions

What cuts should be analysed ?

1. Perennial ryegrass

The method of calculating the digestibility coefficient on an annual basis, DCA or DCA' affects the correlations.

In general this is a valid advice.

If one can analyse only a single cut one should consider the **last** one and never the second. A combination of two cuts improves the result : the combinations **3+4** and **4+5** being the best if one mows 5 times a year or **1+4** or **3+4** if four cuts are taken. So analysing the last two cuts is never wrong.

Should one analyse 3 cuts out of 5, the last two always should be involved.

2. Italian ryegrass

DCA and DCA' were very similar. If one can analyse only a single cut one should select the **fourth**. Any combination with cuts **2+4** is a better choice.

The correlation : DCA-DMY

We found in nearly all trials a negative correlation.

Reference

Jones, D.I.H. and Hayward, M.V., 1975. The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. J. Sci. Fd Agric., 26 : 711-718.

Table 1 : Average correlation coefficient between the digestibility coefficient of single cuts or combinations of cuts and the digestibility on an annual basis
 Perennial ryegrass ; 10 designs ; 329 genotypes
 5 cuts/year; 8 designs.years; 264 genotypes
 4 cuts/year; 3 designs.years; 65 genotypes

Cuts	A.m.	R.	Range	W.m.	R.	Range	A.m.	R.	Range	W.m.	R.	Range
1	0.63		0.57-0.81	0.62		0.49-0.78	0.65		0.49-0.88	0.59		0.32-0.92
2	0.36		-0.09-0.63	0.42		0.09-0.72	0.34		0.18-0.57	0.38		0.11-0.71
3	0.65		0.37-0.81	0.56		0.02-0.69	0.60		0.54-0.65	0.51		0.37-0.64
4	0.69		0.52-0.84	0.74		0.50-0.91	0.65		0.57-0.70	0.70		0.67-0.72
5	0.76		0.66-0.91	0.64		0.33-0.78						
1+2	0.67	10	0.40-0.80	0.70	10	0.43-0.84	0.77	4	0.71-0.89	0.81	3	0.74-0.93
1+3	0.77	7	0.59-0.89	0.74	7	0.40-0.84	0.70	5	0.59-0.85	0.64	6	0.48-0.89
1+4	0.80	6	0.74-0.88	0.85	1	0.73-0.93	0.90	1	0.85-0.93	0.91	1	0.84-0.96
1+5	0.86	2	0.75-0.94	0.76	5	0.62-0.87						
2+3	0.73	8	0.57-0.83	0.71	9	0.47-0.85	0.82	2	0.79-0.84	0.78	4	0.70-0.82
2+4	0.71	9	0.48-0.87	0.82	3	0.66-0.93	0.56	6	0.51-0.66	0.66	5	0.51-0.83
2+5	0.81	5	0.63-0.93	0.75	6	0.49-0.90						
3+4	0.85	3	0.76-0.92	0.82	3	0.71-0.93	0.82	2	0.78-0.86	0.82	2	0.79-0.87
3+5	0.85	3	0.74-0.94	0.73	8	0.41-0.87						
4+5	0.88	1	0.77-0.96	0.85	1	0.72-0.94						
1+2+3	0.83	9	0.71-0.89	0.83	8	0.67-0.91	0.89	3	0.78-0.97	0.89	2	0.80-0.97
1+2+4	0.83	9	0.76-0.92	0.90	4	0.81-0.97	0.90	1	0.84-0.92	0.93	1	0.87-0.96
1+2+5	0.90	5	0.86-0.97	0.84	7	0.75-0.91						
1+3+4	0.89	6	0.85-0.96	0.92	1	0.85-0.98	0.90	1	0.83-0.94	0.89	2	0.79-0.95
1+3+5	0.91	3	0.83-0.96	0.82	9	0.53-0.97						
1+4+5	0.93	2	0.89-0.98	0.92	1	0.85-0.98						
2+3+4	0.88	8	0.83-0.93	0.89	6	0.81-0.95	0.89	3	0.85-0.91	0.89	2	0.86-0.91
2+3+5	0.89	6	0.84-0.96	0.82	9	0.62-0.91						
2+4+5	0.91	3	0.75-0.97	0.91	3	0.74-0.98						
3+4+5	0.94	1	0.87-0.98	0.90	4	0.79-0.95						

A.m. : Arithmetic mean ; W.m. : Weighted mean ; R. : Rank

Table 2 : Average correlation coefficient between the digestibility coefficient of single cuts or combinations of cuts and the digestibility on an annual basis (arithmetic mean)

Italian ryegrass ; 3 designs ; 116 genotypes

5 cuts/year 2 designs.years 44 genotypes			4 cuts/year 2 designs.years 96 genotypes	
Cuts	A.m.	Rank	A.m.	Rank
1	0.57	2	0.55	4
2	0.51	4	0.68	3
3	0.53	3	0.79	2
4	0.65	1	0.80	1
5	0.65	1		
1+2	0.70	8	0.76	6
1+3	0.68	9	0.89	3
1+4	0.83	1	0.84	5
1+5	0.75	6		
2+3	0.65	10	0.91	2
2+4	0.83	1	0.92	1
2+5	0.78	4		
3+4	0.78	4	0.88	4
3+5	0.81	3		
4+5	0.73	7		
1+2+3	0.76	10	0.96	2
1+2+4	0.94	1	0.93	4
1+2+5	0.84	7		
1+3+4	0.90	2	0.95	3
1+3+5	0.86	5		
1+4+5	0.86	5		
2+3+4	0.88	4	0.97	1
2+3+5	0.83	8		
2+4+5	0.90	2		
3+4+5	0.83	8		

A.m. : Arithmetic mean

Table 3 : Correlation coefficient between the digestibility coefficient on an annual basis (arithmetic mean) and the dry matter yield

Trial	Harvest year	Number of cuts	Number of entries	Correlation coefficient
ah 90.6	1991	5	24	-0.50 **
ah 92.1	1993	5	38	-0.37 *
aw 90.3	1992	4	16	0.25
aw 90.36	1991	5	24	0.21
aw 90.36	1992	5	24	-0.05
aw 91.2	1992	5	47	-0.36 *
aw 91.2	1993	4	29	-0.28
aw 91.3	1993	4	20	-0.29
aw 92.2	1993	5	23	0.06
aw 92.3	1993	5	48	-0.20
aw 92.4	1993	5	31	-0.24
aw 92.5	1993	5	29	0.57 *
b 90.40	1991	4	24	-0.77 **
b 90.40	1992	5	24	-0.77 **
b 90.40	1993	4	24	-0.57 **
b 91.2	1993	5	20	0.19
b 92.1	1992	4	72	0.41 **

SESSION 3 : Amenity grasses

QUALITY IN TURFGRASS BREEDING - INFLUENCE OF RED THREAD (*Laetisaria fuciformis* (McAlp.))

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Summary

Resistance against diseases is an important component of quality in turfgrasses. In recent years more attention has been paid to red thread *Laetisaria fuciformis* (Mc Alp.) in the cultivar lists in France, Germany and Great Britain (STRI). Regression analysis of cultivars listed in France, Germany and United Kingdom reveals no or even negative selection progress for red thread diseases during the last 20 years. During a 3 years trial period in Lusignan (France) significant effects for cultivars were only be recorded in 30 % of the assessments and the critical range on a 1-9 scale between 2 means was as high as 1.0-2.1 for single scores and 0.8-1.0 for analysis over all years. Correlation analysis of trials at Verneuil (France) and Le Chatelier (France) showed no significant correlation coefficients between the two replicates in the trials. Significant correlations could be found between assessments at the same location within the same year but not between years and locations. On the basis of this investigation it is concluded that natural incidence of red thread is difficult to assess accurately. It is proposed that observations must be statistically analyzed and only then used to differentiate cultivars.

Introduction

Quality in turfgrass is primarily defined on the basis of the visual appearance of a cultivar and therefore concerns aesthetic aspects. The definition of quality in turfgrass breeding is thus completely different to that of forage plant breeding. In the case of forage quality the different constituents of a plant can be analysed by different analytical methods. They may give slightly different results from lab to lab, but since they are obtained according to a clear protocol they can be checked by anyone. In turfgrass, quality is described mainly by visual inspections. Furthermore the definition of quality in turfgrasses differs a little bit from country to country and different characters are examined. In different cultivars of turfgrasses the health of the plants plays an important role. Suceptibility for certain diseases in general means a worse rating for quality. Recently, red thread is of increasing interest in the examination of turfgrass cultivars. In France the majority of all turfgrass plots are infected with red thread. In some years we find this character also considered in the cultivar lists of Germany and Great Britain (STRI). Clearly the breeders have also worked more with this disease during the last few years. It occurs in many different species but is most obvious in perennial ryegrasses. Nevertheless a study of the national lists shows that there was no positive change in the resistance level for red thread (Feuerstein *et al.*, 1993).

The aim of this presentation is to initiate a discussion between breeders, scientists and turfgrass testing authorities about red thread, i.e. whether it can be recorded consistently, and whether it should remain as a feature in the cultivar lists.

Materials and methods

Disease

Red thread (also called as corticium disease) is caused by the fungus *Laetisaria fuciformis* (McAlp.), previously *Corticium fuciforme* (Berk.). The disease is extremely common, and is recorded in most western European countries. Whilst red thread may occur alone, it often occurs as a disease complex with pink patch (*Limonomyces roseipellis*) (Stalpers & Loerakker) (after Baldwin, 1990).

Analysis of the National Lists

The change of the resistance level was estimated on the basis of cultivars of perennial ryegrass (*Lolium perenne* L.) in the National Lists of France, Germany and the United Kingdom (Anonymous, 1993 ; Bundessortenamt, 1991 and STRI, 1993). For France and Germany the sample regression coefficient was calculated between the year of release and the disease score of all registered cultivars of perennial ryegrass. The result was transformed to the change of resistance in the last 20 years. As there is no year of release from the STRI in UK only cultivars which are also described in Germany and/or France were used. Here the first year of listing was used (after Feuerstein *et al.*, 1993).

Experiments from DP

Trials of ryegrass were sown in Lusignan France in April 1992. The trials were sown as randomised block trials with 2 or 3 replicates and 15 or 18 entries comprising 3 check cultivars in each trial (table 2). All together 10 trials were sown totalling 486 plots. A severe attack of red thread was observed in November 1992, in May 1993 and in August 1994. The plots were scored on a 1 to 9 scale where 9 = cultivar with no red thread. Data from 1992, 1993, 1994 and mean over 1992 - 1994 were analyzed with the GLM procedure in the SAS software package. Cultivar means were separated with Duncan's Multiple Range Test and residuals were checked by graphical means.

Experiments from Semunion Gazon

Trials of ryegrass were sown in Verneuil and Le Chatelier France in April 1991. The trials were sown with 2 replicates in Verneuil with 125 or 69 entries and in Le Chatelier with 75 or 141 entries comprising 3 check cultivars in each trial. All together 4 trials were sown totalling 410 plots. 165 of the entries stood at both locations. A moderate attack of red thread was observed in November 1992 and a severe attack was observed in autumn 1993. The plots were scored on a 1 to 9 scale where 9 = cultivar with no red thread.

From the data of 1992 and 1993 correlation coefficients were calculated between the replicates and between locations. For 1993 the correlation coefficients between the different scores were also calculated.

Results

Analysis of the National Lists

For an estimation of the success of selection procedures for red thread resistance during last 20 years the sample coefficient of regression between the scores and the years of release was calculated based on the cultivar lists from France, Germany and the United Kingdom. The results are shown in table 1. It is obvious that in France there was no selection progress for red thread resistance over the 59 cultivars examined. The slight increase of 0.2 of a score point was not significant.

In Germany 71 cultivars were evaluated. For red thread there had been a significant decrease in resistance of 0.6 in the last 20 years. Finally in the United Kingdom the older cultivars show the same susceptibility of red thread as the more recent ones.

Results from DP

Examination of the residuals showed that cultivar means were without any data transformation in most of the trials normally distributed.

The results of the analysis of variance on untransformed data are given in table 2 and the critical range between 2 means in Duncan's test is given in table 3.

Analysis of variance was performed on pooled data for the control cultivars since block effects generally were insignificant. The F-test calculated on the average of 2 years data revealed significance at 0.1 % level for cultivars and the Duncan separation of 2 means was calculated as 0.44. The control cultivars could be separated as follows :

Means with the same letter are not significantly different.

Duncan Grouping	Mean	Number of plots	Cultivar

A	7.64	12	"DANILO"
B	6.90	16	"BARRAGE"
B	6.63	28	"TROUBADOUR"
B	6.55	28	"ELKA "

Results from Semunion Gazon

The analysis of the data from the moderate infection of 1992 shows for Verneuil a correlation coefficient between both replicates for the two experiments of 0.06. In Le

Chatelier the correlation coefficients were 0.01 and 0.18 respectively (Table 4). All were not significant.

For 1992 and 1993 the average of the two replicates were correlated between the different scores at different locations and between the different locations. In table 5 the correlation coefficients between the different scores are shown.

Table 5 shows that there is no correlation at Verneuil between red thread in the first and second year of the experiment (1992 and 1993), correlation coefficients are 0.19 and 0.10 respectively. However, assessments made in October and November in 1993 were correlated. The same result was found at Le Chatelier. Correlation coefficients between assessments made in 1991 and the other years of the trial were -0.10, 0.19 and 0.19. There was a slightly increased coefficient between assessments made in 1992 and 1993, and there was a significant correlation (0.67) between assessments made in 1993.

Table 6 shows the results of the correlation coefficients between the two locations. No combination shows significant correlation.

Discussion

These results illustrate the difficulty in carrying out selection work for resistance against red thread. There has been no selection progress in red thread for the last 20 years in Germany, France or Great Britain. The reasons are clear - it is difficult to observe any effects of cultivars (table 2). In the DP trials at Lusignan only 10 out of 30 cases in the 3 years trial period showed significant effects of cultivars. In the cases of significant cultivar effects Duncan separations of the means showed that the accuracy of the scoring had been quite poor with critical ranges varying from 1.0 to 2.1 in the 1-9 scale for the single assessment and 0.8 - 1.0 for the analysis over the 3 years (table 3). Even when the number of replicates was increased from 3 to 12-28, as were calculated for the control cultivars, the critical range was still large. The relative high critical range is in accordance with observations in the National Perennial Ryegrass test 1987-1990 in the USA (NTEP, 1990). In these trials which included 65 cultivars at 10 different locations LSD values varied between 0.9 - 2.4 (on a 1-9 scale) at different locations. For the mean value over all the locations and years the LSD value was as high as 0.6.

The results of the Semunion Gazon trials in tables 4 - 6 confirm the results of the DP-trials. There is no correlation between the different replicates of one experiment (table 4). There is no correlation between the different years of one experiment (table 5) and there is also no correlation between the different locations of the experiments (table 6). This result is found for years with moderate or severe attacks of red thread.

On basis of the Semunion Gazon, DP and the NTEP trials it can be concluded that natural incidence of red thread is very hard to score accurately. Observations must be statistically analysed and only then used to differentiate cultivars. Development of reproducible methods for infection of red thread in the field, the lab or the greenhouse would be a great advantage in order to make selection for resistant or tolerant cultivars possible.

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Table 1 : 20 years change (1973-1993) on a 9 to 1 scale of disease resistance of perennial ryegrass

	France	Germany	United Kingdom
Number of cultivars	59	71	40
Red thread	0,2	- 0,6*	- 0,02

Table 2 : Number of reps, entries and effect of blocks and varieties in 10 ryegrass trials sown in 1992.

Trial no	Reps	En-tries	Effect of blocks				Effects of Cultivars			
			92	93	94	92-94	92	93	94	92-94
1501	3	18	ns	ns	ns	ns	ns	ns	*	ns
1502	3	18	ns	ns	ns	ns	**	*	ns	ns
1503	3	18	ns	ns	ns	ns	**	ns	***	***
1504	3	18	ns	ns	ns	*	ns	**	ns	**
1505	3	18	ns	ns	ns	ns	ns	**	ns	**
1506	3	18	**	ns	ns	**	*	ns	ns	*
1507	2	18	ns	ns	ns	ns	ns	ns	ns	ns
1508	2	18	ns	ns	ns	ns	ns	ns	ns	ns
1701	3	18	ns	ns	ns	**	*	ns	ns	**
1702	3	18	ns	ns	ns	ns	**	ns	ns	*

where ns = not significant *, **, *** significance at 5, 1 and 0,1 % level, respectively.

Table 3 : Critical range among 2 means for trials showing significant cultivar effects.

Trial no	Critical range among 2 means			
	92	93	94	92-94
1501	ns	ns	1.0	ns
1502	2.0	1.1	ns	ns
1503	2.1	ns	0.9	1.0
1504	ns	1.0	ns	0.9
1505	ns	1.0	ns	0.9
1506	1.0	ns	ns	0.9
1507	ns	ns	ns	ns
1508	ns	ns	ns	ns
1701	1.7	ns	ns	0.8
1702	1.9	ns	ns	0.9

Table 4 : Sample correlation coefficient (r) between two replicates and two locations of red thread resistance of perennial ryegrass

Location	number of lines	correlation coefficient
Verneuil	125	0.06
	69	0.06
Le Chatelier	75	0.01
	141	0.18

Table 5 : Sample correlation coefficient (r) between the different scores of red thread at Verneuil and at Le Chatelier

Verneuil (70 couples)			
	92/3 ¹	93/10	
93/10	0.19		
93/11	0.10	0.70*	
Le Chatelier (70 couples)			
	91/12	92/11	93/9
92/11	- 0.10		
93/9	0.19	0.37	
93/11	0.19	0.34	0.67*

¹ year and month of scoring

Table 6 : Sample correlation coefficient (r) of red thread between the two locations of the experiment Verneuil and Le Chatelier (70 couples)

Verneuil	92/3*	93/10	93/11
Le Chatelier			
91/12	0.07	0.11	0.22
92/11	0.17	-0.13	-0.07
93/9	0.02	-0.07	0.03
93/11	0.25	0.10	0.13

* year and month of scoring

RED THREAD DISEASE (*LAETISARIA FUCIFORMIS*) IN *LOLIUM PERENNE* AND *FESTUCA RUBRA* BY ARTIFICIAL INOCULATION

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Summary

Red thread is a fungal disease of grass characterized by pink to pale red mycelial structures. In the Netherlands the disease is predominantly caused by *Laetisaria fuciformis*. An inoculum production method has been developed by mycelium production in 100 ml liquid medium in 300 ml Erlenmeyers at 18°C under continuous weak light of 1 W/m² and 100 rotations per minute. A 1 % malt extract medium appeared to be a better medium for mycelium production than a medium consisting of 33.4 g/l Czapek Dox and 1 g/l yeast extract. Inoculation of *Lolium perenne* and *Festuca rubra* in seedling boxes by spraying a solution of mycelium did not result in high infection levels.

Introduction

The symptoms of the red thread disease are circular or irregularly shaped, small to large patches of infected grass, reducing the quality of a turf. Only the foliage is infected, and death usually proceeds from the leaf tip downward. With high humidity the pathogen produces pink to pale red mycelial structures called red threads. Red thread is especially prevalent during the spring and autumn on slow-growing, nitrogen-deficient turf.

Three reddish corticaceous fungi have been identified as parasites on grasses. In the Netherlands *Laetisaria fuciformis* represents the causal organism of the red thread disease. The other two species are *Limonimyces roseipellis* and *Limonimyces culmigenus* (Stalpers & Loerakker, 1982).

In grass breeding programmes, selection for resistance to this disease depends on natural epidemics which occur erratic in time and over locations. Therefore, in a collaboration programme between grass breeding companies (Barenbrug Holland BV, Cebeco Zaden BV, J. Joordens Zaadhandel, Limagrains Genetics, Mommersteeg International BV, Zelder BV) and CPRO-DLO, an artificial inoculation method is now being developed.

Materials and methods

Inoculum production

From four locations in the Netherlands, 14 red thread isolates from *Lolium perenne* and *Festuca rubra* were collected and stored on SNA, a nutrient-poor medium, which conserves well the vitality (Nirenberg, 1981). Based on their mycelium growth on 2 % malt agar plates, two types of isolates can be identified : one type that forms web like, antler like,

fast growing cultures forming strands and one of a slow growing floccose type. Two isolates, one of the fast growing type forming strands (isolate 93-80) and one of the slow growing floccose type (isolate 93-4) were selected for inoculum production experiments.

Experiment 1

300 ml Erlenmeyers with 125 ml liquid medium were inoculated with one 50 mg mycelium disc from a red thread culture on a 2 % malt agar plate. Incubation took place during 13 days at 18°C under continuous weak light of 1 W/m² and 75 rotations per minute. In this experiment a 1 % malt extract medium was compared with a medium consisting of 33.4 g/l Czapek Dox and 1 g/l yeast extract (Bahuon, 1986). The experiment was carried out in three replicates.

Experiment 2

300 ml Erlenmeyers with 100 ml medium were each inoculated with 10 mycelium discs of 32 mg from a red thread culture on a 2 % malt agar plate. The cultures were incubated for 6, 9 and 12 days for a first trial and 7, 11, 14 and 17 days for a second trial. Incubation was at 100 rotations per minute under temperature and light conditions as described above. One medium was used for this experiment based on the results of experiment 1. The experiment was carried out in three replicates.

Inoculation test

A total of 14 varieties and populations of *Lolium perenne* and *Festuca rubra* with different resistance levels was sown in rows in seedling boxes with an inter-row distance of 2.5 cm and a density of 0.5 g/m. The plants were trimmed about twice a week to promote turf development at a temperature regime of 15/10°C. Three months after sowing the grass turfs were inoculated by spraying a solution of mycelium and water in a ratio of 1:2 using 1 l per 10 m². After inoculation the boxes were incubated at 18°C and at a relative humidity of 95 % under natural day light in spring. The experiment was repeated three times. As a control the inoculum was also sprayed on 2 % malt agar plates.

Results

Inoculum production

Experiment 1

After 14 days one compact ball of mycelium was formed. Fresh weight data are given in Table 1. A 1 % malt extract medium appeared to be a better medium for mycelium production than a medium consisting of Czapek Dox and yeast extract. Dry matter content of the mycelium after 13 days incubation was 3.2 % for which no difference existed between isolates or media. Pieces of the produced mycelium plated on 2 % malt agar produced the same type of mycelium, strands forming or floccose, which was used as starting material. Isolate 93-4, which is a slow grower on 2 % malt agar, was found to be a fast grower in liquid culture.

Experiment 2

Based on the results of experiment 1, a 1 % malt extract medium was used in this experiment. During the incubation 10 balls of mycelium or a cluster of balls was formed in the medium. Growth of mycelium expressed in g fresh weight is shown in Figure 1. The growth of mycelium over a period of 17 days was linear with the number of days incubation with a regression coefficient of 0.95 g/day for isolate 93-4 and 0.66 g/day for isolate 93-80.

Inoculation test

None of the inoculation trials resulted in a significant level of infection. On the inoculated control malt agar plates the same type of mycelium which was used as starting material was found.

Discussion

Inoculum production of red thread by mycelium production in a liquid culture looks very promising. Starting with an initial 0.3 g only, the multiplication for a fast growing isolate was about 1 g a day. Whether the plant inoculation tests failed either because of non-optimal incubation conditions or loss of aggressiveness of the inoculum is under study.

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Table 1 : Fresh weight (g) of mycelium of two red thread isolates after 13 days incubation in liquid medium

	isolate	
	93-4	93-80
malt extract	10.54	7.07
Czapec Dox/yeast extract	1.01	1.28

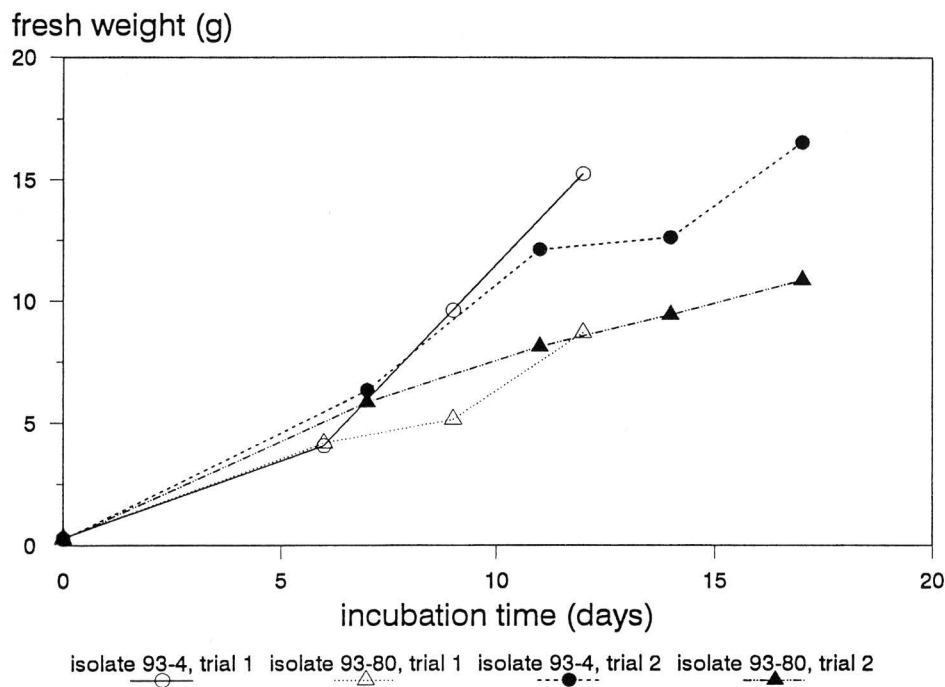


Figure 1 : Growth of mycelium in a liquid 1% malt extract medium.

LOOKING FOR SHADE TOLERANCE IN LAWN GRASSES

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Summary

Evaluations of turfgrass in park conditions were performed in the Central Poland in 1992-1994.

Festuca rubra, *Festuca ovina*, *Festuca arundinacea*, *Festuca heterophylla*, *Lolium perenne*, *Poa pratensis*, *Poa nemoralis*, *Deschampsia caespitosa*, *Deschampsia flexuosa* were represented by 26 varieties and ecotypes which were selected for lawn, mostly in Poland. The variability of varieties have been shown in connection to the diseases problem. The best varieties have been selected : *Festuca rubra commutata* "Nimba" cv., *Deschampsia caespitosa* "Brok" ecotype, *Festuca arundinacea* "Bartes" and "Villageoise" cv. The most injurious diseases were : powdery mildew, autumn - winter complex and leaf spot.

Results of general performance of species have been compared to the data from The Netherlands and France.

Introduction

The lawns very often are located at some shade situation (trees, building, walls etc.). Generally it is not comfortable for the most turfgrasses. However it is know significant variability exists among of species and varieties (Bakker & Vos, 1976 ; Funk, 1981 ; ASPA Turf News, 1988).

In Poland the program for breeding tolerant shade turfgrass started in 1988. Some results were presented at Eucarpia 1993 (Prończuk). The aim of this study was to investigate the species and varieties/ecotypes in context of the general aspects (performance), stability and diseases problem in shade condition.

Materials and methods

Evaluation of 26 varieties/ecotypes in 7 species have been done in nature park in Radzików (central Poland) during 1992 - 1994 (Tab. 1).

The restriction of day light on the lawn was from 10 % in the morning to 80 % in the late afternoon.

Management data rulers : cuts 10-12 times per year, mowing height 5-7 cm, nitrogen fertilization - 60 kg/ha, no irrigation.

Used varieties have not been bred for shade tolerance.

The data of the General aspect (GA) was evaluated in seasons: spring, summer and autumn.

General aspect of species have been compared to the corresponding data from The Netherlands and France.

Results

Varieties/ecotypes with best GA have been selected: *Festuca rubra* (0) Nimba (Polish variety), *Deschampsia coespitosa* RA-Brok (ecotype), *Festuca arundinacea* Villageaise (French variety), Bartes (Dutch variety) and *Lolium perenne* Stadion (Polish variety) (tab. 1). Stability of GA in years and seasons was also rather good (c.v. 11-13 %). The results of GA were influenced by diseases: winter disease complex mainly pink snow mould (*Microdochium nivale*) and pink patch (*Limonomyces roseipellis*).

The poorest lawn have been found for *Poa pratensis* varieties severely all infested by powdery mildew (*Erisiphe graminis*) and leaf spot (*Drechslera* spp.).

The performance of turfgrass from some varieties was different in different seasons and years (Figure 1 and 2).

When data of all species were compared to corresponding data from The Netherlands and France the better agreement of Polish data was found with that from France then that from The Netherlands (Tab. 2). Extremely hot summers 1992-1994 in Poland and maybe the set of tested varieties were responsible for such results.

Conclusion

- It has been found that the common lawn varieties can differ significantly in shade condition. The results by seasons and years can change dramatically.
- Some new species, for example *Deschampsia caespitosa*, can be useful for shaded lawns.
- The main diseases in Polish park lawn can be: autumn - winter complex (pink patch and pink snow mould), powdery mildew and leaf spot.

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Table 1 : Performance of varieties in shade conditions, Radzików 1992-1994

Varieties/ecotypes	GA n = 24	Coefficient of variation by seasons and years	Powdery mildew n = 9	Autumn -winter disease complex * n = 6	Leaf spot n = 9
<i>Festuca rubra</i> : Leo (1) (0) Nimba (1/2) Dawson (0) Lifalla (0) RA-24 (0) RA-25 (0) RA-41 (0)	5.3 EFG 7.5 A 5.3 EFG 6.6 C 6.3 CD 5.7 DF 6.8 ABC	18.9 11.8 18.9 19.4 22.1 18.2 19.7	8.8 A 8.8 A A A A A A	7.3 ABC 6.7 ABCD 6.2 BCD 5.7 CD 6.2 BCD BCD BCD	8.4 A 8.4 A A A A A A
<i>Poa nemoralis</i> : KRH-80** RA-187**	5.4 EFG 4.5 HJ	35.5 35.7	A A	A A	A A
<i>Poa pratensis</i> : Alicia Gol RA-213**	3.0 J 3.3 J 4.7 GHI	35.3 32.8 20.5	3.9 F 4.8 E 6.4 D	A A A	7.3 BC 6.7 C 4.8 D
<i>Lolium perenne</i> :RAH 391 (Stadion) Nira Gazon	6.8 ABC 5.8 DE 5.3 EFG	12.9 16.3 21.1	A A A	7.3 ABC ABC ABC	A A A
<i>Festuca ovina</i> : Sima Niko KRH-11 Rożas**	5.8 DF 5.3 EFG 5.5 EF 5.7 DF	25.4 31.1 27.3 28.9	A A A A	5.7 CD 6.2 BCD 5.8 CD 5.0 D	A A A A
<i>Festuca arundinacea</i> :Sinfonia Bartes Villageoise	6.7 BC 6.8 ABC 7.0 ABC	14.4 13.5 11.1	A A A	6.7 ABCD 6.2 BCD 6.3 BCD	A A A
<i>Festuca heterophylla</i> : Sawa	5.1 EFGH	31.3	A	A	A
<i>Deschampsia caespitosa</i> : RA-Brok**	7.4 AB	11.9	A	A	A
<i>Deschampsia flex.</i> : Ostrołka**	4.2 I	33.5	A	A	A
LSD 0.05 ABC... Duncan test	0.71		0.71	1.87	0.76

* Pink snow mould and pink patch ; ** wood ecotypes

Table 2 : Shade tolerance of turfgrasses in countries (the best performance - 9 or + + +)

Turfgrasses	Poland R- adzików 1992-94	Netherlands C.R.L*	France R.A.G.T**
<i>Festuca rubra</i> L.	6.2	8.0	++
<i>Festuca ovina</i> L.	5.6	6.0	++
<i>Festuca arundinacea</i> Schreb.	6.8	6.0	+++
<i>Lolium perenne</i> L.	6.0	4.0	+
<i>Poa pratensis</i> L.	3.6	5.0	-
<i>Poa nemoralis</i> L.	5.0	7.0	?
<i>Poa trivialis</i> L.	?	7.0	+++
<i>Agrostis tenuis</i> Sibth.	?	6.0	-
<i>Deschampsia caespitosa</i> L.	7.4	?	?
<i>Deschampsia flexuosa</i> L.	4.2	?	?

* 67e Beschrijvende Ressenlijst

** Turfgrass seed, R.A.G.T.

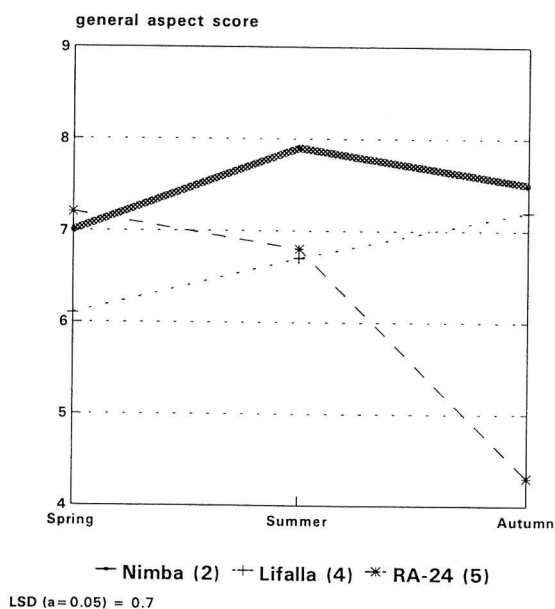


Figure 1 : General aspect (GA) of *Festuca rubra* lawn varieties as influenced by seasons

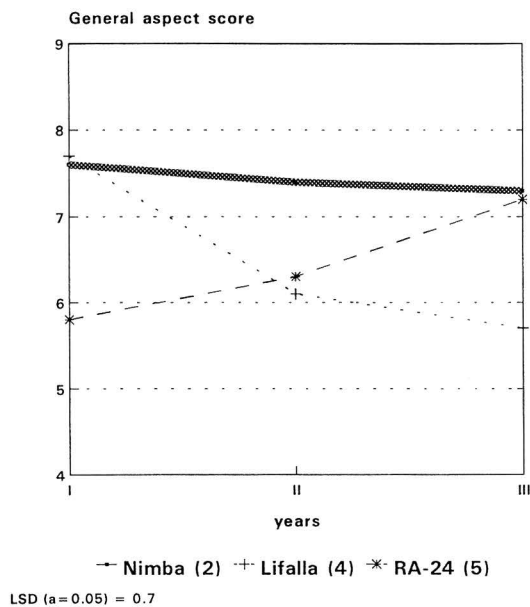


Figure 2 : General aspect (GA) of *Festuca rubra* lawn varieties as influenced by years

EVALUATION OF DIFFERENT AMENITY GRASSES IN CENTRAL ITALY¹

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Introduction

With the aim to point out the main features of the cool grasses most utilized in Italy, 16 varieties of *Lolium perenne* L., 16 of *Poa pratensis* L., 3 of *Festuca rubra* L. subsp. *rubra*, 6 of *Festuca rubra* L. subsp. *commutata* Gaud., 3 of *Festuca rubra* L. subsp. *trichophylla* (Gaud.) Richter, 6 of *Festuca arundinacea* Schreb. and 3 of *Agrostis palustris* Huds. were sown in Perugia (Central Italy) on March 3, 1991 and evaluated from April 1991 to September 1994 for establishment, number of shoots per 100 cm², turf colour and thatch layer. The experimental design was a randomized block with 3 replicates. Plot size was 10 m².

Results

F-significance relative to the anova for the analyzed traits is reported in Table 1.

L. perenne constantly showed higher values for establishment than any other species; however, all entries reached an adequate level of cover three weeks after sowing (Table 2). As far as the number of shoots per 100 cm² is concerned (Table 3), *F. arundinacea* showed a lower performance than the other species, thus confirming to be unsuitable for quality turfs. *L. perenne*, *P. pratensis* and *F. rubra* spp. were characterized by a quite constant behaviour; *A. palustris* appeared to be very interesting in this respect, reaching a far higher number of shoots than any other species at the end of the experiment.

L. perenne, *P. pratensis* and *F. rubra* spp. presented an effective colour for a large portion of the year maintaining it at acceptable levels during the winter, while *A. palustris* was characterized by a marked bleaching in winter and *F. arundinacea* by a drop of colour which started in mid-summer and was maintained for all of the following winter months. *A. palustris* and *P. pratensis* showed a clear attitude to produce thatch with time while the thatch layer of *L. perenne* tended to remain constant and low; intermediate behaviour has been shown by *F. arundinacea* and *F. rubra* spp.

¹Research supported by the *Ministero dell'Università e della Ricerca Scientifica e Tecnologica* (60 %, year 1994) ; subject : *Amenity grasses in Central Italy* ; project leader : Prof. Mario Falcinelli

Conclusions

On the basis of the significance of the "Varieties within species x Survey" source of variation for both establishment and number of shoots per 100 cm², it seems appropriate to look more closely to these two traits to obtain information suitable to be used in the production of a list of varieties recommended for Central Italy.

Table 1 : F-significance relative to the analysis of variance for establishment, number of shoots per 100 cm², turf colour and thatch layer

Source of variation	Establishment d.f. F	N° of shoots d.f. F	Turf colour d.f. F	Thatch layer d.f. F
Replicates	2	2	2	2
Entries (A)	52	52	52	52
Surveys (B)	8	3	4	2
A x B	416**	156**	208**	104**
Species x B	48**	18**	24**	12**
Varieties within species x B	368**	138**	184 < 1	92 < 1
Error	952	422	528	316
Total	1430	635	794	476

** Significant at $P < 0.01$

Table 2 : Establishment (0 = bare soil ; 9 = complete)

SPECIES	Days from sowing								
	3	7	9	13	17	21	25	29	33
<i>L. perenne</i>	1.6	2.7	3.9	4.8	5.9	6.5	7.6	7.9	8.8
<i>P. pratensis</i>	-	0.8	2.4	3.5	4.4	5.2	5.6	6.0	6.5
<i>F. rubra</i> spp.*	-	0.4	1.7	3.0	3.8	4.9	5.7	6.1	6.3
<i>F. arundinacea</i>	-	1.7	2.5	3.6	4.4	5.3	6.1	6.5	7.0
<i>A. palustris</i>	-	0.6	1.2	2.1	3.8	4.9	5.7	6.4	6.5
LSD _{0.05}	-	-	-	-	-	0.8	1.2	0.9	-
LSD _{0.01}	-	0.6	0.8	1.0	1.2	-	-	-	1.2

* *F. rubra* + *F. rubra commutata* + *F. rubra trichophylla*

Table 3 : Number of shoots per 100 cm²

SPECIES	Shoots per 100 cm ²			
	10-Jun-91	12-Aug-91	10-Jun-93	27-Sep-94
<i>L. perenne</i>	280	250	260	223
<i>P. pratensis</i>	300	320	270	229
<i>F. rubra</i> spp.*	408	400	445	365
<i>F. arundinacea</i>	185	205	210	165
<i>A. palustris</i>	370	400	490	580
LSD _{0.01}	45	47	66	42

* *F. rubra* + *F. rubra commutata* + *F. rubra trichophylla*

**SESSION 4 : Germplasm screening and genetic variation
for quality characteristics**

EVALUATION OF ALFALFA GENETIC RESOURCES

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Introduction

At the Malchow branch station of the German Genebank of IPK Gatersleben, about 25 % of the accessions belong to the Fabaceae. Among them, there are 520 cultivars and breeder lines of *Medicago x varia* Martyn. Most of them have been described for morphological characters. A set of 20 accessions were evaluated in field plot trials to investigate persistency, yield and quality.

Material and methods

The genetic material originates mainly from East European countries (see appendix). The entries were sown in 1984 in complete block design with four replications and a plot size of 4.1 sqm. In the year of establishment two cuts, and in the three successive years three cuts per year could be made, respectively. Samples were taken to determine dry matter (%) and crude protein content (after Kjeldahl). Dry matter yields (DMY) and protein yields (CPY) were calculated. Several characters of agronomic importance have been scored (data not given).

Results

According to their yielding ability in the 4 years of investigation (see tables 1 to 4), the entries could be classified into four different groups :

Group I (short rotation type)

High yielding in the first year followed by a decreasing performance in the successive years due to low persistence (i.e., cv. Lutetia).

Group II (not well adopted)

Entries of this group performed below average in all years (i.e., 'Szentesi Rona').

Group III (high yielding, less persistent)

Entries of this group are slow in establishment but outyielded all other group in the second year. They are able to maintain a rather high level of performance in the following years, though inferior to group IV entries (i.e., 'Vertibenda').

Group IV (high yielding, highly persistent)

Above average in the first year. Yields increase from year to year because of fast tiller development and high persistence (i.e., 'Palava').

Besides DMY (see Fig. 1), due to the positive correlation between DMY and CPY the same situation occurs for CPY (Fig. 2). The grouping of all 20 entries can be seen from the appendix.

Conclusion

For the growing conditions of Northeast Germany, group I accessions are only suitable for short rotation practise. Group II is the most inferior due to lack of adaptation. Group IV material is by far the best, both in terms of yielding ability and yield stability (i.e., persistence).

Tabel 1 : Average performance of dry matter yield

Year	Mean	Min.	Max.
1	5.52	4.58	6.61
2	16.03	14.72	18.31
3	14.12	11.37	16.01
4	12.57	10.65	15.59

Tabel 2 : Average performance of crude protein yield

Year	Mean	Min.	Max.
1	1.03	0.81	1.23
2	3.07	2.76	3.52
3	2.98	2.49	3.45
4	2.52	1.61	3.16

Table 3 : Dry matter yield (t/ha)

Cultivar	year 1	year 2	year 3	year 4
Ovari Kuszo	5.50	15.93	14.52	12.13
Szentesi Rona	4.58	14.72	11.90	12.10
Pleven 1	4.99	14.84	14.64	12.18
Pleven 6	5.78	17.04	15.85	13.58
TB 10	5.21	17.38	15.09	15.59
Palava	5.69	17.56	16.06	14.89
Fundulea 652	5.81	15.17	14.50	11.27
Luxin	5.15	15.41	15.84	12.42
Verko	5.29	15.59	15.38	14.06
Vertibenda	4.98	18.31	16.01	13.56
Lada	5.69	15.36	12.94	10.84
Lutetia	6.55	15.45	11.37	8.45
Gloria	6.36	15.55	12.91	12.75
Syn A-77	6.61	16.03	12.68	12.05
Syn MR	5.95	15.98	12.89	12.46
F 77-80	6.18	16.94	14.63	10.65
Saponex	4.77	15.73	12.78	12.84
CF	5.09	16.39	15.08	13.12
MEV 7601	5.33	15.76	14.43	12.81
Erecta	4.90	15.53	12.98	13.71
Average	5.52	16.03	14.12	12.57

Table 4 : Crude protein yield (t/ha)

Cultivar	year 1	year 2	year 3	year 4
Ovari Kuszo	1.00	3.11	3.09	2.49
Szentesi Rona	0.81	2.92	2.65	2.45
Pleven 1	1.07	2.88	3.06	2.40
Pleven 6	1.05	3.23	3.27	2.76
TB 10	0.92	3.43	3.12	3.16
Palava	0.99	3.17	3.45	2.96
Fundulea 652	0.98	3.02	2.98	2.28
Luxin	0.94	2.76	3.43	2.54
Verko	0.94	2.83	3.18	2.78
Vertibenda	1.07	3.52	3.36	2.81
Lada	1.10	3.00	3.00	2.35
Lutetia	1.23	2.98	2.49	1.61
Gloria	1.21	3.00	2.62	2.40
Syn A-77	1.17	3.09	2.56	2.40
Syn MR	1.13	3.02	2.69	2.41
F 77-80	1.11	3.22	3.22	2.14
Saponex	0.91	2.95	2.68	2.49
CF	0.90	3.02	3.12	2.72
MEV 7601	1.04	3.35	3.05	2.64
Erecta	0.95	2.81	2.55	2.61
Average	1.03	3.07	2.98	2.52

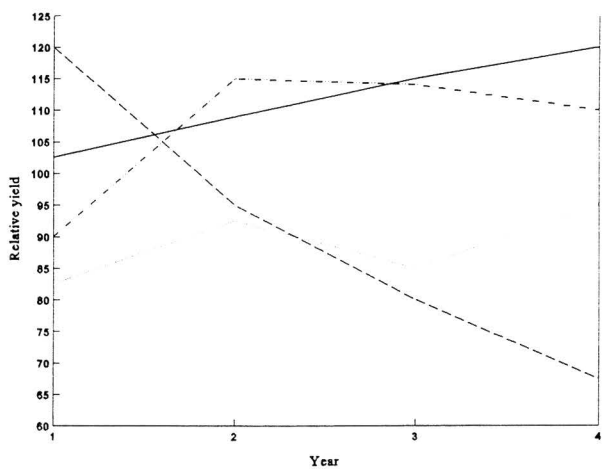


Figure 1 : Dry matter yield (DMY) of 4 varieties :
--- Lutetia, ... Szentesi Rona, - Vertibenda, — Palava

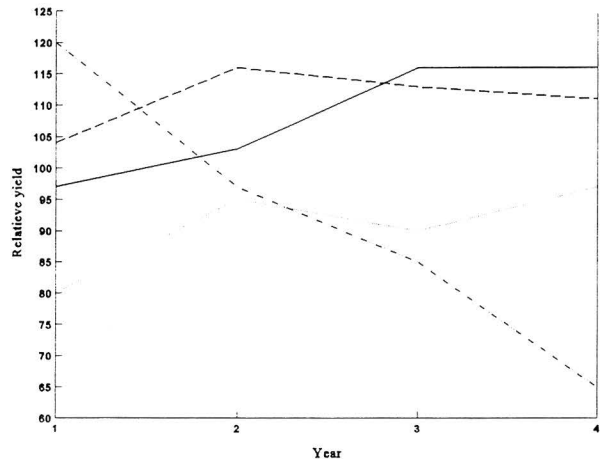


Figure 2 : Crude protein yield (CPY) of 4 varieties :
--- Lutetia, ... Szentesi Rona, -.-.- Vertibenda, — Palava

SOME OBSERVATIONS ON THE QUALITATIVE CHARACTERISTICS OF GRAZING-TYPE LUCERNE

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Summary

A collection of lucerne germplasm belonging to different taxa of the *Medicago sativa* complex and to distinct morphological models possibly adapted to grazing was evaluated for qualitative characters. Significant variation was found among genotypes, taxonomic groups and plant models. Selection of grazing-type lucerne seems compatible with the maintenance of satisfactory qualitative features.

Introduction

Lucerne (*Medicago sativa* complex) has a potential as pasture legume for many classes of livestock. Morpho-physiological traits such as creeping-rootedness, rhizomatous stems or large and deep-set crown, all contributing to subsurface budding and spatial proliferation of the shoots, improve persistence of swards under grazing (Heinrichs, 1963). Plants with these characteristics are rather frequent in *M. sativa* ssp. *falcata* (L.) Arcang. and *M. sativa* ssp. *X varia* Martin, and may occur in *M. sativa* ssp. *sativa* (L.) L. & L. or in artificial crosses among these taxa (Heinrichs, 1963). The rhizomatous character has also been reported in wild populations of *M. sativa* ssp. *sativa* from Spain called "mielga" (Ben Chabaane, 1990). In a breeding programme of grazing tolerant lucerne varieties, a collection pertaining to all the above-mentioned germplasm groups was evaluated at Lodi, leading to the phenotypic selection of about 130 individual plants for their top-growth morphology and forage yield. As described in a previous work (Piano *et al.*, 1994), the plants pertained to 4 different morphological models of the aerial part (Fig. 1) which largely corresponded to rhizomatous (D1), creeping (D2) and deep-crowned (D4) types ; D3 plants were somewhat intermediate between D1 and D4. Selected genotypes were cloned and entered the second phase of the programme which will include progeny testing and further selection. The present study refers to the evaluation of the cloned genotypes for both chemical components related to nutritive value and presence of antinutritional factors.

Materials and methods

Top-growth from two field replications of each selected genotype grown in plots of 9 clonal plants was harvested thrice at the green bud stage, respectively on May 3, May 26 and June 24. The oven-dried, ground material was analysed by near infrared reflectance spectroscopy (NIRS). Predicted values were obtained from calibration equations already in use at our Institute for a range of forage legumes. Crude protein (CP) content, neutral

detergent fibre (NDF) content and its components acid detergent fibre (ADF) and acid detergent lignin (ADL) were estimated by NIRS. The average plot values across the three cuts were considered in the statistical analyses. Sub-samples of ground leaves from the third cut were used to estimate the content of toxic lucerne saponins by the hemolytic micromethod (Jurzysta, 1979). The hemolytic ring area of the meal extract from each plot (average of 4 spots) was standardised over the area hemolysed by the 1 % solution of commercial saponin commonly used throughout the plates. The hemolysis of each plot was then expressed as "hemolysis relative to the standard" (HRS). Statistical comparisons were made among mean values of the genotypes, of the germplasm groups here examined, and of the different plant models shown in Fig. 1.

Results and discussion

Wide and significant ($P \leq 0.001$) variation was found among selected clones for all the recorded traits. Ranges of individual mean values were respectively : 22.5-30.6 % for CP ; 29.0-40.0 % for NDF ; 17.6-25.7 % for ADF ; 5.0-7.2 % for ADL ; 0-2.72 for HRS.

A common, hay-type cultivar of good qualitative characteristics ('Equipe') was harvested from the same trial and used as a reference. Its character mean values across cuts were : 22.8 % for CP, 33.4 % for NDF, 21.1 % for ADF and 6.4 % for ADL, while its HRS mean value was 1.11. A comparison made by Dunnett's test between individual clone mean values and 'Equipe' showed that 35 clones had significantly ($P \leq 0.05$) higher CP, while only few had higher fibre content and one higher saponin content. The analysis of variance revealed significant ($P \leq 0.05$) variation among the groups of lucerne germplasm. *M. sativa* ssp. *falcata* had the highest values of protein, NDF and HRS while *M. sativa* ssp. *sativa* tended to have an opposite behaviour (Table 1). "Mielga" populations had higher NDF than the cultivated ssp. *sativa* types and lower HRS than the morphologically closer (Piano *et al.*, 1994) ssp. *falcata* populations. A substantial variation was found for all traits within each taxonomic group. The different morphological categories (top-growth models) shown in Fig. 1 were characterised by significant ($P \leq 0.05$) differences for CP and NDF. The variation was not significant for HRS, although true creeping plants (D2) showed a lower mean value for this trait. Rhizomatous types, either prostrate or semi-erect (D1 and D3), tended to have higher protein content (Table 1). This could be related to their apparently higher leaf/stem ratio.

The results suggest that morphological plant models conferring adaptation to grazing can also be characterised by qualitative features similar to, or even better than those found in common hay-type varieties. As regards the taxonomic groups the ssp. *falcata* shows a relatively high saponin content. However, also in this case the wide variation found may allow selection for low saponin levels in all the top-growth models individuated in this taxon.

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Table 1 : Mean values and mean separation of different germplasm groups and plant morphological models for crude protein % (CP), neutral detergent fibre % (NDF) and saponin hemolysis relative to the standard (HRS) in a lucerne germplasm collection.

Germplasm group	CP	NDF	HRS	Plant model	CP	NDF	HRS
<i>M. sativa</i> ssp. <i>sativa</i>	25.8 b	33.1 b	0.82 b	D1	27.1 a	35.8 a	0.99 a
<i>M. sativa</i> ssp. <i>falcata</i>	27.6 a	35.0 a	1.24 a	D2	25.9 b	35.0 ab	0.68 a
<i>M. sativa</i> ssp. <i>X varia</i>	26.8 ab 27.0 a	35.3 a	0.96 ab	D3	27.1	34.5 ab	0.93 a
Artificial crosses "Mielga" populations	26.5 ab	34.3 ab 34.7 a	0.87 b 0.70 b	D4	26.5 ab	33.7 b	0.92 a

Means followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple range test.

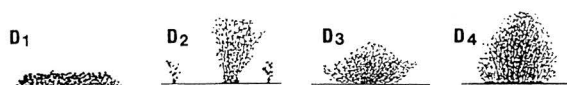


Fig. 1 : Schematic representation of four top-growth categories (models) of deep-crowned plants individuated in a lucerne germplasm collection.

VARIATION IN CRUDE PROTEIN AND FIBRE CONTENT : THEIR RELATIONSHIP WITH DRY MATTER AND ITS COMPONENTS, IN ALFALFA CULTIVARS, IN MEDITERRANEAN ENVIRONMENT

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Introduction

Alfalfa (*Medicago sativa* L.) is a high-yielding forage crop with high nutritive value which is widely grown in Italy. The climatic and edaphic conditions of the environments affect dry matter yield and nutritive value of forage. Herbage quality is related to the chemical composition and digestibility, closely associated to the variation of the fibre components. Studies have reported that alfalfa quality is largely influenced by leaf-to-stem ratio and varies with the phenological stage of growth (Demment *et al.*, 1986 ; Kepart *et al.*, 1989 ; Rotili *et al.*, 1993). Furthermore, quality of the stems is more influenced than that of the leaves with increasing the maturity of the plant (Terry and Tilley, 1964 ; Hill and Barnes, 1977).

Fibre and crude protein content of leaves and stems were compared in ten alfalfa cultivars in order to determine the yield potential of the cultivars and to compare the forage quality parameters of leaves and stems with dry matter yield and its components.

Materials and methods

The trial was carried out during 1987-1991 at the Forage Crop Institute, located in Foggia (southern Italy), using ten alfalfa genotypes (three ecotypes : Ascolana, Romagnola and Friulana) and seven registered cultivars (Delta, Equipe, La Rocca, Magalì, Robot, Tornese and Turrane), grown under irrigated conditions and harvested at early flowering growth stage (10 % of flowers). The experimental design was a randomized complete block with 8 replications. On each plot and at harvest of the years in question, the traits evaluated were : dry matter yield (DMY, t ha⁻¹), regrowth at 14th day (RGW, cm), plant height at cut (PLH, cm), plant density (TIL, tillers m⁻²) and leaf-stem ratio (LSR, %).

Chemical analysis were performed only on leaf and stem samples from the first two harvests of the years 1990 and 1991. A random sample of forage for quality analysis (50 tillers) was taken from each plot, dried in a forced air oven at 60°C for 48 h and then ground in a Cyclotec mill to pass a 1-mm screen. Neutral detergent fibre (NDF, %), acid detergent fibre (ADF, %) and acid detergent lignin (ADL, %) were determined by methods of Goering and Van Soest (1970). Hemicellulose (HCL, %) and cellulose (CL, %) were estimated as NDF minus ADF and ADF minus ADL, respectively. Crude protein (CP, %) was obtained multiplying the N percent obtained by micro-Kjeldahl analysis by 6.25.

Data were analyzed using the analysis of variance techniques. Where F-tests were significant, LSD at 0.05 level of probability were assessed, to determine differences among

cultivars and years. The performance of the cultivars through the cuts were computed according to Eberhart and Russel (1966). Furthermore, the cultivars best adapted to the environmental conditions according to productivity parameters, were identified by cluster method procedure (Scott and Knott, 1974).

Results and discussion

Dry matter yield and its components

DMY and LSR of cultivars as mean of all harvests during the five years clearly showed yield and leaf-to-stem ratio stability and performance through the cuts. Demment *et al.*, 1986, suggested that LSR is a major contributor to quality improvement, after selection for highly reduced nitrogen concentration. Cluster analysis identified 7 more productive cultivars. Because leaf-stem ratio and tiller density are the main agronomical traits influencing the productivity and quality of alfalfa, they are used to identify the cultivars best adapted to the environment. The cultivars with higher values for both traits were Equipe and La Rocca, while Romagnola and Friulana displayed higher values for LSR, and Tornese and Robot for TIL. Cluster analysis showed cultivars with higher performance as having more than 3 traits in the higher cluster group (La Rocca, Romagnola, Friulana, Equipe and Robot).

Protein and fibre components

Differences among yearly means of the chemical composition in leaves were statistically significant for all traits, except for CP in the second year. By contrast, differences among years were not significant. Nevertheless, in the first year the cultivars showed higher values in all chemical parameters, except in NDF and HCL. As regards the differences among cultivars in the stems, they were similar to those observed in the leaves ; statistically significant differences between years were observed in the CP, NDF and HCL parameters. Moreover, data showed that protein leaves had a higher range of variation (24.4-26.0 %) than in stems (10.8-11.5 %). In agreement with Buxton *et al.* (1985), leaves have substantially higher CP and lower fibre components than the stems at nearly all developmental stages. The values obtained for CP were 25.2 % in leaves and 11.2 % in stems, 24.7 % for NDF, 15.9 % for ADF and 3.2 % for ADL in the leaves and 55.2, 44.1 and 8.5 % for NDF, ADF and ADL in the stems, respectively. Similar results were obtained for HCL and CL components. The results of cluster analysis (Table 1) showed 3 better cultivars as having higher values of CP in leaves (Ascolana, Friulana and Robot) and cultivars with higher values in CP and lower NDF, ADF and ADL, in the stems (Equipe, Magali and La Rocca). Data showed that crude protein and cell wall components are related to dry matter yield : among the high-yielding cultivars, Ascolana and Friulana had higher values for CP in leaves and stems and Robot for CP leaves, only. The cvs. La Rocca and Equipe had the lowest values for NDF and ADF in leaves and stems, and Romagnola for ADL. Furthermore, an adverse relationship was observed among leaf CP and fibre components : cultivars with higher NDF, ADF, or ADL, in leaves, stems or in both. No relationship was found among cultivars with higher leaves CP and those with higher LSR.

Conclusions

The variability observed in the cultivars, for yield and quality parameters, may provide indications as to cultivar characteristics and their performance so as to cope better with the Mediterranean environment. The study underlined that selection for quality traits concerning high leaf protein and low stem fibre content, associated with increased yield, is a difficult target to achieve. However three high yielding cultivars with higher leaf CP (Ascolana, Friulana and Robot) and two with lower NDF, ADF and ADL in leaves and stems (Equipe and La Rocca) could be used as parents in breeding programs.

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Table 1 : Selected homogeneous cluster group varieties for dry matter yield (DMY), crude protein and cell wall components in leaf (L) and stem (S) : mean of two years

CULTIVARS	DMY	CP		NDF		ADF		ADL		HCL		CL	
		L	S	L	S	L	S	L	S	L	S	L	S
	(g m ⁻²)	(%)											
TURRENA	--	--	--	--	56.2	16.3	45.5	--	9.0	--	--	13.2	36.6
EQUIPE	27.4	--	11.5	--	--	--	--	--	--	9.3	--	--	--
MAGALI'	--	--	11.0	--	--	16.5	--	--	--	--	--	13.3	35.4
DELTA	--	--	--	--	--	--	44.5	--	--	--	--	--	36.0
LA ROCCA	28.7	--	11.2	--	--	--	--	--	--	--	--	--	--
ASSOLANA	27.8	26.0	11.3	--	55.9	16.0	44.7	--	--	--	11.2	12.7	36.2
ROMAGNOLA	27.6	--	--	--	55.8	--	44.1	--	--	--	11.7	--	35.6
TORNESE	27.9	--	11.5	27.5	56.3	17.3	--	3.6	--	10.2	12.6	13.7	35.5
FRIULANA	27.5	25.9	11.4	25.6	56.4	15.9	45.0	3.5	8.7	9.7	11.4	--	36.2
ROBOT	28.3	25.7	--	--	--	16.1	44.3	3.7	8.7	--	11.5	--	35.7
Mean	27.9	25.9	11.3	26.6	56.1	16.4	44.7	3.6	8.8	9.7	11.7	13.2	35.9

SCREENING *Atriplex halimus* AND *Medicago arborea* POPULATIONS FOR SAPONIN CONTENT

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Abstract

In order to acquire information on the genetic potential of shrub species suitable for grazing in semi-arid areas of the Mediterranean region, genotypes from progeny clones belonging to three natural populations of saltbush (*Atriplex halimus*) from Sicily (Italy), and from ten tree-medic (*Medicago arborea*) populations from different Mediterranean countries have been studied. Hemolytic saponins have been considered as representative of the antinutrients affecting intake and preference of grazing animals. Wide and significant variation was found among the tree-medic genotypes, while the saltbush clones did not give rise to hemolysis from their extracts. Genotypes with satisfactory qualitative features (zero-low saponin content) can be easily selected among the best performing tree-medics under study.

Introduction

One of the most serious problems of forage production in rainfed areas of the Mediterranean environment is the high seasonability of grazeable resources. For long periods of the year, livestock farmers must break off grazing and resort to hay, ensilage, stubble, etc. with a considerable increase of stock-farming costs. Woody forage plants, particularly shrubs, could represent a useful tool to fill up the periods of grazing shortage. Among them, saltbush (*Atriplex halimus*) and tree-medic (*Medicago arborea*) are probably the most interesting shrubs for many semi-arid areas of the Mediterranean basin (Stringi *et al.*, 1987 ; Martiniello and Baviello, 1993) ; in particular the good regrowth ability soon after the summer stasis may allow forage productions to be obtained during the autumn-winter period which represents one of the critical moments of feed shortage for the livestock grazing in the Mediterranean environment. In such conditions it is furthermore important to assess the nutritional value and the degree of utilization of these species by grazing animals, to determine their potential fodder value. So digestibility, palatability and voluntary intake related to chemical composition of the edible biomass from these shrubs might be assessed. A study has been undertaken in order to acquire more information on the genetic potential of these species through the characterization of different ecotypes for qualitative traits together with morpho-physiological and productive traits.

Materials and methods

As much as 81 genotypes belonging to three natural populations of *Atriplex halimus* from Sicily, and 871 plants belonging to ten *Medicago arborea* populations from different areas of the Mediterranean basin have been studied. The presence of antinutrients affecting palatability has been monitored in the individual plants at the third year after plantation. Hemolytic saponins have been considered as representative of the most important saponin fractions influencing intake and preference of grazing animals, as well as responsible for the toxic effects (growth inhibition and possibly bloating) reported in ruminants and monogastric animals. The hemolytic micromethod (Jurzysta, 1979) for rapid estimation of toxic saponins has been applied, using dried samples of leaves collected from individual plants at the same growth stage. The hemolytic ring area of the meal extract or leaf disc from each plant was standardised over the area hemolysed by a 1 % solution of commercial saponin, and expressed as "hemolysis relative to the standard" (HRS).

Results

No hemolytic saponins have been detected in the saltbush samples analysed, although crude saponins, mainly soyasaponins, can be chemically extracted from these materials, and have been quantified in few plants (data not reported). On the contrary, a wide and significant ($P \leq 0.001$) variation in the hemolytic saponin content has been revealed by the ten *Medicago arborea* populations, with individual mean values of the hemolytic index (HRS) ranging from 0 to 6.6. Table 1 shows that the mean values of hemolytic saponin content of the tree-medics tested vary significantly among populations. Possible correlations between saponin level and site of origin of the various genotypes could therefore be hypothesized. The Sicilian (Pietranera and Casteltermini) populations and 3 out of 4 Greek populations scored the highest saponin indices, ranging from as much as roughly once to twice the saponin content (1 %) of the standard solution used in the hemolytic test. The results obtained confirmed however the usefulness of the hemolytic micromethod for breeding purposes, due to its ability to reveal the individual plants to be selected for low/zero saponin content.

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Table 1 : Mean HRS values of the ten tree-medic populations screened.

Population	Origin	N°of Plants	HRS
Naxos	Greece	84	2.02 a
Attica	Greece	81	1.49 b
Pietranera	Italy	98	1.34 bc
Casteltermini	Italy	93	1.18 c
Hellas	Greece	84	0.97 d
Atene	Greece	73	0.73 e
Melagon	Algeria	86	0.73 e
Bari	Italy	98	0.68 e
Batna	Algeria	83	0.63 e
Cap Ferrat	France	91	0.60 e

Means followed by the same letter are not different at $P \leq 0.05$ according to Duncan's multiple range test.

OCCURRENCE AND NATURE OF ENDOPHYTIC FUNGI IN NATURAL POPULATIONS OF TALL FESCUE FROM SARDINIA, ITALY

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Abstract

Tall fescue natural populations from Sardinia (Italy) resulted all infected with *Acremonium* fungal endophytes. The isolates obtained were examined for morpho-physiological characters. They could be ascribed to a short-conidia taxonomic group different from *A. coenophialum*, not producing loline alkaloids in the host, and probably specific to Mediterranean tall fescue populations. Appreciable variation was found among isolates.

Introduction

Many grasses are asymptomatic host to seed-borne fungal endophytes of the genus *Acremonium* Link sect. *Albo-lanosa* Morgan-Jones et Gams. Tall fescue (*Festuca arundinacea* Schreb.) is known to be a host to *A. coenophialum* Morgan-Jones et Gams. The endophyte lives entirely within the plant, do not produce fruiting structures in the host, and is apparently transmitted only through infected seed. The relationship between the fungus and the plant is regarded as mutualistic with the host benefiting of the infection through enhanced plant growth and fertility, better drought tolerance, increased resistance to insects and, possibly, diseases. In contrast with these beneficial effects the ingestion of endophyte-infected plants has been associated to a range of toxicoses in grazing animals. Evidence indicates that the effect of endophyte on both grass performance and animal production are mediated by a range of secondary metabolites, including different toxic alkaloids, produced by the plant-fungus interaction. Following the great variation recently found among endophyte strains for the nature and amount of secondary metabolites they produce (Christensen *et al.*, 1993), the selection of naturally occurring strains, able to maintain the beneficial effects on pasture performance without producing negative effects on animals, is now being investigated. As a great part of the variation seems associated to the phylogenetic diversity of the *Festuca* genus and possible endophyte-host plant coevolution has been advocated (Leuchtmann, 1993), it could be expected that different endophyte forms are associated with tall fescues of different genetic and geographic origin. This paper reports on the presence, nature and morpho-physiological variation of endophytic fungi in 15 natural populations of tall fescue from Sardinia, Italy.

Materials and methods

Seeds from each population were stained with 0.1% cotton blue in lacto-phenol after digestion in a 3 % NaOH solution and observed under the microscope for the presence of endophytic fungal hyphae. For each population isolates of the fungus were obtained on potato-dextrose agar (PDA), incubated at 22°C in the dark, from seed surface-sterilised with 50 % sulfuric acid and then with 10 % sodium hypochlorite. Sub-cultures from marginal fragments of the colonies, grown on PDA from seed, were obtained and compared with the strains TF31 of *A. coenophialum*, TF33 of *Acremonium* sp. from cv Au-Triumph and three other *Acremonium* isolates obtained from cv Tanit, a variety originating from Sardinian tall fescue populations. Using already described methodology (Christensen *et al.*, 1991) with minor modifications, two or three isolates per population and reference isolates were assessed for colony appearance, mean length of conidia (μm), radial growth of colonies (mm/d), sensitivity to concentrations of Benomyl from 1 to 10 $\mu\text{g/ml}$, and *in vitro* antibiosis with the fungi *Drechslera erythrospila* (Drechsler) Shoemaker, *D. graminea* (Rabenh. ex Schlecht.) Shoemaker and *D. dyctioides* (Drechsler) Shoemaker. Production of loline alkaloids by endophyte naturally infected plants of 'Tanit' was assessed by aphid bioassay. When appropriate, differences among isolates were statistically tested with the ANOVA.

Results and discussion

All populations resulted infected with *Acremonium*-type hyphae, the infection ranging from 42 to 88 % of the examined seeds. The infection rate in cv Tanit was 53 %. The isolates from the populations morphologically resembled the isolates from cv Tanit while being clearly distinct from the strain TF31. They were typical in bearing conidia smaller than reported for *A. coenophialum*, with conidia mean length always significantly shorter than in TF31. Growth rates also tended to be slightly slower than in both TF31 and TF33 (Table 1). Differences among isolates within populations were evident for both characters. Isolates differed in their antibiotic effects on test fungi. Most of them exhibited inhibitory activity against growth of both *D. graminea* and *D. erythrospila*, which, however, varied in extent. Differences also occurred for inhibition against *D. dyctioides*. TF31 did not inhibit any of the test fungi. Sensitivity to Benomyl also varied among isolates but there was evidence of acquired tolerance, since mycelial growth could develop from plugs placed at Benomyl concentrations which had previously prevented growth. Aphid colonies of *Rhopalosiphum padi* L. became established on both infected and not infected plants of 'Tanit', the lack of feeding deterrence suggesting that endophyte infection did not induce loline production by the host.

The widespread endophyte infection found in the examined materials suggests that the endophyte presence leads to some adaptive benefit in tall fescue natural populations. The isolates obtained do not exactly fit the morphological characteristics of *A. coenophialum*. Recent studies on *Acremonium* endophytes from tall fescue have revealed a morpho-physiological variation much greater than previously assumed and the occurrence of distinct taxonomic groups (TG) other than *A. coenophialum*, each containing sub-groups differing in isozyme phenotype and alkaloid production (Christensen *et al.*, 1993). The isolates from Sardinian populations could probably be accommodated in a short-conidia

group referred to as FaTG2 which is also distinct for the lack of production of loline alkaloids. Interestingly, isolates of FaTG2 and the other short-conidia group FaTG3, which produces loline derivatives, have been previously found only in plants of Mediterranean origin (Christensen *et al.*, 1993), a "specificity" of host-endophyte association within tall fescue which seems corroborated by the present results. This study has further indicated that appreciable variation can be found among endophyte isolates, which can lead to development and selection of strains that could confer useful characteristics to infected plants.

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Table 1 : Characteristics of the *Acremonium* isolates in comparison with reference strains

Isolates	Length of conidia (μm)		Growth rate (mm/d)	
	Mean	Range(1,2)	Mean	Range(1)
From populations	6.0	5.2 - 6.9	0.50	0.33 - 0.70
From Tanit	5.9	4.5 - 7.0	0.50	0.35 - 0.68
TF31	10.4	----	0.66	----
TF33	7.1	----	0.77	----

(1) among isolates

(2) all isolates different from TF31 at $P \leq 0.05$ according to Dunnett's test.

A SURVEY OF *ACREMONIUM*-ENDOPHYTES IN WILD POPULATIONS OF PERENNIAL RYEGRASS COLLECTED IN FRANCE

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Abstract

A survey of *Acremonium* endophytes occurrence has been carried out in 178 French populations of perennial ryegrass. 114 accessions were found to be infected, with rates ranging from 5 % to 100 %. Although there is no clear-cut geographic pattern of distribution, the infection rates seem to be higher in the warmest and driest regions.

Introduction

Perennial ryegrass (*Lolium perenne*) is one of many grass species that can be infected by symptomless, seed transmitted endophytic fungi (*Acremonium spp*) belonging to the tribe *Balansiae* of the *Clavicipitaceae* (Siegel *et al.*, 1987).

Recently, mutualism between clavicipitaceous fungal endophyte and host grasses has been recognized (Clay, 1986). Indeed endophyte infection is reported to enhance host plant fitness characters such as drought tolerance (Elmi *et al.*, 1989), establishment, tillering or dry matter yield (Latch *et al.*, 1985). Similar results have been found in France (Ravel *et al.*, 1994), at least in the driest locations.

On the other hand, perennial ryegrass endophyte (*Acremonium lolii*) produces several alkaloids which are active against herbivores: 1) Lolitrems and ergovaline are deleterious to livestock (Gallagher *et al.*, 1984 ; Bacon, 1986). 2) Other ergopeptines and peramine are deterrent or toxic to some insects (Rowan and Gaynor, 1986).

Siegel *et al.* (1987) suggested an European origin for endophytes, since perennial ryegrass and tall fescue are native plants from Europe introduced worldwide, however most research on endophytes have been carried out in the U.S.A. and New Zealand where toxicosis due to endophyte alkaloids are important. Some toxicosis due to the endophyte were reported in European conditions were first mentioned by Sampson (1935) in the U.K. Later, Raynal (1991) mentioned some case of gangrena on bovines in France, and Huizing *et al.* (1991) reported some cases of 'solare eczema' among lambs in the Netherlands. Nevertheless, toxicoses seem to be less important in Europe than in the New world, where climatic conditions and nutrition of cattle are different.

Therefore, in order to study the impact of *Acremonium lolii* in France, we carried out a survey of the occurrence of this endophyte in a large collection of wild populations (Charmet *et al.*, 1990), as previously done by Lewis and Clements (1986) in the U.K..

Results and discussion

Out of 178 populations examined, 114 (64 %) were found to be infected, with infection rates ranging from 5 % to 100 %. The results are shown on Figure 1. This map shows that it is possible to find *Acremonium lolii* everywhere in France.

Observing 20 seeds per population leads to an estimation of the infected rate with a large confidence interval. In spite of this lack of accuracy, the most highly infected populations seem to grow in the warmest and driest regions of the Mediterranean and the Rhone valley. However endophyte infected material can also be found in the North-East of France, and a more precise study of the relationships between infection rate and climatic factors is needed.

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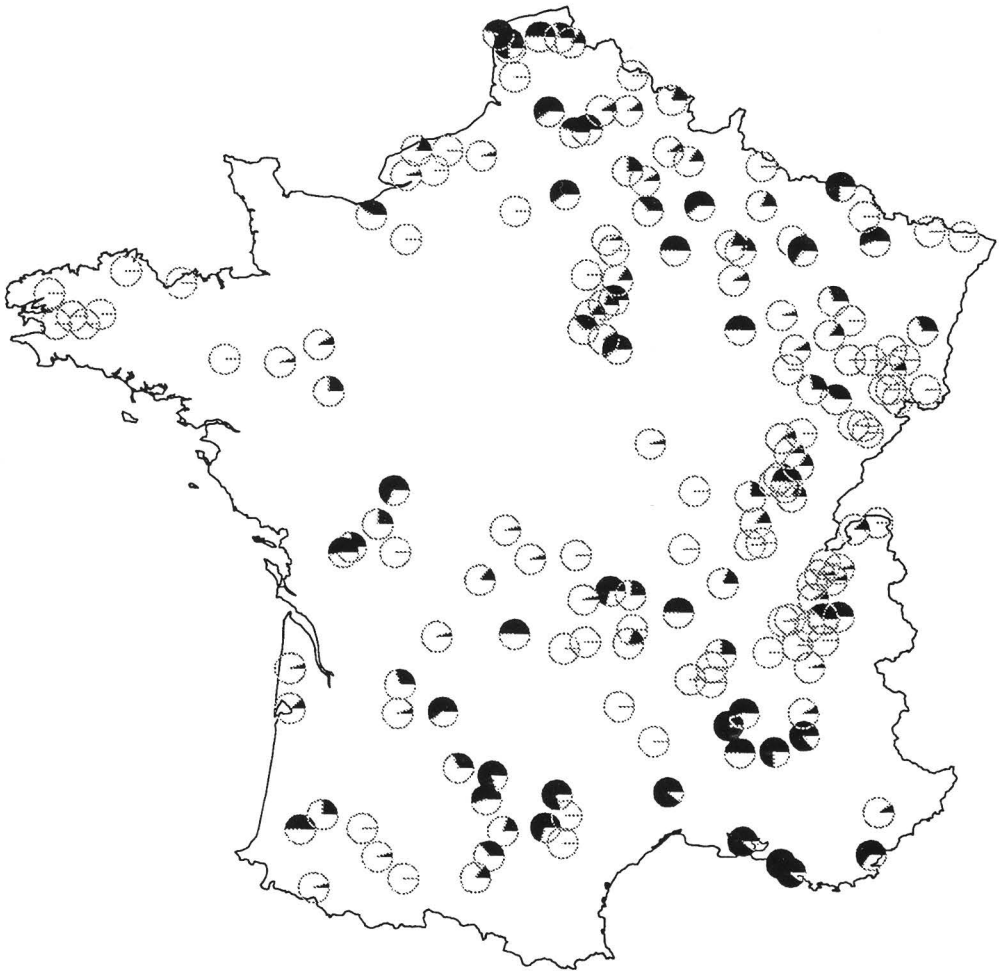


Figure 1 : Infection rate of wild french populations

SESSION 5 : Breeding research

DRY MATTER CONTENT OF INDUCED TETRAPLOID FORAGES

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Summary

The dry matter content (DMc) of the leaves of tetraploid fodder crops is about 7.5 % lower than the DMc of the diploids. The variation of the DMc within tetraploids is equal to that within diploids. The DMc is negatively correlated with the yield. This negative correlation is stronger for the tetraploids than for the diploids.

A high DMc of perennial ryegrass has a positive but rather slight effect on animal intake, digestibility and prewilting time.

The heritability of the DMc is high but, due to the negative correlation with yield, the expected advance of the DMc is limited. It is advised to start the breeding of high DMc tetraploids from high DMc diploids.

In mixtures with diploid perennial ryegrass, tetraploids persist well particularly when frequently cut.

Introduction

In general the dry matter content of the vegetative parts of tetraploid crops is lower than of diploids.

Tetraploid cells contain more DNA than diploids.

The amount of DNA is positively correlated both with the duration of the mitosis and with the cell volume (Cavalier-Smith, 1978 ; Evans and Rees, 1971). So, tetraploid cells divide less frequently and their volume is greater than of diploids. A greater cell volume also results in a higher cell content/cell wall ratio. Tetraploids contain less cell wall components and more water. The transpiration rate of diploids and tetraploids is almost the same. All this may explain the lower dry matter content of the tetraploids.

Warner and Edwards (1993) also put: "The amount of DNA per cell is correlated with the photosynthetic rate per cell. The photosynthetic rate per unit leaf area is the product of the rate per cell times the number of photosynthetic cells per unit leaf area. Therefore, the photosynthetic rate per unit leaf area will increase if there is a less than proportional increase in cell volume at higher ploidy levels, or if cell packing is altered to allow more cells per unit leaf area."

As a consequence of these theories it is very inviting to look for tetraploids with a high dry matter content. But what does practice show ?

Variation of the dry matter content in and between diploid and tetraploid fodder crops

The effect of the ploidy on the dry matter content (DMc) is indicated in table 1. The table shows the components of variance of yield and DMc in a trial with 5 diploid and 5 derived tetraploid populations of perennial ryegrass. The plots, in 3 replicates, were cut 11 times over 3 years. The effect of the ploidy on the DMc is almost as high as the effect of the cut. The ploidy hardly affects the yield.

The variation of the DMc in and between diploids and derived tetraploids of several forages in their vegetative stage is illustrated in table 2. On an average the DMc of tetraploid leaves is relatively about 7.5 % lower than the DMc of diploids. The storage parts of beet and turnip differed less. The variation of the DMc within tetraploids is equal to that within diploids.

The DMc of perennial ryegrass clones in the vegetative stage was positively but weakly correlated with their DMc in the year after vernalization. The latter DMc was positively correlated with the earliness. After correction for this cofactor the correlation between the DMc of the vegetative clones and their DMc after vernalization improved considerably.

The absolute differences of the DMc between diploid and derived tetraploid populations over cuts and years is highly influenced by the level of the DMc and by the difference in fresh yield.

Dry matter content and agricultural value

Yield

Table 3 shows the coefficients of regression between DMc and yield of some diploid and derived tetraploid fodder crops. These coefficients also are the coefficients of correlation between DMc and yield because they have been calculated based on standardized values. Yield and DMc are negatively correlated. The coefficients of the tetraploids are more negative than these of the diploids.

Plant-animal interaction

Dry matter intake

A brief review of literature on the relationship between dry matter intake and DMc of perennial ryegrass is presented in table 4. A higher DMc favours the dry matter intake to a lesser or larger extent, depending on the reference.

Eating time per kg dry matter intake decreases when DMc increases. This decrease is partly cancelled by an increase in ruminating time. If the dry matter content (in %) decreases by 1 unit, a cow of 600 kg spends only 15 minutes longer on eating 15 kg of dry matter.

Water intake

Figure 1 shows the relationship between the DMc of perennial ryegrass and the total water intake by the animals. The total water is the sum of the herbage moisture and the drinking water. From a DMc of 15 % onwards the animals compensate the decrease in water content of the grass by drinking more water. At a DMc lower than 15 % animals

don't drink anymore and consume more water, as herbage moisture, than they really need. The excess of water takes rumen volume and has to be processed by the animal.

Digestibility

One trial with 32 vegetative clones both of diploid and of tetraploid perennial ryegrass indicated a slight increase of digestibility when increasing DMc (figure 2).

Drying rate

During wilting, the dry matter content of varieties of perennial ryegrass, varying in DMc at mowing, rises almost at the same rate. Suppose: a drying rate of 2 units DMc (in %) per hour and a ryegrass variety A that contains at mowing 1 unit of DMc more than a variety B. The variety A needs only half an hour less drying time to meet a desired level of DMc than the variety B.

Breeding for a high dry matter content

Figures of broad and narrow sense heritability of both DMc and yield of diploid and tetraploid perennial ryegrass are shown in table 5. The heritability of DMc is as good for tetraploids as for diploids. The expected advance when breeding for a high dry matter content seems promising. However, the snake in the grass is the negative correlation between DMc and yield.

Figures 3 and 4 show the predicted advance for DMc and fresh yield when applying index selection to a diploid and a derived tetraploid perennial ryegrass population. Depending on the relative importance of DMc and yield, their expected advances change. Only in the triangle above the 0-line, there is a gain of both DMc and yield. The basis of this triangle is broader for the diploid than for the tetraploid population. In other words, the breeder is more flexible towards the importance of the DMc in the index, when he selects on the diploid than when he selects on the tetraploid level. In the right corner of the triangle you may expect the greatest advance of DMc without losing yield. This expected advance of DMc is higher for tetraploids than for diploids.

So, it is better to start the breeding of tetraploids with a high dry matter content at the diploid level. Moreover, the heritability of DMc from one ploidy level to another seems to be rather high (Feuerstein and Paul, 1991; Van Bockstaele, 1990).

The persistence of tetraploid perennial ryegrass in mixtures with diploid perennial ryegrass

Is the game worth the candle to improve the DMc of tetraploid perennial ryegrass knowing that diploid and tetraploid varieties often are offered to the farmer as a mixture. This was the basic assumption to look at the persistence of tetraploids in a mixture with diploids.

About 5000 individual perennial ryegrass plants of different mixtures, ages and management modes (table 6) were analysed by a flow cytometer to determine the ploidy level. In the short run (up to 5 years after sowing) tetraploids persist fairly well compared to the diploids, particularly when they are frequently cut.

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Table 1 : Components of variance (%) of fresh yield (FY), dry matter yield (DMY) and dry matter content (DMc) of perennial ryegrass

variance component	FY	DMY	DMc
cut	81	82	42
replicate	0	0	0
population	3	5	0
ploidy	1	0	33
population x ploidy	2	1	4
cut x population	6	4	5
cut x ploidy	5	4	8
cut x population x ploidy	0	0	0
error	3	5	9

Table 2 : The variation of dry matter content (%) of diploid and derived tetraploid vegetative fodder crops

crop	n genotypes	n cuts	n reps	DMc \pm se 2x	DMc \pm se 4x
perennial ryegrass	32	3	4	19.1 \pm 1.0	17.9 \pm 1.0
italian ryegrass	16	4	4	15.8 \pm 0.5	14.6 \pm 0.3
red clover	48	4	1	17.5 \pm 0.9	16.3 \pm 0.9
fodder beet	160	1	1		
top				12.5 \pm 1.1	11.5 \pm 1.5
beet				10.5 \pm 1.3	10.2 \pm 1.0
stubble turnip	120	1	1		
top				10.8 \pm 0.8	10.1 \pm 0.6
turnip				8.9 \pm 0.7	8.5 \pm 0.6
fodder radish	180	1	1	7.4 \pm 0.3	6.9 \pm 0.3
white mustard	180	1	1	8.0 \pm 0.3	7.4 \pm 0.4

Table 3 : Coefficients of regression of fresh yield on dry matter content of diploid and derived tetraploid fodder crops

crop	diploid	tetraploid
perennial ryegrass	-0.51 **	-0.72 ***
italian ryegrass	-0.24 ns	-0.87 ***
red clover	-0.11 ns	-0.42 **
fodder beet root	-0.25 **	-0.32 ***
top	-0.22 **	-0.46 ***
stubble turnip	-0.32 ***	-0.27 *
top	-0.29 **	0.01 ns

Table 4 : Change of DM intake of fresh ryegrass when DMc rises with 1 %

reference	animals	increase of dry matter intake (g/kg metabolic weight)
Baert (1994)	steers	+ 0.3
Bruins (1990)	dairy cows	+ 1.8
Butris and Philips (1987)	steers	+ 2.5
Meissner et al. (1992)	sheeps	+ 4.0
Vérité and Journet (1970)	dairy cows	+ 1.6

Table 5 : Broad (Hb) and narrow (Hn) sense heritabilities of dry matter yield (DMY) and dry matter content (DMc) of diploid and tetraploid perennial ryegrass

reference	diploid				tetraploid			
	DMY		DMc		DMY		DMc	
	Hb	Hn	Hb	Hn	Hb	Hn	Hb	Hn
Baert (1994)	0.4	0.9	0.7	1.0	0.7	0.9	0.8	0.7
Frandsen (1986)	0.4	0.4	0.8	0.6	-	-	-	-
Reheul and Baert (1991)	-	-	-	-	0.9	-	0.7	-

Table 6 : % of tetraploid perennial ryegrass in mixtures with diploid perennial ryegrass in different years after sowing

n years after sowing	sowing ratio 4x/2x	n mixtures	n analyses	management					
				grazing	1 cut/ grazing	2 cuts/ grazing	mowing		
0	50/50	2	191				61		
1	50/50	2	293				66	50	66
		4	608						
		2	197						
2	50/50	2	298				50	58	
		4	571						
3	50/50	2	325		34		49		
		4	300						
		4	300						
5	100/0	4	300		94	93			
		5	248						
10	100/0	5	255	22	66				
		4	200						
12	100/0	4	200	21	63				
		4	271						
		4	150						

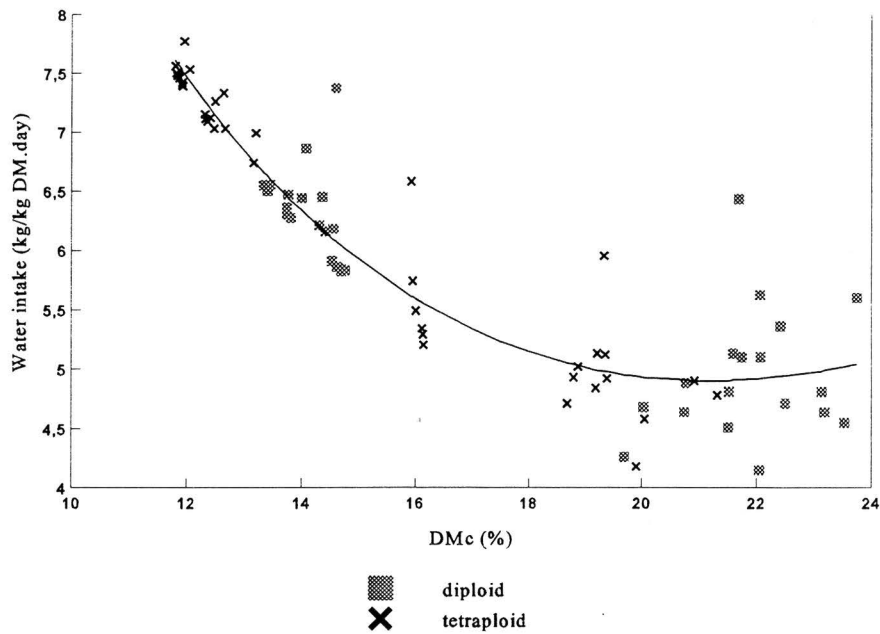


Figure 1 : Relationship between dry matter content (DMc) and total water intake by steers, fed on fresh diploid and tetraploid perennial ryegrass
 $y = 100/x + 0.79 - 0.28x + 0.01x^2$ $R^2=0.69$ ***

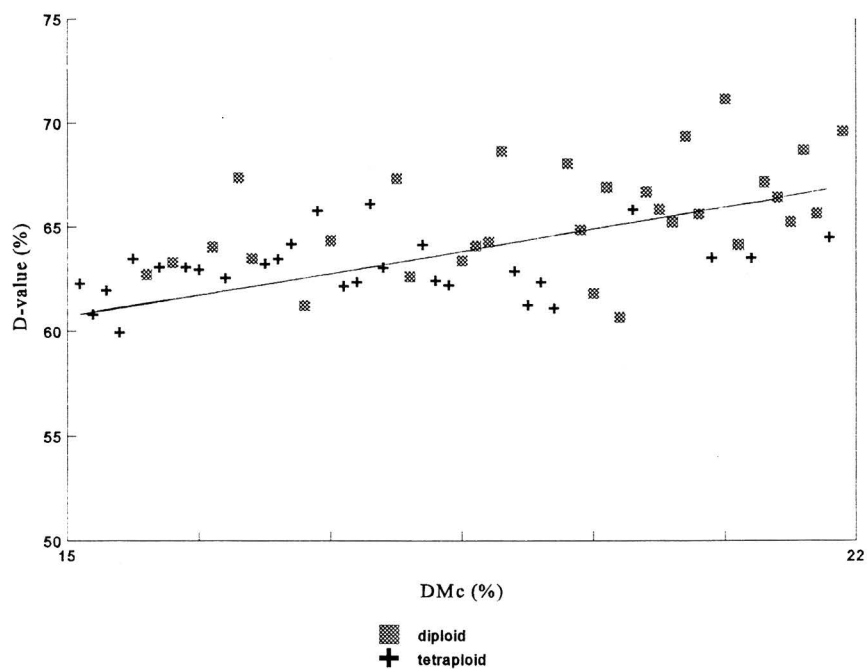


Figure 2 : Relationship between DMc and D-value of 32 diploid and 32 derived tetraploid clones of perennial ryegrass, in the vegetative stage
 $y = 42.5 + 1.2 x$ $r = 0.32 ***$

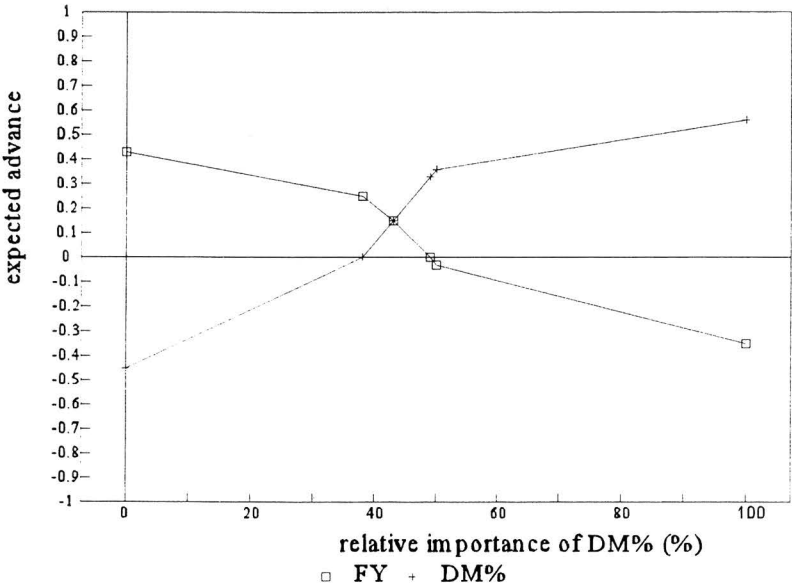


Figure 3 : Expected advance (expressed in units standard deviation) for DMc and FY of a diploid population of perennial ryegrass as a function of the relative importance of DMc and a selection intensity of 5 %.
 $\sigma_{DMc}=2.5\%$; $\sigma_{FY}=43.6g$

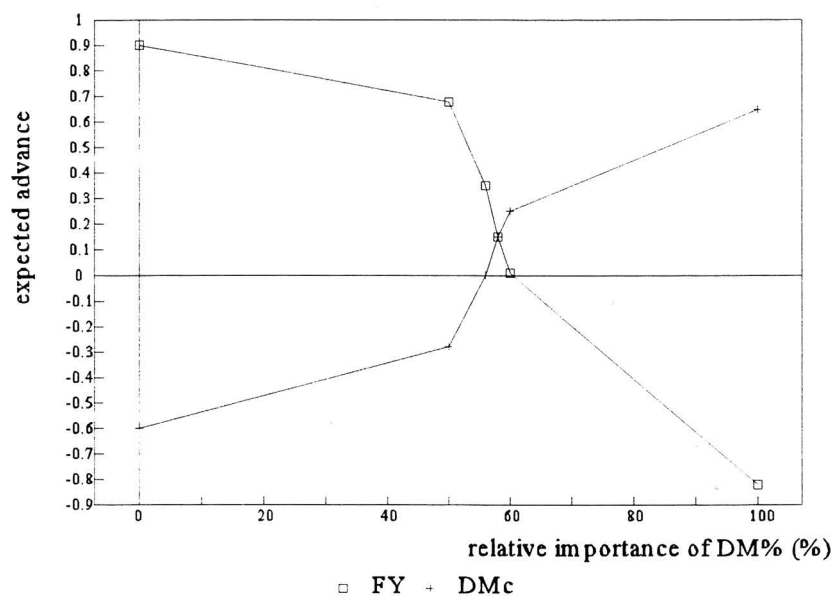


Figure 4 : Expected advance (expressed in units standard deviation) for DMc and FY of a tetraploid population of perennial ryegrass as a function of the relative importance of DMc and a selection intensity of 5 %.
 $\sigma_{DMc}=2.5\%$; $\sigma_{FY}=43.6g$

SELECTION PARAMETERS OF QUALITY TRAITS IN PERENNIAL RYEGRASS

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Summary

A set of 25 hybrids of perennial ryegrass originating from factorial crosses of 10 partial inbred lines was evaluated for 3 years at 2 locations for dry matter yield (DMY), digestibility (DMD), and protein content (PC). For these traits and the resultant digestible yield (DMDY) and protein yield (PY) variance components have been estimated. Genetic variance was largest for yield and digestible yield. Heritability (h^2_b) varied from 0.39 (DMD) to 0.57 (PC). Gain of selection was highest for yield and yield related traits. The hybrids were equal to their inbred parents in quality content components, but were superior by 5 to 6 % in all 3 yield traits. Correlation coefficients between yield traits were rather high ($r = 0.77$ to 0.94) and always positive, while those between yield traits and both quality parameters (DMD, PC) were negative. The implications of these results to practical breeding work is discussed.

Introduction

The main purpose of forage grasses is to provide inexpensive food for ruminants. Since long time grass breeders have been aware of the importance of forage quality on improving animal performance. The term forage quality is a broad term referring to a number of factors that affect nutritive value of a forage. Among these factors, dry matter digestibility is considered to be the most important one. Protein content seems to be of less importance than non-structural carbohydrates. (Wheeler & Corbett, 1989). A comprehensive review on the various aspects of quality in forage grasses was given by Marten (1989). Forage quality can be affected by management, environmental or genetic factors. Breeders are aware, that genetic variation must be present for a particular quality factor if breeding progress is to be successful (Hanna, 1993).

In perennial ryegrass (*Lolium perenne* L.) significant genetic variation for quality factors like *in vitro* digestibility and protein content was demonstrated by Rogers & Thomson (1970), Cooper (1973) and Posselt (1985). Heritability estimates varied according to the genetic material, evaluating conditions (spaced plants or dense sward), and type of statistical analysis (Rogers & Thomson, 1970 ; Harrison *et al.*, 1984 ; Posselt, 1985 ; Frandsen, 1986). Genetic variation and thus heritability were inconsistent over years (Rogers & Thomson, 1970 ; Frandsen, 1986 ; Posselt, 1985). In most studies, a negative correlation between yield and quality parameters has been found (Frandsen, 1986). According to this negative correlation it was ineffective to increase protein yield via protein content (Arcioni *et al.*, 1983). A set of hybrids were analysed to investigate selection parameters (variance components, heritability, gain of selection) of yield and quality components under field plot conditions.

Material and Methods

Genetic material

From a genetical broad base population 30 genotypes had been selected and selfed. The S_1 -lines were multiplied in isolation by open pollination. 3 sets of 5 x 5 factorial crosses were produced. The resultant SI-Hybrids, together with their parental lines were tested for 3 years in 2 locations in 5 sqm plots with 2 replications in a lattice design. (For details see Posselt, 1993). In this study only one set will be considered.

Quality analysis

Harvest time of the first cut was at the beginning of heading (on av. 23 % dry matter content). During harvest with a Haldrup a chopped sample of each plot was collected, weighted and dried at 70°C. After coarse grinding with a Brabender mill, fine grinding was done using a Cyclotec mill with 0.5 mm mesh size. Quality parameters like dry matter digestibility (DMD) and protein content (PC) were analysed by means of NIRS with a Technicon 400 R. Details of the calibration have been described elsewhere (Posselt, 1985). On the basis of the dry matter yields (DMY) of the first cut, digestible dry matter yield (DMDY) and protein yield (PY) were calculated.

Statistical analysis

Analysis of variance (ANOVA) was carried out using the adjusted means from the single harvest analysis. A split-plot in time model with years as subplots was applied to estimate variance components. Negative estimates were set to zero. Heritability was calculated on an entry mean basis (h^2_b), and as parent-offspring (PO) regression with $2b_{po} = h^2_n$ (narrow sense heritability). Gain of selection (G_s) was predicted according to $G_s = i \times h^2_b \times \sigma_{ph}$, with a selection intensity of $i = 1.345$ (i.e. 5 out of 25) and σ_{ph} being the phenotypic standard deviation (Falconer, 1981). The statistical analysis was done with PLABSTAT (Utz, 1989).

Results

Means and variances

In table 1 the mean values and the range of the 25 hybrids on average of 3 harvest years and 2 locations are given for the 5 traits under consideration. Except for PY, significant differences between entries could be found for all other traits.

In the ANOVA (see table 2) the genetic variance components - except for PY - were significant. At one of the locations (Ob. Lindenhof) almost none of the genetic variance components were significant, while at Hohenheim they were mostly much larger and highly significant. ($DMY = 10.38^{xx}$, $DMD = 0.38$, $PC = 0.27^{xx}$, $DMDY = 4.14^{xx}$ and $PY = 0.08^x$). Regarding DMY, both interaction components ($G \times L$ and $G \times Y$) were significant. In the single location analysis years were by far the largest source of variation. The year in location variance in table 2 indicates large environmental variation. For DMD most variance

component had negative estimates. PC was the only trait, where the 3-year-average differed between the two locations. For DMDY years were the largest source of variation. Genetic variance of DMDY was about two third of that of DMY.

Heritabilities & gain of selection

Broad sense heritability (h^2_b) and gain of selection (G_s) are given in table 3. All h^2 -estimates are of medium size with PC having the highest value. The estimates for DMY and DMDY are almost identical. Gain of selection is given as absolute (G_s) and relative ($G_{s\%}$) values. Both, DMY and DMDY have a comparable large phenotypic standard deviation and thus yielding the highest gain of selection. However, expressed as $G_{s\%}$ DMDY is superior compared to DMY. Because of the much greater standard deviation of DMD, the G_s with 0.56 % is somewhat higher as for PC. However, due to the very different mean values, $G_{s\%}$ of PC is four times greater than DMD. Because a negative variance component for PY was obtained, no heritability estimate was available and thus G_s could not be calculated.

Heritability of HS-families and by parent-offspring regression (b_{op})

Since the tested genotypes are fullsib families (FS), the marginal means correspond to halfsib (HS) family means. Besides parent-offspring regression, phenotypic (r_{ph}) and genotypic (r_g) correlation between the 10 parental lines and 10 HS-family means could be calculated. From the data in table 4 it can be seen, that the h^2_{HS} -values are always greater than those from the FS-families (see table 3). Heritability from PO-regression is rather high for DMY. For all other traits h^2_n is always inferior to h^2_b .

All phenotypic correlation coefficients (see table 4) are of a low size. Among the genotypic correlations only those for the 2 quality components seem to represent a realistic size.

Correlations between traits

From table 5 it is obvious, that all yield related traits are positively correlated. On the other hand, both quality traits (DMD, PC) are negatively correlated with yield. In table 5, only weak correlation coefficients between DMD and DMY or DMDY occur. However, in the single location (Hohenheim) analysis, a much stronger relation between these traits could be found ($r = -.56^{xx}$ for DMD vs DMY, and $r = -.47^x$ for DMD vs DDMY).

Parents vs hybrids

As can be seen from table 6, in yield and yield dependant traits, the hybrids are outyielding their inbred parents. While the crosses are slightly better in DMD (0.4 %) the opposite was found in PC, where the parents were superior (1.2 %).

Discussion

Among the forage grasses grown under the temperate conditions of NW-Europa perennial ryegrass is probably the most important one. Besides high yielding performance its

quality is believed to be better than in most other grass species. It has been well established that the content of the different quality factors (i.e. digestibility, protein content) in the dry matter are very much dependant of the growth stage of the crop. In this study, all genetic material belonged to the same maturity group (early to medium). The first cut was taken at the beginning of heading (i.e. conservation cut) with yields being about 50 % of the total annual yield. Although no preselection for any of the traits under consideration took place, large differences in the amount of genetic variance occurred. The variance components of yield and digestible yield were about 10 times greater than those of digestibility and protein content. Though in the combined analysis negative estimates were obtained for DMY and DMD, in general, years are the greatest source of variation. Genotype \times location and genotype \times year interaction for DMY were of the same order and twice that of the genetic variance, respectively, while none of these interactions were of any importance for DMD and PC. These findings correspond well with the summarized results given by Cherney & Volence (1992). Heritability on an entry mean basis (h^2_b) were of medium size (0.39 to 0.57) for the traits investigated. Frandsen (1986) too, found heritabilities for comparable traits in the range of 0.4 to 0.8 for drilled plots, while those from spaced plants were on a higher level (0.7 to 0.9).

Heritability estimates on the basis of HS-family means (see table 4) were always higher than those from the crosses (full sibs). The estimates (h^2_n) from parent-offspring regression were somewhat lower than the former. The estimate for DMD with $h^2_n = 0.30$ falls between those given by Harrison *et al.* (1984) with 0.06 and 0.14, and 0.63 (Frandsen, 1986). For DMDY Frandsen (1986) found very low h^2_n values, mostly below 0.1, while in this study $h^2_n = 0.24$ could be estimated. Compared to these rather low estimates, it should be mentioned, that from the single location experiment h^2_n was greater than 0.79 for all traits.

Using h^2_b (see table 3) gain of selection was predicted. For both quality factors only little genetic advance seems to be possible, though expressed as % of the mean, $G_{s\%}$ of PC was four times higher as compared to DMD. The G_s for both, DMY and DMDY were in the same order. However, no data are yet available to proof, whether these gains can be realized. In meadow fescue, Aastveit and Aastveit (1989) predicted a gain of 10 % for DMY, however, for spaced plant material. The correlation coefficients, presented in table 4, describe the relationship between S_1 -line performance *per se* and the performance of HS-families. For all yield traits very poor estimates have been obtained. The quality factors differ in sign, with PC having the highest coefficient of correlation. Regarding DMD, Harrison *et al.* (1984), too, found very low ($r = 0.22$), and non significant correlations. (The same situation occurred on the basis of all 30 S_1 -lines correlated to their respective HS-families). The conclusion which has to be drawn is, that it is not feasible to predict the performance of the progenies. It should be added, that in the single location experiment all r_g were close to 1. However, from this limited amount of data ($n = 10$) no meaningful conclusion can be drawn.

The interrelationship between traits (see table 5) is mostly as expected. The correlation coefficients between the yield traits are all positive and highly significant. Frandsen (1986) too, found a close relationship ($r = 0.99$) between DMY and DMDY. The correlation between DMD and DMY (DMDY) is unexpected low. However, Frandsen (1986) too, obtained very low correlation coefficients between these traits from a plot experiment, while those from the spaced plants were above $r = -.74$.

In this study for all three yield traits the hybrids were superior to their inbred parents i.e. heterosis occurred. Since heterosis and inbreeding depression are two sides of the same coin, the results agree well with the data from literature. Frandsen (1986), who compared F_1 with F_2 found inbreeding depression in F_2 for DMD and DMDY. Rogers & Thomson (1970) compared crosses with the S_1 and found inbreeding depression for all yield traits, and similar to this study a higher PC in the S_1 while the crosses were slightly better for DMD. For both quality factors Rogers & Thomson (1970) found, that sca was more important than gca. In this study too, for PC sca was as important as gca. For all other traits (data not given) sca was of minor importance.

Conclusion

The selection parameters found in this study cannot lead to overall recommendations, since they are only valid for the genetic material they derived from.

The only exception from generalisation is the negative correlation between quality factors and yield, which seems to be universal.

If in the crop of interest, high digestibility is already available, one should consider to regard digestibility a threshold character and improve DMDY by increasing yield.

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Table 1 : Mean values of yield and quality parameters of 25 hybrids (av. of 3 years & 2 locations, first cut)

	DMY dt/ha	DMD %	PC %	DMDY dt/ha	PY dt/ha
Min.	45.4	729	113	342	5.7
Max.	56.4	764	135	431	6.7
Mean	50.9	747	127	378	6.4
LSD5	5.9	24	9	48	1.0

Table 2 : Variance components of quality and yield parameters

	d f	D M Y	D M D	P C	D M D Y	P Y
Loc. (L)	1	0.39	0	3.21 ^{xx}	29	0.88 ^{xx}
Genotypes (G)	24	3.59 ⁺	0.45 ⁺	0.13 ^x	2.26 ⁺	0
G : L	24	3.02 ⁺	0	2	142	0.15 ^{xx}
Years (Y)	2	0 ¹⁾	0	5.27 ^x	14.94 ^x	86
Y : L	2	44.17 ^{xx}	62.71 ^{xx}	0.40 ^{xx}	1.40 ^x	0.81 ^{xx}
G x Y	48	6.83 ^x	0	4	197	7
G x Y x L	48	16.04	846	51	1205	36

⁺, ^x, ^{xx} significant at P = 0.1, 0.05 and 0.01

¹⁾ negative estimate

Table 3 : Heritability (h_b^2) and gain of selection (G_s , $G_{s\%}$) of yield and quality parameters ($i = 20\%$)

	h_b^2	G_s	$G_{s\%}$
D M Y	0.46	172 kg	3.37
D M D	0.39	0.56 %	0.74
D M D Y	0.45	135 kg	3.58
P C	0.57	0.36 %	2.83

Table 4 : Heritability estimates of 10 HS-families (h^2_b), by PO-regression (h^2_n), and PO-correlation (av. of 3 years & 2 loc.)

	$h^2_{b(HS)}$	$h^2_n = 2b_{PO}$	r_{ph}	r_g
D M Y	0.70	0.58	0.32	> 1.0
D M D	0.55	0.30	0.24	.33
P C	0.81	⁻¹⁾	-.08	-.63
D M D Y	0.63	0.24	0.13	< .01
P Y	0 ¹⁾	0.42	0.34	< .01

¹⁾ negative value

Table 5 : Correlation coefficients between traits (25 hybrids, 3 years, 2 locations)

	DMD	PC	DMDY	PY
D M Y	-.17	-.67 ^{xx}	.94 ^{xx}	.79 ^{xx}
D M D		.28	-.03	-.16
P C			-.70 ^{xx}	-.21
D M D Y				.77 ^{xx}

^{xx} significant at $P = 0.01$

Table 6 : Parents vs. hybrids

Trait	Parents	Hybrids	rel.
D M Y dt/ha	47.79	50.81	106.0
D M D %	74.45	74.77	100.4
P C %	12.86	12.70	98.8
D M D Y dt/ha	35.45	37.82	106.0
P Y dt/ha	6.13	6.42	104.7

IMPROVED DIGESTIBILITY AND PROTEIN CONTENT AS BREEDING PROBLEMS IN NORWEGIAN TIMOTHY (*Phleum pratense* L.) AND COCKSFOOT (*Dactylis glomerata* L.).

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Introduction

Breeding for quality has a relatively short history in forage grass breeding compared with breeding for productivity and persistency. It is well established that digestibility and voluntary intake of dry matter are the two major components of quality of fodder crops. Based on extensive experimental data, Ingalls *et al.* (1965) and Crampton *et al.* (1969) concluded that about 70 % of the variation in production potential among forages could be attributed to differences in voluntary intake, while 30 % could be explained by differences in digestibility. Grasses are often deficient in protein when they are cut for conservation. However, under pasture conditions the reverse is generally true.

The general primary characters, i.e. characters of direct economic interest, are dry matter yield (DM-yield), digestibility of total or organic dry matter, protein content, voluntary intake, persistency, stability and seed yield. Other characters, i.e. secondary characters, are of interest only if they can improve the primary characters through indirect selection. Our opinion is that the individual primary characters should be looked upon as components of a super-character, in index-selection terminology called the total economic value. The goal of breeding should be to improve this value. The most common breeding method in temperate grasses is multi-trait selection between and within populations constructed in one way or another. Progress of breeding for total economic value will then depend on the size of the phenotypic and genetic variation for each primary character, phenotypic and genetic correlations among the characters, environmental stability and economic weights associated with each character.

In this presentation we will report results from series of sward trials with half-sib families (HS) and local populations of timothy and cocksfoot conducted at four locations in Norway with very diverse climatic conditions. The objectives have been to estimate genetic variation and environmental stability of quality characters, and genetic relationships between quality characteristics and other characters as a basis for developing index selection procedures. We have also studied the differential changes of quality characteristics in cocksfoot as affected by plant developmental stage.

Materials and methods

A total number of 88 polycross progeny HS-families and 2 commercial cultivars (cv. 'Engmo' and cv. 'Grindstad') of timothy, and 76 cocksfoot HS-families, commercial

cultivars and local populations were laid out in sward trials at three locations in Norway ; Ås (60°N) and Løken (61°N) in South-East Norway, and Alta (77°N) in North-Norway. Each trial had 4 replications, and conservation cuts were taken 2-3 times in each of 3 growing seasons. Within each trial and cut, all plots were harvested at the same date. It is wellknown that both digestibility and protein content is highly influenced by the developmental stage of the plants. Differences in quality among entries cut at the same date will therefore partly be due to differences in heading time. In order to estimate the effect of developmental stage we conducted a separate trial with a sample of local populations and commercial cultivars of cocksfoot using small plots (1 m²). In this trial samples of each entry were taken at heading (anthers visible on 50 % of tillers), and 7, 14 and 21 days after heading. In order to remove the confounding effect of developmental stage, data from the sward trials was also corrected by means of covariance analyses, using heading time as an independent variable.

The Near Infrared Reflectance (NIR) method was used to measure the quality characters, i.e. *in vitro* digestibility of dry matter (IVDDM-%), crude protein content (CP-%) and crude fibre content (CF-%). The NIR-methods used have been described by Marum (1990).

Results and discussion

Sward trials

The combined analyses of variance over populations, years and locations demonstrated highly significant variation ($P \leq 0.001$) among populations both in timothy and cocksfoot for DM-yield, IVDDM-% and CP-%, except for DM-yield in cocksfoot ($P \leq 0.05$). Both timothy and cocksfoot had a higher stability over locations and years for the two quality characters than for DM-yield.

A detailed account of all the results from the sward trials will not be possible to present here. We will therefore present some selected parts of the results, mainly from the trials with timothy populations.

Table 1 gives the mean values and range of variation for DM-yield, digestibility and protein content observed for timothy at each location. The range of variation was about 5 tons/ha for DM-yield, 3-4 percentage points for IVDDM-% and 2-3 percentage points for CP-% at all locations. Table 2 presents heritabilities, and phenotypic and genotypic correlations, estimated for timothy at Ås. The heritabilities were high for DM-yield as well as for the quality characters. The genotypic correlations between DM-yield on one hand and digestibility and protein content on the other, were strongly negative, as expected. Both the strong positive genotypic correlations between digestibility and protein content, and the relatively high genotypic correlations between the quality measures and heading time are general findings representative for these plant materials. These correlations can partly be explained by the effect of developmental stage on changes in quality, since all families were harvested on the same day in these trials.

Effect of developmental stage on quality.

The overall differences between the 4 sampling dates were highly significant in both species for all the quality characters studied. Digestibility and protein content decreased by delayed cutting, while crude fibre content increased. Regression analyses showed that the linear as well as the quadratic components of the variation between the overall means at different sampling dates were highly significant in both species both for digestibility and protein content. The linear component did, however, in all cases take up more than 90 % of the sum of squares due to the differences between overall sampling dates. The following equations were estimated for cocksfoot :

$$\begin{aligned}\text{Protein (\%)} &= 20.68 - 3.71 t + 0.36 t^2 \\ \text{IVDDM (\%)} &= 80.40 - 3.28 t + 0.17 t^2 \\ \text{Crude fibre (\%)} &= 24.65 + 2.87 t + 0.22 t^2\end{aligned}$$

In these equations $1t = 7$ days.

The analysis of variance showed that the heterogeneity of the linear regression components was significant for digestibility of cocksfoot. The linear regression coefficients for IVDDM-% on heading time ranged from -0.2 to -0.50, demonstrating that the linear decrease in digestibility was different for different local populations. In Fig. 1 the observed decreases in IVDDM-% are presented for the two most extreme populations of cocksfoot in each direction. Population 12 and 16 exhibit a loss of digestibility of only 2-3 % over the 3 weeks period after heading, while population 1 and 28 are losing about 10 % of the digestibility in the same period. Clearly this ability to keep the quality after heading could be utilized in breeding for quality. There was a tendency to differential linear regressions for digestibility also among the timothy populations. The variance ratio for this item did, however, not reach the 5 % level of significance ($P \leq 0.07$).

Selection indices

Quality characters are no doubt important in the breeding of forage grasses. However, it is necessary to consider quality in relation to other characters, first of all DM-yield. In order to aid selection for increased economic potential, we have estimated optimal index values (see Baker, 1986) I_i , where

$$I_i = b_1X_1 + b_2X_2 + \text{-----} + b_nX_n.$$

In this equation, $X_1, X_2 \text{-----} X_n$ are phenotypic values of the characters. The index weights (b-values) are estimated on the basis of the relative economic weights associated with each character, and phenotypic and genotypic values, in such a way that the correlation between the index values and genotypic, economic potentials becomes optimal. The relative economic weights will vary with time and place, and as a preliminary test we have estimated index values based on four selection indices with different relative economic weights, as presented in Table 3. Index 1 is a pure selection for DM-yield, index 2 is a selection for digestibility with the relative weights on IVDDM-% : DM-yield : CP-% of

10:1:0. Index 3 is most in favour of quality with relative weights of IVDDM-% : CP-% : DM-yield of 100:100:1, while index 4 is similar to index 3, but with less emphasis on protein and more on dry matter yield.

The mean of the 10 families with the highest index values would give an indication of the properties of synthetic populations constructed by using these selection indices. This is demonstrated in Table 4 which shows the effect of different selection indices on DM-yield, digestibility and protein content, averaged over the 10 families with the highest index values. This selection is based on the data for timothy at the southern location Ås. Table 4 shows that selection indices 1, 2 and 4 would have only small effects on the three characters as compared with the dominating commercial cultivar 'Grindstad'. Index 3 would, on the other hand, have relatively drastic effects with an increase in digestibility and protein content of about 2-3 %. However, the better quality would have to be paid for by a reduction in DM-yield of nearly 3 tons/ha.

Index values were also estimated for each location separately. Table 5 shows the correlations between locations for index values. It can be seen that the correlations between Ås and Løken are quite good for selection indices 1, 2 and 4, while not for index 3. The correlations between Alta and the two other locations was very low, demonstrating the necessity of breeding cultivars with specific adaptation to the northern region of our country.

Adjusting for the effect of heading date by covariance analyses

Adjusting for the effect of heading date by covariance analyses, led to reduced heritabilities, reduced phenotypic and genotypic correlations (Table 6), and led to changes in the index values. The main conclusions are, however, the same.

Conclusions

Timothy and cocksfoot behave in a very similar manner as regards quality characteristics. There was a tendency towards higher phenotypic stability of digestibility and protein content, estimated over locations and years, than for DM-yield. Highly significant variation in digestibility and protein content were found between local populations harvested at the same developmental stage. The same was true for HS-families corrected by covariance analyses. The decreases in digestibility and protein content after the stage of heading can be described by a non-linear equation of second degree. Heritabilities of digestibility, protein content and DM-yield in the individual trials were of the same size. Significant interactions between families/populations and developmental stage were found for digestibility as well as for protein content, indicating the possibility of selecting for a low rate of decrease in quality after heading. Very high positive genotypic correlations were found between digestibility and protein content. Equally high negative genotypic correlations seems to exist in both species between DM-yield on one hand, and digestibility and protein content on the other. In order to predict the effect of selection, optimum selection indices have been constructed for four sets of economic weights. The results show clearly that it is possible to obtain significant responses to selection for digestibility and protein content, but it has to be paid for by reduction in DM-yield.

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Table 1 : Mean values and ranges for DM-yield (kg/decare), IVDDM-%, CP-%, coefficient of variation (CV) and R-squares (R²) for timothy at three locations in Norway.

	Ås			Løken			Alta		
	DM-yield	IVDDM	CP	DM-yield	IVDDM	CP	DM-yield	IVDDM	CP
Mean	1166	72.6	13.2	1345	72.1	14.0	1261	73.3	12.9
Range, from	914	70.4	11.8	1142	70.4	13.1	871	71.9	11.5
to	1406	74.5	14.8	1623	73.4	15.3	1455	75.4	14.6
CV	6.3	1.4	5.2	7.5	2.9	5.1	10.4	1.5	8.6
R ²	0.97	0.83	0.97	0.90	0.80	0.91	0.48	0.34	0.37

Table 2 : Genetic (above diagonal) and phenotypic (below diagonal) correlations and heritability estimated for timothy at Ås.

Characters	DM-yield			IVDDM-%			CP-%			Heading date	Heritability
	Cut 1	Cut 2	Total	Cut 1	Cut 2	Total	Cut 1	Cut 2	Total		
DM-yield											
Cut 1		0.62	0.73	-0.58	-0.32	-0.44	-0.46	-0.55	-0.49	0.09	0.45
Cut 2	0.43		0.98	-0.73	-0.87	-0.91	-1.00	-0.95	-0.97	-0.32	0.95
Total	0.64	0.97		-0.75	-0.87	-0.88	-1.00	-0.95	-0.97	-0.27	0.92
IVDDM-%											
Cut 1	-0.24	-0.55	-0.54		0.78	0.88	0.66	0.69	0.66	0.60	0.56
Cut 2	-0.19	-0.79	-0.72	0.56		0.98	1.00	0.97	0.95	0.37	0.87
CP-%											
Cut 1	-0.37	-0.84	-0.82	0.51	0.67	0.73		1.00	1.00	0.65	0.45
Cut 2	-0.34	-0.89	-0.85	0.48	0.86	0.83	0.86		1.00	0.32	0.85
Heading date	0.03	-0.24	-0.19	0.37	0.28	0.35	0.34	0.23	0.28		0.67

Table 3 : Economic weights for different selection indices.

Characters	Index no.			
	1	2	3	4
DM-yield	1	0.1	0.01	0.025
IVDDM-%	0	1	1	1
CP-%	0	0	1	0.5

Table 4 : Comparisons of the averages the 10 highest ranking timothy HS-families selected with each index and the dominating commercial cultivar, estimated from the trial at Ås.

Index	DM-yield	IVDDM-%	CP-%
1	1329	71.1	12.2
2	1332	71.4	12.2
3	1052	74.1	14.3
4	1328	71.5	12.2
cv. 'Grindstad'	1305	70.8	12.5

Table 5 : Correlations between index values for timothy at different locations.

Correlation between:	Index			
	1	2	3	4
Ås - Løken	0.78***	0.78***	-0.62***	0.67***
Ås - Alta	0.17	0.17	0.12	0.18
Løken - Alta	0.13	0.14	-0.07	0.13

Table 6 : Effects of covariate adjustments for heading date in timothy on heritability and genotypic (above diagonal) and phenotypic (below diagonal) correlations.

Characters	Heritability	Correlations		
		DM-yield	IVDDM-%	CP-%
<i>Not adjusted</i>				
DM-yield	0.92		-0.88	-0.97
IVDDM-%	0.88	-0.78		0.93
CP-%	0.89	-0.90	0.86	
<i>Adjusted</i>				
DM-yield	0.44		-0.58	-0.46
IVDDM-%	0.56	-0.24		0.66
CP-%	0.46	-0.37	0.51	

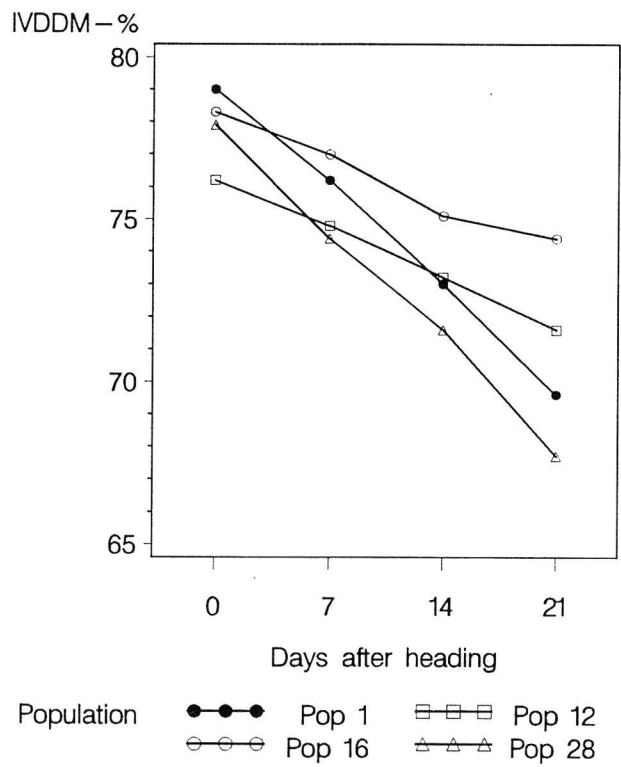


Figure 1 : Differences between 4 cocksfoot populations for rate of decrease in digestibility after heading

VARIATION AND INHERITANCE OF QUALITY OF *DACTYLIS GLOMERATA* VARIETIES WHICH WERE OBTAINED BY DIFFERENT BREEDING METHODS

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Summary

Our experiments tried to define the quality of forage of *Dactylis glomerata* by : carbohydrate, crude protein and cellulose. The biological creations were : synthetic varieties, hybrids and mixture hybrids. The hybrids which were obtained from male sterile sources seemed to be better than the synthetic varieties concerning crude protein and cellulose. The breeding method of hybrid mixture permitted to obtain improved varieties concerning dry matter yield and carbohydrates. Because broad sense heritability for crude protein was high, new breeding methods and biological materials for the improvement of this feature by genetical way could be investigated.

Introduction

The quality of the forage of different varieties of *Dactylis glomerata* is an amplified feature, which is defined by the digestibility of the forage, its palatability, the content of protein and cellulose, carbohydrates, Mg, Ca, Na content. On the other hand, the quality of the forage is indirectly defined also by the degree of the infection with different foliar diseases ; Schmidt's (1985) and Mika's experiences (1988) are conclusive in this sense.

The improvement of the forage quality has been tried in all perennial grasses species and also in *Dactylis glomerata* by increasing the content in Mg, Ca, Na (Sleeper, 1977 ; Saige *et al.*, 1989 ; Lentz and Buxton, 1991). These experiments showed that the variability of the content in these elements is an additive matter, their increase by genetic way being possible. Mika (1988) underlines the fact that the heritability for crude protein and carbohydrates is higher than for the green matter and dry matter and for all that, the breeding of these traits seems difficult enough.

At the present time, obtaining improved varieties of these species represents a major objective, because till now the improving of the forage yield was the most important aspect.

Material and methods

Our experiment tries to define the quality of the forage of different biological creations of *Dactylis glomerata* (the synthetic variety, hybrids obtained through cytoplasmic male

sterile plants and hybrid mixtures) by measuring the contents of carbohydrates, crude protein and cellulose.

The experiments were developed, in plots of 12 m² and in four replicates, in the field during 1987-1993, during two periods. The soil was very good provided with P, K and in every year uniform fertilisation was applied with 50 kg N/ha after each cut.

Two natural cytoplasmic male sterile plants were pollinated with a common pollinator, their fertility being restored.

The seeds of those two crosses represented the first generation of two hybrids which were compared with the variety 'Poiana' concerning the forage yield, the seed production, crude protein and cellulose (1987-1988). After the method of the hybrids mixture represented by Christie (1973) this became an other breeding method of these species, influencing directly the production in the first, second and following generations. So, four simple hybrids obtained by pollinisation of four clones with the pollinator 'Poiana' were mechanically mixed in equal proportion, obtaining the first (F₁) generation of a hybrid mixture. This creation was compared with the synthetic variety, which was obtained from the same mother clones, but by polycross method.

The pollinator 'Poiana' and the two biological creations were evaluated in plots of 6 m² in four replications as regards the forage yield and its quality in carbohydrates, crude protein and cellulose. The broad sense heritability was estimated by the formula proposed by Frandsen (1986).

$$h^2_B = \sigma^2_F (\sigma^2_F + \frac{\sigma^2_{FY}}{Y} + \frac{\sigma^2_{FC}}{C} + \frac{\sigma^3_{FYZ}}{YC} + \frac{\sigma^3_E}{YCR})$$

where σ^2_F , σ^2_{FY} , σ^2_{FC} , σ^2_E are variance components for families, families x years, families x cuts, families x years x cuts and error, respectively.

To underline the variability of the content of the carbohydrates, these were determined in 1992 at the third cut and in 1993 at the second cut.

Results

Analysing the data from the first table, the average of the three cuts, we observed that in 1987 the hybrids created by cytoplasmic male sterility had a content in crude cellulose lower than the synthetic variety 'Poiana' (at the same precocity). In 1988 the differences were more obvious between the hybrid A₄₁ and the synthetic variety 'Poiana', the limits of variation being from 28.1 % to 32.7 % (average of three cuts).

The crude cellulose as well as the digestibility being influenced by the number of the generative tillers of the first cut, the literature recommends for this feature the analysis of the second and the third cuts.

On the average at the second and the third cuts, the hybrids by cytoplasmic male sterility also had in 1987 and 1988 a lower content of crude cellulose than 'Poiana', what means that the forage is improved in this respect.

As regards the content in crude protein, the hybrid A₄₁ seems to produce a qualitative improved forage, the content of the average at the three cuts being superior to the synthetic variety 'Poiana', in both investigation years (table 1). The hybrid A₄₂ was situated lower than the value of the synthetic variety in both years.

As a result we are permitted to conclude that the forage production of the synthetic variety 'Poiana' could be overtaken with 6 % respectively 9 % (table 1) by the hybrids created by cytoplasmic male sterility. Their quality appreciated through crude protein is more difficult to be realised.

Mika (1988) underlines the fact that the tendency to increase the concentration of nitrate substances in the green forage by selection seems doubtful. This can be assured by agro-technical proceedings. Although the ecological protection of the agricultural ecosystems is a matter of actuality, that gives us the right to state that the improvement of the content in crude protein of the *Dactylis glomerata* varieties brings the support to this protective idea.

It's worthwhile to look for new hybrid combinations with these male sterile sources, which besides the forage production should also present obvious qualitative features.

In the second part of the experiment the quality of the forage offered by the mixture of simple hybrids and the synthetic varieties was studied through the value of the carbohydrates, crude protein and cellulose. Though the concentration of the carbohydrates is strongly influenced by the environment conditions, there is a special interest to increase their concentration by genetic way.

As can be seen in figure 1, the soluble and total carbohydrates alternated from one year to another as also between the different biological creations. The cumulative character can be noticed both at the soluble and the total carbohydrates. The limits of variation (soluble carbohydrates) were situated between 10.5 % to 11 % in 1992 and between 15.5 % tot 19 % in 1993. Because the soluble carbohydrates play an important part in the quality of the forage these will be more discussed.

In 1992 and 1993 the differences between the three biological creations in the respect of the soluble carbohydrates were not statistically assured. The differences between both biological creations which had the same mother clones were insignificant, that means that the paternal form and the additive effects probably had not an important part in the determination of the character.

On the total carbohydrates, the differences between the three biological creations, again were not statistically assured, in the sepearte years and in the average of the two years.

The possibility of improvement of this trait (soluble carbohydrates) by a genetic way seems to be possible taking in consideration the broad sense heritability (table 3).

The content in crude cellulose and crude protein of the biological creations with the common mother-form (the S-synthetic and the mixture of the simple hybrids) were very similar (table 2).

This aspect permits us to conclude that these traits had been determined mostly by the maternal line. The paternal form (the synthetic 'Poiana') contributed only to the improvement of the production forage, what was more obvious in 1993.

Especially in 1993 the mixture of simple hybrids had a lower content in crude protein with almost 1 % compared with the paternal form (15.7-14.5), meaning that in the situation of the analysed germplasm the effects of additivity probably had not been very important in determination of this character. In the same time, the content in crude cellulose increased with 1 % in the mixture of the simple hybrids, compared with the paternal pollinator.

The present experience let us to conclude that the improvement of the yield of the forage with one percent is much easier than the improvement of the contents including proteins.

The analysis of variance proved there were no significant differences between the three biological creations for cellulose and protein content. Nevertheless the analysed biological material is very valuable as a source of germoplasm, since the broad sense heritability varied between 0.11-0.584 for crude cellulose and protein (table 3).

Conclusions

The first part of the present experiment demonstrated that the use of the male sterile sources in breeding of these species is possible, obtaining improved varieties in respect of forage yield but the quality (protein) is more difficult.

The method of mixing the hybrids seems possible in the practice of breeding these species with the aim of increasing the production of forage but less in its qualitative improvement (protein, cellulose and carbohydrates).

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Table 1 : The variation of crude protein and cellulose of *Dactylis glomerata* varieties

Biological creation	DM t/ha		Rel. %		Average cuts 1987				Average cuts 1988			
					1 : 2 : 3		2 : 3		1 : 2 : 3		2 : 3	
	1987	1988	1987	1988	CC	CP	CC	CP	CC	CP	CC	CP
A41 x P	12.1	11.0	108	106	30.3	12.8	30.1	14.4	28.1	12.0	26.7	13.5
A42 x P	12.2	11.3	109	109	30.6	11.7	30.5	12.8	31.0	10.5	30.1	11.8
Poiana	11.2	10.4	100	100	32.4	12.0	31.3	13.6	32.7	11.6	31.9	12.8

CC = crude cellulose
CP = crude protein
A41 x P ; A42 x P = hybrids with male sterile
Poiana = synthetic variety

Tabel 2 : Forage yield, crude protein content and cellulose of *Dactylis glomerata* creations

Biological creation	DM t/ha		Rel. %		Average cuts : 2 and 3			
					1992		1993	
	1992	1993	1992	1993	CC	CP	CC	CP
Poiana	9.0	5.3	100	100	27.6	15.9	29.1	15.7
Synthetic S	9.1	6.7	101	126	28.6	15.4	30.0	15.0
Mixtures	9.4	6.3	104	119	28.7	15.6	30.1	14.5

CC = crude cellulose

CP = crude protein

Table 3 : Broad sense heritability for crude protein, cellulose and carbohydrates of *Dactylis glomerata* varieties in 1992-1993

Feature analyzed	(year x cut x rep.) broad sense heritability
Crude cellulose	0.100
Crude protein	0.724
Soluble carbohydrate	0.584

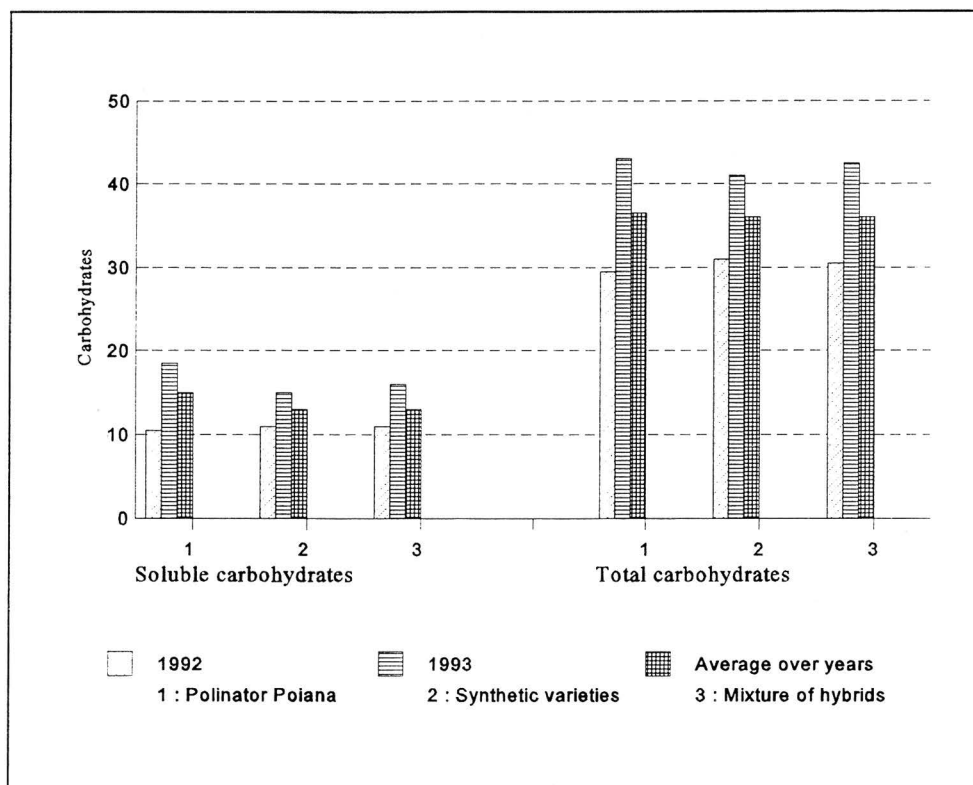


Figure 1 : The variation of carbohydrates of *Dactylis glomerata* varieties

VARIABILITY AND SELECTION OF GENOTYPES OF PERENNIAL RYEGRASS FOR NUTRITIVE VALUE USING NIRS

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Summary

An artificial perennial ryegrass population of Galician origin was evaluated in order to study the variation in forage yield and forage quality. Thirty five half-sib families were evaluated in a split-split-plot design (2 locations and 2 harvests). Significant differences were found between families in dry matter yield (DMY) as well as NIRS-predicted crude protein (CP), acid detergent fibre (ADF) and in vitro digestibility (IVOMD). Narrow sense heritabilities over all families were estimated to 0.37, 0.61, 0.65 and 0.47 for the characters DMY, CP, ADF and IVOMD, respectively. High negative genotypic correlations were obtained both between DMY and NIRS-CP and between NIRS-IVOMD and NIRS-ADF.

Key words : *Lolium perenne* L., nutritive value, yield, NIRS, heritability, correlations.

Introduction

The importance of the forage quality in improving animal production and the utilisation of fast analytical techniques have permitted the consideration of nutritive value as an objective in perennial ryegrass breeding. Near Infrared Reflectance Spectroscopy (NIRS) has been proposed as a tool to predict the nutritive value of forages in breeding programmes (Ostrem, 1988). Forage digestibility might be one of the most promising selection parameters for improved animal productivity from grassland since considerable variation has been found both between and within species (Harrison *et al.*, 1984). The aim of the present study was to determine the heritabilities of dry matter yield (DMY), NIRS-predicted in vitro organic dry matter (IVOMD), crude protein (CP) and acid detergent fibre (ADF) and the genetic correlations among these characters in a set of half-sib families.

Material and methods

The artificial population studied consisted of 35 half-sib families derived from late flowering plants taken from 17 wild Galician populations. All these families were evaluated in plot trials at two locations in Galicia : Mabegondo on the Atlantic coast and Puebla de Brollón in the interior. A split-split-plot design was used, with locations as main plots, harvests as split-plots and genotypes as split-split-plots. In this study only the harvests made on 3 May and 20 June of 1990 were considered. The harvests were taken at the start of inflorescence emergence. The samples were dried for 24 hours at 80°C. The dried samples were weighed and milled through a 1-mm sieve in a "Christy & Norris" hammer mill.

NIRS calibration equations were obtained by a stepwise procedure (Castro and Oliveira, 1994). In addition, all families were analyzed for laboratory IVOMD, CP and ADF.

The characters considered in this study were : DMY = Dry matter yield (t/ha), NIRS-CP = Crude protein-NIRS (%), NIRS-ADF = Acid detergent fibre-NIRS (%), NIRS-IVOMD = In vitro organic dry matter digestibility-NIRS (%). The data were subjected to analysis of variance and covariance, from which components of variance and covariance, heritabilities and genetic correlations were computed.

Results and discussion

Significant differences were found between the families in all characters. Limited ranges of variation were revealed between families. Narrow sense heritabilities estimates ranged from 0.37-0.65. High negative genotypic correlations were found between NIRS-CP and DMY (-0.70) and between NIRS-IVOMD and NIRS-ADF (-0.66). The negative correlation between NIRS-CP and DMY suggest the necessity of using a selection index, with the breeding objective being to increase simultaneously the crude protein and the yield. The high negative correlation observed between NIRS-IVOMD and NIRS-ADF indicates that any of these characters can be used to increase digestibility in perennial ryegrass. NIRS is an aid in breeding perennial ryegrass because it provides to the breeder a valuable screening tool for quality characters. However, it will always remain necessary to confirm NIRS results of promising genotypes by in vitro analysis.

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Table 1 : Means, range of variation, phenotypic (VP) and genotypic (VG) variances, narrow sense family heritabilities (h^2N) and coefficient of variation (CV) for dry matter yield and quality characters.

Character	Mean	Range	CV	VP	VG	h^2N
DMY	1.61	1.08-2.30	19.62	0.032	0.012	0.37
NIRS-CP	11.12	8.99-12.97	7.73	0.153	0.094	0.61
NIRS-ADF	24.81	22.61-27.32	3.09	0.193	0.126	0.65
NIRS-IVOMD	77.53	73.14-81.82	1.20	0.167	0.079	0.47

NITROGEN X GENOTYPE INTERACTIONS FOR NITROGEN USE EFFICIENCY IN PERENNIAL RYEGRASS (*Lolium perenne* L.)

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Introduction

With intensive dairy farming, nitrogen utilisation of nitrogen from perennial ryegrass by dairy cattle is too low, mainly because the protein content of grass is higher than is needed for meeting the protein requirement of dairy cattle. Reducing the protein content of grass, can help to increase the nitrogen utilisation by cattle. This will lead to lower emissions of nitrogen to the environment. A reduction of the protein content of grass can simply be achieved by a reduction of the nitrogen application rate. However, this will reduce dry matter yields of grass considerably and is therefore a costly method of reducing nitrogen surpluses of intensive dairy farming.

Plant breeding may offer an alternative way of reducing protein contents while avoiding negative effects of a lower protein content on dry matter yield by increasing the production of harvestable dry matter per kg of nitrogen in the harvestable dry matter, i.e. by increasing the nitrogen use efficiency. High economic returns and high reductions of nitrogen surpluses may be expected when plant breeding should improve nitrogen use efficiency (Van Loo & Vellinga, 1994).

Until now ranking of genotypes or populations with respect to nitrogen use efficiency or components nitrogen use efficiency was similar at low and high nitrogen levels (e.g. Van Loo, Schapendonk & de Vos, 1992). From this it was concluded that selection for high productivity at low nitrogen inputs may be done at high nitrogen levels. However, here we will show that for some components of nitrogen use efficiency nitrogen level x genotype-interaction exists that warrant assessment of nitrogen use efficiency at high and low nitrogen levels.

Material and methods

Six populations collected at various sites in Europe were examined for cumulative dry matter production of 5 cuts of roots and shoots, tillering, leaf extension, leaf width, specific leaf area and nitrogen content. Specific leaf area is the leaf area per leaf dry weight. These populations were grown on hydroponics using the system of nitrogen addition as described by Schapendonk *et al.* (1990) and Van Loo *et al.* (1992). Two nitrogen levels were created : a high nitrogen level aiming at 4 % N and a low nitrogen level aiming at 2.7 % N. Nitrogen was added proportional to the expected average growth rate calculated with a simulation model. Cuts were taken at 2 cm at four-weekly interval.

The first cut was taken 8 weeks after sowing. At each cut not only shoots were harvested, but also roots. After the fifth cut, shoots and roots were harvested after 2 weeks. Leaf weight ratio was calculated from shoot and root production determined after the fifth cut as weight of harvested shoot divided by total harvested dry matter.

Results

Actual nitrogen contents in the leaves were slightly lower than the setpoint for the nitrogen addition, because actual growth rates were slightly higher than expected. Still, the aim of this method of nitrogen addition was reached, namely to create two different levels of nitrogen in the leaves without large differences in nitrogen content in the leaves between populations.

Large differences between the populations were found in the relation between leaf nitrogen content and harvested shoot dry matter (Fig. 1), leaf weight ratio (Fig. 2), specific leaf area (Fig. 3) and leaf area increase during the first 7 days after cut 5. At the high nitrogen level, population 4 shows the highest nitrogen use efficiency, since it reached the lowest nitrogen content and the highest harvestable dry matter production. However at the low nitrogen level populations 8 and 28 are performing similarly or even are superior to population 4, with respect to dry matter production, leaf weight ratio, specific leaf area and leaf area increase during the first 7 days after cut 5. Also, the nitrogen content of population 28 at the low nitrogen level was lower than that of population 4. This indicates that population 28 had a higher nitrogen use efficiency than population 4 at the low nitrogen level. Also, for components of nitrogen use efficiency like leaf weight ratio, specific leaf area and leaf area increase just after cutting such population x nitrogen level-interactions were found.

Conclusions

The present study shows again that considerable genetic variation exists for nitrogen use efficiency and its components. Further it was shown that the best populations at low nitrogen would not have been selected when only performance at a high nitrogen level would have been measured. So, selection for nitrogen use efficiency should not only be done at one, high nitrogen level, but also information on the performance under low nitrogen should be assessed.

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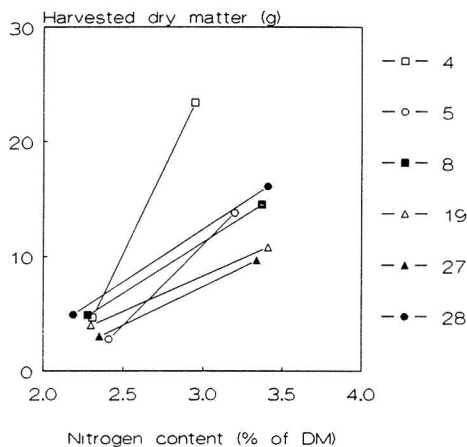


Figure 1 : Differences between 6 populations in cumulative harvested shoot dry matter of 5 cuts in g per 4 plants as a function of leaf nitrogen content. In all figures, points at the lower nitrogen contents are from the low nitrogen level and at the higher nitrogen contents from the high nitrogen level. Different symbols denote different populations.

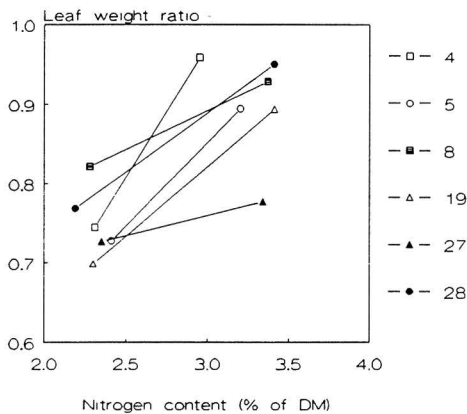


Figure 2 : Population differences in leaf weight ratio of cut 6 after a three week regrowth as a function of leaf nitrogen content.

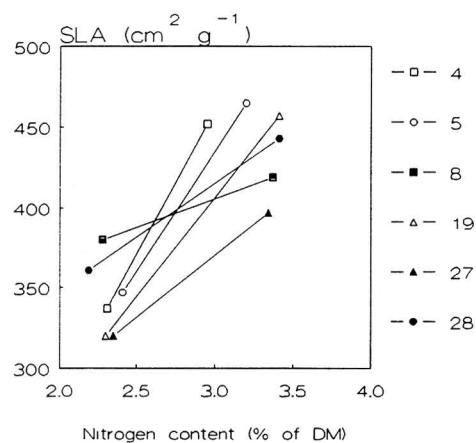


Figure 3 : Population differences in specific leaf area at cut 5, as a function of leaf nitrogen content.

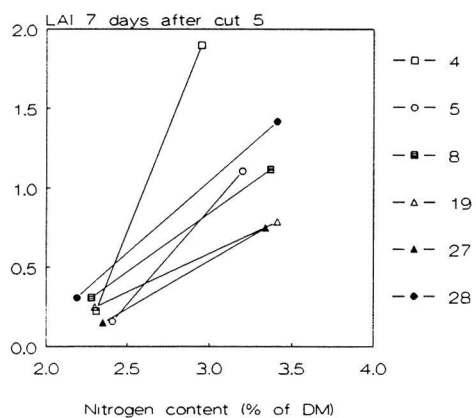


Figure 4 : Population differences in leaf area index 7 days after cut 5, as a function of leaf nitrogen content.

MULTITRAIT SELECTION IN A BREEDING POPULATION OF TALL FESCUE WITH FLEXIBLE LEAVES

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Abstract

A breeding population of tall fescue was developed by polycrossing 24 plants with flexible leaves of various origins. Half-sib families were evaluated for dry matter yield, nursery traits and digestibility. Genetic parameters allow the prediction of expected response to multitrait selection. Digestibility can be improved by about 1 % per cycle while keeping yield and seed production at the level of the best controls.

Introduction

Tall fescue is used as a forage and turf grass, particularly adapted to warm and dry area in Europe and in the USA. It is appreciated by farmers for its high dry matter yield and its good tolerance to both water deficiency and excess. However animal production is lower on tall fescue than on other grasses or legumes. This poor forage quality can be ascribed, at least partially, to the presence of the endophyte fungus *Acremonium coenophialum* (Fribourg *et al.*, 1991). But even among endophyte free material, genotypic differences do exist for palatability (Jadas-Hacart, 1982) or *in vitro* digestibility (Bughrara *et al.*, 1991). The aim of this study was to estimate heritability and genetic correlations among both quality and production traits, in order to predict the expected response to multitrait index selection.

Material and methods

A breeding population of tall fescue was made by polycrossing 24 elite plants, selected from a range of semi-inbred lines developed from various French natural populations. 2 to 4 generations of selfing were used to fix the most heritable traits such as crown rust tolerance or leaf flexibility, as suggested by Buckner and Fergus (1962). Leaf flexibility has been shown to be correlated with palatability and digestibility (Gillet and Jadas-Hecart, 1965).

The 24 half-sib families were evaluated in replicated plots for dry matter yield, leaf flexibility, digestibility and persistence, and as spaced plants for crown rust, heading date and seed production. *In vitro* digestibility of Tilley and Terry (1963) was predicted by multiple regression from Near Infra Red Spectrometry data according to Riviere *et al.* (1989).

Results and discussion

One half-sib family out of 24 was determined to be infected by *Acremonium coenophialum*. This progeny gave the highest yield and was discarded from further analyses. Evaluation data of the 23 remaining families and of the two control cultivars are presented in table 1 : the breeding population shows intermediate values for yield, persistence and digestibility, while it compares with the best control for leaf flexibility or seed production. The most significant correlations are between spring and summer-autumn yields (0.69) and between yields and persistence (0.71 and 0.87, respectively). Digestibility is only slightly correlated to heading date (0.32) and seed production (-0.37), while leaf flexibility is negatively correlated to heading date (-0.50) and to rust tolerance (-0.56). These last three correlation are not favorable to breeding objectives. It would therefore be possible to simultaneously improve dry matter and seed yields as well as quality traits, but problems may occur with rust tolerance and leaf flexibility.

Table 1 shows the values of heritabilities and of the expected responses to a 30 % family selection and to a 10 % mass selection, respectively for plot traits and single plant traits, using a Smith-Hazel index with equal weights.

All traits have high heritability values, of the same magnitude as those reported by Nguyen *et al.* (1982) or Bughrara *et al.* (1991), except rust tolerance and seed production. The expected responses to selection are moderate for yields and seed production (this latter in the wrong sense), and fairly good for persistence, heading date and digestibility.

The expected value of the progenies of selected plants indicate that it is possible to combine high digestibility and palatability with dry matter and seed yields at the level of the best controls, presently available in different cultivars.

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Table 1

	PLOT CHARACTERS						NURSERY CHARACTERS		
	Spring DM yield t/ha	Summer Au- tumn DMY t/ha	IVDMD (NIRS) %	Leaf flexibility 1-9 score	PER- SISTENCE 1-9 score	Crown rust susceptib. 1-9 score	Heading date	Seed production g/plant (gramme)	
CLARINE (control for yield)	3.95	6.49	48.9	4.7	6.0	2.6	06 May	67	
SOPLINE (control for quality)	3.79	4.64	51.9	6.0	4.7	14.1	10 May	58	
Average 23 Families	3.79	5.60	50.66	6.0	5.3	3.0	06 May	70	
Heritability	0.85	0.74	0.88	0.76	0.78	0.06	0.75	0.39	
Expected response to :									
- Family selection (30 %)	0.32	0.22	1.17	-0.05	0.87	-0.11	1.4	2	
- Mass selection (10 %)						+0.16	3.5	-5	
Expected average									
Next cycle progenies :	4.11	5.82	51.83	5.95	6.17	3.05	11 May	67	
- in % of CLARINE	104	90	106	126	103	117		100	
- in % of SOPLINE	108	125	100	100	131	74		115	

SESSION 6 : Laboratory techniques

VARIATION IN THE CARBOHYDRATE AND PROTEIN CONTENT OF RYEGRASSES : POTENTIAL FOR GENETIC MANIPULATION

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Summary

Animal nutritionists have emphasised the need for grass breeders to pay more attention to the composition of cell walls and cell contents to improve the nutritive quality of grasses. Water soluble carbohydrates (WSC) and crude protein (CP) are the main components of herbage cell contents. The range of genetic variation found among perennial ryegrasses for WSC is greater than that for CP or DMD and WSC often has a higher heritability than other quality traits. High WSC helps to buffer the loss of digestibility which occurs during summer. Also successful silage depends on the presence of sufficient WSC to allow good fermentation. High WSC promotes production of propionate during rumen fermentation which benefits animal growth and production. It also helps to ensure that readily available energy within the rumen are at levels which minimise loss of nitrogen to the environment. Genetic manipulation of protein content in grasses is complicated by strong interactions with nitrogen availability and uptake. However, potential exists to reduce proteolysis either by protecting proteins using secondary compounds or by reducing the activity of proteases. Genetic variation in the natural processes of leaf senescence may also be useful.

Introduction

Efforts by plant breeders to improve the nutritive quality of grasses have concentrated mainly on increasing overall digestibility (DMD). This is a complex trait and various strategies have been employed to produce varieties with improved DMD. For example, leaf/stem ratio can be a major determining factor in the digestibility of both primary reproductive growth and secondary regrowth as stems tend to have a higher lignified fibre content compared to leaves. An increase in leaf content has concerned breeders since the early days of ryegrass improvement (Stapledon *et al.*, 1927) and it continues to be an important objective in breeding improved varieties of Italian ryegrass (Hides, Lovatt and Hayward, 1983 ; Wilman *et al.*, 1992), hybrid ryegrass (Jones, pers. comm.) and perennial ryegrass (Wilkins, pers. comm.). Cell wall/cell content ratio also affects DMD and a larger cell size is probably an important factor in the generally higher nutritive quality of tetraploid ryegrasses (Lantinga, 1988 ; Baert and Carlier, 1988).

Recently, however, animal nutritionists have emphasised the need for grass breeders to pay more attention to the composition of cell walls and cell contents rather than just their relative proportions (Beever, 1993 ; Clark and Wilson, 1993). This is because efficient

utilisation of nitrogen by ruminant animals largely depends on the synchronisation of energy and protein release in the rumen to optimise microbial protein synthesis. This paper considers genetic variation in amount and composition of cell contents and reports on progress that has been made in breeding ryegrasses with increased WSC. The value of increased WSC in grassland and animal production and in promoting the efficient use of nitrogen by ruminants is discussed. Possibilities for the genetic manipulation of the protein content of herbage are also considered briefly. While recognising that great potential also exists for the genetic manipulation of cell wall structural carbohydrates and their association with secondary compounds such as lignin, space does not allow consideration of this complex subject within the present paper.

Variation in water soluble carbohydrate content

Water soluble carbohydrates (WSC) and crude protein (CP) are the main components of herbage cell contents. The range of genetic variation for WSC found among perennial ryegrass accessions was greater than that found for CP or DMD (Humphreys, 1989c) and WSC showed generally good heritability. Variation in average WSC content among 6 perennial ryegrass accessions is shown in Table 1. It is important to distinguish between changes in WSC caused by environmental effects (management and climate) and those arising from genetic differences. Environmentally caused variation in WSC is primarily due to adjustment of source-sink relationships and there is often a strong negative relationship between growth rate and WSC in this context. Cut means for the 6 varieties in Table 1 show that WSC contents fall rapidly in early spring as plant growth uses reserves stored in the previous autumn. During primary reproductive growth WSC accumulates in the lower stem internodes. In post-reproductive recovery growth levels fall again as new tillers emerge, although WSC may accumulate if growth is restricted by dry conditions. Despite seasonal fluctuations in WSC, genetic differences among varieties (Table 1) remain consistent and it appears possible to manipulate WSC levels independently of growth traits. The very early perennial ryegrass cultivar Aurora, based on an accession from the Zurich uplands of Switzerland, was identified as a particularly good genetic resource for high WSC (Humphreys, 1989a). Although there is a slight reduction in the CP content of this high WSC material, it is small compared to the increase in WSC so that the overall amount of cell content in the herbage is increased.

Manipulation of genetic variation in WSC

It has proved possible to transfer expression of high WSC from Aurora into later heading perennial ryegrasses without sacrificing dry matter yield (Humphreys, 1989b). The trait was also successfully incorporated into tetraploid ryegrass hybrids (Jones and Humphreys, 1993). A consistent genetic association was found between WSC content and genotype at the PGI-2 locus in *L. perenne* (Table 2, see also Humphreys, 1992) which could be useful for marker-assisted selection (Hayward and McAdam, 1988). This association between WSC and PGI-2 was also detected during introgression of genes from meadow fescue into Italian ryegrass (Table 3). Work is in progress using other genetic markers to elucidate this relationship further. It could be due to close linkage or, possibly, to a direct effect of PGI-2 which catalyses an essential reaction (the reversible isomerizati-

on of glucose-6-phosphate and fructose-6-phosphate) preceding the synthesis of sucrose and subsequent carbohydrate metabolism in plants.

The adaptive value of high WSC in plants

Stored WSC is used by plants to provide the energy and structural carbohydrate required for growth when demand cannot be met by contemporary photosynthesis e.g. regrowth after defoliation, recovery from drought and persistency during periods of low light intensity in winter, including snow cover. WSC may also be important in maintaining the osmotic potential necessary for water uptake during dry periods and in saline conditions. The role of WSC in freezing tolerance is still open to question and theories that it simply acts as an anti-freeze are now largely discounted. However the proximity of WSC within cells as a readily available and easily transported source of energy for metabolism is likely to be advantageous in cold as well as dry conditions. In general, good storage of WSC is necessary to maintain perenniality.

The value of high WSC for silage

The success of silage in conserving digestible energy and protein depends on good fermentation of readily available WSC by lactic acid bacteria. A minimum of 3.7 % WSC in fresh grass (i.e. about 15 % of the dry weight) is required to ensure good fermentation without additives (Haigh, 1990). As the grass crop matures during primary reproductive growth, WSC accumulates in the lower stem internodes while the structural carbohydrates of stems become more lignified. This reduces cell wall digestibility and hence reduces the availability of structural carbohydrates as an energy source for the animal. An optimum balance between accumulation of dry matter and WSC and a decline in cell wall digestibility must be achieved in the timing of silage cuts. Work carried out by Wilman *et al.* (1992) showed that the Italian ryegrass Tribune has higher stem and leaf WSC compared to the control variety RvP (Lemtal) and that Tribune silage is more digestible and has a lower neutral detergent fibre content and fibrosity index than RvP silage. Hides, Lovatt and Hayward (1983) showed that the rate of decline of DMD in stems of a Tribune precursor (Bb1277) was slower than in RvP. High WSC concentrations can also buffer decreasing DMD due to declining neutral detergent fibre digestibility during summer growth of perennial ryegrass (Radojevic *et al.*, 1994). Therefore high leaf and/or stem WSC enables good quality silage to be made over a longer period during crop maturation. It also allows good quality silage to be made from cuts later in the growing season when WSC levels tend to fall.

The value of high wsc in grazed pastures

This is a complex area of animal nutrition. Together, WSC and CP comprise the cell content fraction of herbage which is completely digested by animals. The remaining cell wall fraction is more or less available as a source of energy for the animal depending on its digestibility. There is clear evidence that the performance of grazing animals (live-weight gain, milk yield etc.) is consistently related to digestibility and intake of herbage (Munro and Walters, 1986). Intake (eating rate and amount eaten) depends, to some extent, on

digestibility but other factors are also important. For example palatability, indicated by animal preference, can have a significant effect with increased consumption of grass containing high levels of WSC (Jones and Roberts, 1991). Another factor is that a high proportion of cell content to cell wall increases the degradability of grass during the early stages of digestion. This promotes rapid transfer of material out of the rumen and thus increases intake (Moseley and Antuna Manendez, 1989). These factors have been important in demonstrating the value of tetraploid ryegrasses for modern European grassland farming. Similar attributes are found in high WSC diploid ryegrasses, although they have a generally higher dry matter content compared to tetraploids which further increases ruminant intake of dry matter.

Much of the grass protein which enters the rumen is rapidly degraded. The nitrogen released by this process may be recaptured in microbial protein for later digestion if sufficient energy is readily available for microbial synthesis. Thus, efficient utilisation of nitrogen by ruminant animals largely depends on a synchronous supply of rumen degradable protein and readily fermentable carbohydrate to provide the amino acids and energy required for microbial growth (Beever, 1993). WSC is a good source of readily available energy in the rumen and thus helps to minimise loss of nitrogen to the environment. Rates of fermentation of a range of grasses in simulated rumen conditions are strongly associated with WSC content (Davies *et al.*, 1994). Among a range of grasses with WSC contents varying from 5.5 % to 29.6 %, the total volume of gas produced during 8h fermentation ranged from 43.3 ml to 68.4 ml and the rate of fermentation varied between 4.8 ml h⁻¹ to 10.5 ml h⁻¹. Correlations between WSC and gas volume and gas production rate were 0.80 and 0.82 respectively.

In the absence of sufficient carbohydrate, energy may be derived from the degradation of forage protein but this can lead to an excess of non-utilised N, often in the form of ammonia, which is excreted. This may occur in mixed swards of grass and white clover where WSC levels may be depleted during the summer due to increased shading. Evans, Humphreys and Williams (1994) found that, on average, the WSC content of grasses in monoculture was 16.2 % compared to 13.7 % for grasses growing with clover but that there could be up to a 6.3 % difference in summer. They also found that high WSC selections could help to compensate for this deficiency. The relative energy available from WSC and CP in grass/clover and grass only swards containing a high WSC grass (Ba 10727) and a low WSC grass (Ba11318) is shown in Figure 1. Equal availability of WSC and CP would give a digestible energy ratio of 0.67. The grass monocultures generally exceeded this value as did the grass/clover mixture containing Ba10727. However the mixture containing Ba11318 gave a consistently lower ratio which was nearer to 0.30. Although the excess energy available from CP compared to WSC may have little effect on animal production, which is consistently higher on clover based diets, it may result in low N use efficiency by the animal. Fermentation of structural carbohydrates in cell walls may not achieve the synchrony necessary to ensure that the maximum amount of nitrogen is retained as microbial protein by the animal rather than excreted to the environment.

The composition of the volatile fatty acids produced by fermentation in the rumen is also influenced by the availability of WSC in ingested herbage. High levels of WSC tend to

promote the production of propionate (a glucose precursor) which is thought to contribute more to animal growth and production than acetate which is more readily produced during rumen fermentation (Beever *et al.*, 1978). Beever (1993) also suggests that propionate production, stimulated by high WSC grasses, should reduce the loss of digested energy as methane and may substantially increase the protein content of milk.

Genetic manipulation of protein content

Genetic manipulation of protein content in grasses is complicated by strong interactions with nitrogen availability and uptake, although there is evidence that significant genetic improvement in nitrogen use efficiency may be obtained (Wilkins & Lovatt, 1989). Attention is also being paid to possibilities of altering protein composition to reduce rates of protein degradation in silage and in the rumen. Environmental pollution from nitrogenous animal wastes and silage effluent is one of the most intractable problems of livestock farming. A large amount of potential N pollution is derived from autocatalytic post harvest changes in forage. Although high WSC promotes efficient fermentation in the silo and the rumen, protein degradation still occurs at a rate which is generally too high to allow maximum utilisation of nitrogen. Microbial proteases have a major role in this but it is now considered that plant proteases may also be important. Potential exists to use various techniques of genetic manipulation to reduce proteolysis either by protecting proteins using secondary compounds, such as condensed tannins, or by reducing the activity of proteases. Genetic variation in the natural processes of leaf senescence may also be useful. Stay-green lines of grass have been characterised in which the stability of thylakoid membrane protein, which comprises about 40 % of the total leaf protein, is increased (Thomas, 1987). An average 6 % increase in CP was obtained over 8 cuts in stay-green ryegrass and the effect of this trait on protein degradation during ensilage and rumen fermentation is now being investigated.

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Table 1 : Mean WSC content of herbage in 8 successive cuts averaged over 6 cultivars (s.e.d. 0.97)

WSC %	27.4	13.9	21.0	18.0	18.8	25.3	18.4	16.5
Date	13 April	5 May	26 May	16 June	5 July	3 Aug	1 Sept	20 Oct

Mean WSC content of 6 cultivars averaged over 8 successive cuts (s.e.d 0.84)

WSC %	24.2	16.4	21.7	20.2	17.1	19.9
Cult.	Aurora	Majest.	Perma	Melle	S.23	Ba9795

Table 2 : WSC content of parents and F₂ progeny classified according to genotype at 3 isozyme loci

Isozyme genotype	Parent	F ₂ progeny		
		PGI-2	GOT-3	ACP-1
aa	13.3	12.1	13.9	13.7
ab		13.7	13.4	13.4
bb	16.2	14.8	14.3	14.5
aa v. bb	***	*	ns	ns

* P < 0.05; *** P < 0.001

Table 3 : WSC content and digestibility (DMD) of parent species and progeny of backcrosses into Italian ryegrass which retain isozyme markers introgressed from the meadow fescue parent.

		Parents	Backcrossed progeny		
			PGI-2	GOT-3	ACP-1
Italian ryegrass	WSC %	32.8	24.9	27.7	27.6
	DMD %	64.5	62.1	64.5	64.3
Meadow fescue	WSC %	24.6			
	DMD %	62.7			

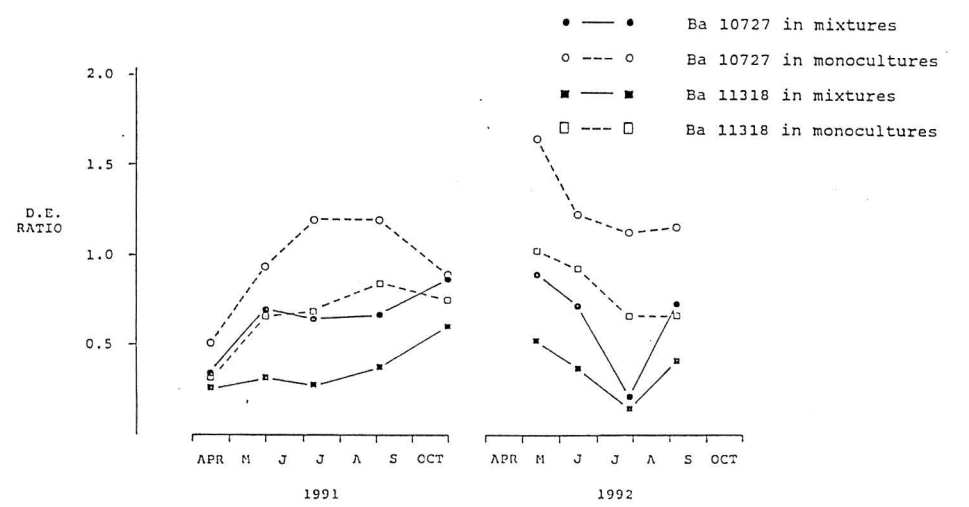


Figure 1 : Ratio of digestible energy (D.E.) of cell contents derived from WSC compared to CP

PURIFICATION, CHARACTERIZATION AND PARTIAL AMINO ACID SEQUENCE OF A PROTEINASE INHIBITOR FROM SNAIL MEDIC (*Medicago scutellata*)

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Summary

High levels of proteinase inhibitors in forage and pasture plants can control damage caused by feeding insects and overcome the use of chemical insecticides, so limiting costs and possible damage to the environment.

In order to test the efficacy of different proteinase inhibitors against phytophagous insects, a study has been conducted on various leguminous forage species of the genus *Medicago*, monitoring the activity of the Trypsin Inhibitor extracted from seed and aerial part of the plant. A Trypsin Inhibitor was purified from seed extracts of snail medic, *M. scutellata*, by ion-exchange and gel-filtration chromatography. This inhibitor belongs to the Bowman-Birk inhibitor family and has the closest homology with the wound-induced Trypsin inhibitor from alfalfa leaves. Due to its high level of inhibitory activity in the seed and to other properties, like thermostability, this molecule could be interesting, after isolation of the corresponding MsTI gene, for a possible future utilization as natural insecticide and in obtaining resistant transgenic plants.

Introduction

Naturally occurring proteinase inhibitors are proteins of low molecular weight, widely distributed in the plant kingdom, that can repress the activity of proteinases by binding into the active site of the enzyme. High concentrations of proteinase inhibitors are often found in plant tissues particularly vulnerable to foreign proteinases such as seeds, tubers, bulbs, particularly in Gramineae, Leguminosae and Solanaceae (Ryan, 1981). Proteinase inhibitors have been reported as insect pest resistance factors that act by repressing the activity of proteinases in the digestive tract of feeding insect larvae, so retarding their development. In some cases proteinase inhibitors are developmentally regulated, in others, as in alfalfa, they are wound-inducible, often leading to a systemic response (Boulter, 1993). A considerable number of these inhibitors has been found in legume seeds, where they represent a large amount of storage proteins (5-10 % in many plant genera). The presence of proteinase inhibitors in seeds used as food or feed and their potential toxic nature is regarded as a major fact contributing to their decreased nutritional value.

High levels of proteinase inhibitors in forage and pasture plants can however control damage caused by feeding insects and overcome the use of chemical insecticides, so

limiting costs and possible damage to the environment. In crop protection recent advances in plant molecular genetics are offering the possibility of a much improved means of biological control : transgenic pest-resistant plants. Proteinase inhibitors from plants proved to be effective insect-resistance factors, and genes coding for these inhibitors have been incorporated in transgenic plants and showed to inhibit the growth of feeding insect larvae (Hilder *et al.*, 1987 ; Mc Manus *et al.*, 1993).

In order to test the efficacy of different proteinase inhibitors against phytophagous insects, a study has been conducted on various leguminous forage species of the genus *Medicago*, monitoring the activity of the Trypsin Inhibitor protein extracted from seed. The highest content has been found in seeds of snail medic (*Medicago scutellata*), an annual pasture species showing high level of insect resistance. The specificity and partial amino acid sequence of the Trypsin Inhibitor purified from *M. scutellata* seed extracts and its homology with other inhibitors are reported, also considering a possible relationship between Trypsin Inhibitor content and insect resistance in this species.

Materials and Methods

Fourteen accessions of different species of the genus *Medicago* showing high levels of resistance to insect pests and environmental stresses have been characterized for the occurrence of Trypsin Inhibitory Activity (TIA) in the seed. The different strains of the genus *Medicago* employed are listed in Table 1, differentiating ploidy level, growth habit, annual or perennial cycle and thousand-kernel weight.

Total soluble proteins have been extracted from seed meal and tested for trypsin inhibitory activity (TIA) using BApNA as chromogenic substrate, measuring the change in absorbance at 405 nm (Kassel, 1970). One Inhibitory Unit (IU) is defined as the amount of inhibitor which inhibits 1 mg of trypsin.

The samples were analyzed by non-denaturing acid-PAGE : TIA was revealed according to Uriel and Berges (1968) as modified by Tomé *et al.* (1981).

Purification of *M. scutellata* Trypsin Inhibitor (MsTI) followed successive steps : ion exchange chromatography in a S-Sepharose Fast Flow preparative column, then gel filtration in an FPLC apparatus. After reduction and carboxymethylation, MsTI was sequenced using an Applied Biosystem Sequencer. The amino acid composition after gas phase hydrolysis was performed using a JASCO HPLC.

Molecular mass of MsTI was determined by mass spectrometry. Similarities between the primary structure of MsTI and other proteins were searched with the Swiss Prot Data Bank.

Results and Discussion

A well detectable Trypsin Inhibitory Activity (TIA) has always been observed in all the seed samples of the *Medicago* genus analyzed. A previous survey on the trypsin inhibitor content in seeds of 25 *Medicago sativa* cultivars of different geographic origin showed wide variation for this seed protein fraction (Odoardi *et al.*, 1991). A wider range of variation has been detected in the present investigation, with significant differences among the species here studied. On the basis of the TIA data reported in Table 2, the 14 genotypes fall into at least three homogeneous groups. The first one comprises cultivars of the *Medicago sativa* complex, with a mean Trypsin Inhibitor content of 70.3 Inhibition

Units/100 g seed. The second group is represented by the two *M. sativa* spp. *falcata* cultivars, with the lowest level of activity, 22.9 IU on average. The third group is represented by the shrub types and the annual species, showing high TIA levels (mean value = 171.4 IU). Among annuals *M. scutellata*, the snail medic, showed the highest TIA (284.9 IU), exceeding more than 12-fold the mean TIA observed in the *falcata* genotypes. It should be noted that the snail medics are the largest seeded cultivars among annual and perennial medics (Piano and Francis, 1992). Also *M. arborea* and *M. truncatula* are fairly large seeded species. Thus the high level of TIA in the accessions belonging to these taxa could be related to the high content of storage proteins per seed and, therefore, to the necessity to protect highly appetizing seeds from insects and other pests.

The decrease in inhibitory activity after 5 min treatment at 100°C is also reported in Table 2. Serine proteinase inhibitors are in fact known as generally rather stable molecules, often resistant to heat, due to the high degree of cross-linking disulphite bridges (Ryan, 1981). Characterization of thermostability of Trypsin Inhibitors from these materials could help in identifying their chemical structure. A general considerable stability against heat denaturation has been found in all sample extracts boiled for 5 minutes. The highest stability (100 % activity recovered) has been detected in both *falcata* types, and in *Medicago sativa* spp. x *varia* cultivars, except Algonquin, that showed, with Sava, the snail medic, still more than 80 % Inhibitory Activity after heat treatment. In all other accessions the decrease of TIA was around 30-50 %, confirming the good level of thermostability usually shown by the trypsin inhibitors from seeds of *Leguminosae*, particularly the Bowman-Birk family of serine proteinase inhibitors (Richardson, 1991).

The *Medicago* accessions have been further characterized for the electrophoretic mobility of the trypsin inhibitors detected in the seed. The samples have been submitted to non-denaturing acid-PAGE, and the resulting banding patterns of the seed extracts from the 14 strains are represented in Figure 1. Differences have been observed for the number of bands showing trypsin-inhibitory activity in each sample, as well as for their relative mobility. Nine out of 14 samples have shown a single polypeptide band with inhibitory activity, though with differentiated mobility. The remaining 5 genotypes showed two or three inhibitory protein bands, with different mobility.

The presence of a particularly interesting Trypsin Inhibitor protein in *M. scutellata*, which showed the highest activity in the seed, together with the highest level of insect resistance in the field (Sorensen *et al.*, 1988), stimulated further studies on this protein.

The proteinase inhibitor present in crude extracts of snail medic has been purified from defatted seed meal following subsequent chromatographic steps. After extraction in acetate buffer (pH 3.8), preparative ion exchange chromatography allowed a 70 % recovery of Trypsin Inhibitor Activity in the soluble protein. The following step, gel filtration in an FPLC apparatus, gave rise to a highly purified Trypsin Inhibitor, now labelled as MsTI, used as such for the biological assay on insects and for the structural characterization.

The amino acid composition determined after acid hydrolysis resulted in good accordance with that of ATI, Alfalfa Trypsin Inhibitor, from *Medicago sativa* leaves (Brown *et al.*, 1985). In fact, the comparison of the two Tripsin Inhibitors (Table 3) confirmed the strong similarity between ATI and MsTI : in particular, 14 cysteine residues have been found in both Inhibitors, in accordance with the classical 7 disulphide bonds characteristic of the Bowman-Birk Trypsin Inhibitor family.

Automated amino-terminal analysis of reduced and carboxymethylated MsTI allowed, thus

far, the identification of the first twenty residues (Figure 2). By comparing the preliminary data of the MsTI sequence with those of other proteins in the Swiss Prot Data Bank, the polypeptide with the greatest similarity to MsTI again resulted the wound-induced Alfalfa Trypsin Inhibitor from *M. sativa* leaves. Of course the real homology will only be assessed when the amino acid sequence of MsTI, now under way, will be completed.

Another similarity of MsTI with the Bowman-Birk Trypsin Inhibitors is the molecular mass determined by Mass spectrometry : it resulted to be 6926 m/z, but the final sequence will confirm this value (Figure 3).

MsTI is a strong inhibitor of trypsin, since the apparent K_d for the formation of Trypsin : MsTI complex is 1.8×10^{-9} M, but it exhibits no measurable inhibitory activity toward chymotrypsin, as expected since the *M. scutellata* crude extract did not show any activity against chymotrypsin in the spectrometric assay.

On the basis of the primary structure so far determined, and from the inhibitory properties and the molecular mass, MsTI seems to belong to the same Bowman-Birk type inhibitors from *Leguminosae*, with the cysteine residues so far sequenced, and the reactive site (Arg16-Ser17) placed in the same position as ATI. Its importance in conferring the high level of insect resistance exhibited by this species must be confirmed through specific assays against the digestive enzymes from different insect species. A preliminary experiment using crude gut extracts from European corn borer (*Ostrinia nubilalis*) seems to evidenciate strong inhibitory activity of the purified sample of MsTI (data not shown).

The complete amino acid sequence and characterization of the MsTI should contribute to knowledge about the interaction of these types of proteinase inhibitors with insect enzymes. This could be useful for crop improvement against insect attack and could contribute toward better understanding of the possible role of some of the components involved in the defense mechanism of plants. Lastly, introduction of proteinase inhibitor genes into forage legumes genome using genetic engineering will lead to agronomically important pasture plants effectively resistant to insect attack, and stimulate deeper investigations on these molecules.

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Table 1 : Description of the 14 genotypes of the genus *Medicago* tested for Trypsin Inhibitory Activity (TIA).

Taxon	Chrom n°	Cultivar	Growth habit/Cycle	Seed weight g/100
<i>M. sativa</i> ssp. <i>sativa</i>	16-32	Delta	common/perennial	2-3
<i>M. sativa</i> ssp. <i>sativa</i>	16-32	Veroia	common/perennial	2-3
<i>M. sativa</i> ssp. <i>X varia</i>	16-32	Algonquin	rhizomatous/perennial	2-3
<i>M. sativa</i> ssp. <i>X varia</i>	16-32	Rambler	creeping/perennial	2-3
<i>M. sativa</i> ssp. <i>X varia</i>	16-32	Spredor	creeping/perennial	2-3
<i>M. sativa</i> ssp. <i>X varia</i>	16-32	Vertibenda	common/perennial	2-3
<i>M. sativa</i> ssp. <i>falcata</i>	16-32	Pavloszkaja-7	creeping/perennial	2-3
<i>M. sativa</i> ssp. <i>falcata</i>	16-32	WIR 44033	creeping/perennial	2-3
<i>M. arborea</i>	32-48	Hellas	shrub/perennial	9-12
<i>M. arborea</i>	32-48	Batna	shrub/perennial	9-12
<i>M. arborea</i>	32-48	Pietranera	shrub/perennial	9-12
<i>M. lupulina</i>	16-32	Virgo P.	common/annual	2-3
<i>M. scutellata</i>	30	Sava	common/annual	16-18
<i>M. truncatula</i>	16	Ascot	common/annual	4-5

Table 2 : Trypsin Inhibitory Activity (TIA) expressed as Inhibitory Units (IU) per 100 g seed and % residual activity of TIA after heat treatment (h.t.) of the *Medicago* genotypes tested.

Taxon	Cultivar	TIA IU/100 g seed	% recovery after h.t.
<i>M. sativa</i> ssp. <i>sativa</i>	Delta	57.6	68
	Veroia	76.2	65
<i>M. sativa</i> ssp. <i>X varia</i>	Algonquin	78.4	84
	Rambler	72.0	100
	Spredor	66.0	100
	Vertibenda	71.7	100
<i>M. sativa</i> ssp. <i>falcata</i>	Pavloszkaja-7	14.5*	100
	Wir 44033	31.3	100
<i>M. arborea</i>	Batna	159.0	57
	Hellas	188.4	53
	Pietranera	85.1	71
<i>M. lupulina</i>	Virgo P.	133.2	50
<i>M. scutellata</i>	Sava	284.9	80
<i>M. truncatula</i>	Ascot	177.6	67

* very low activity, probably underestimated

Table 3 : Amino acid composition of MsTI compared with that of ATI from *Medicago sativa* (Brown *et al.* 1985).

	MsTI from <i>M. scutellata</i>	ATI from <i>M. sativa</i>
Ala	2	3
Arg	4	2
Asp/Asn	4	5
Cys	14	14
Glu/Gln	4	3
Gly	0	1
His	1	2
Ile	3	4
Leu	1	1
Lys	4	3
Met	0	0
Phe	2	2
Pro	6	6
Ser	6	3
Thr	8	8
Trp	0	0
Tyr	2	1
Val	1	0

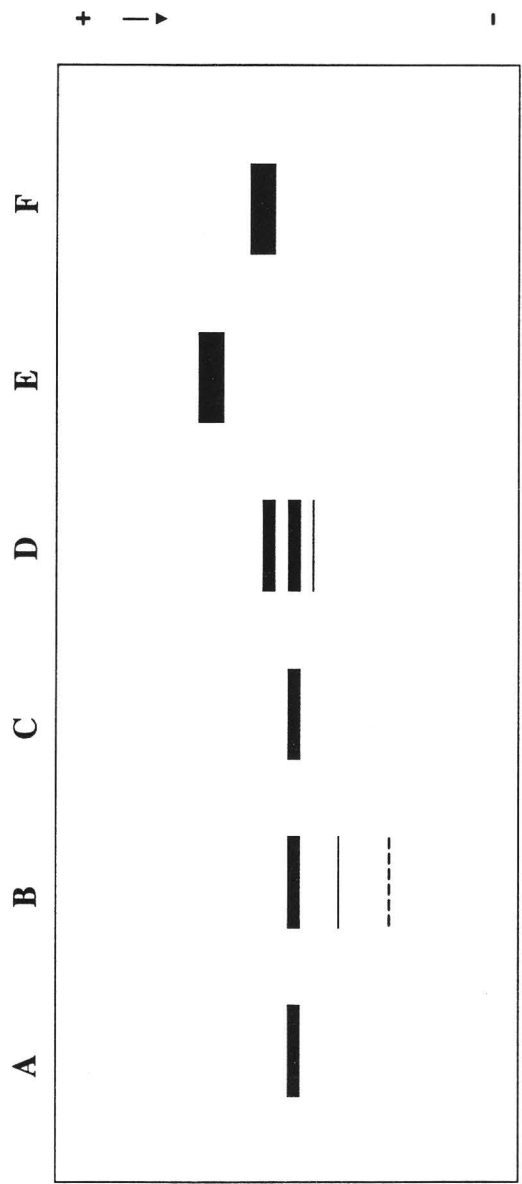


Figure 1 : Electrophoretic patterns of Trypsin Inhibitors of the *Medicago* species by aid-PAGE. Lane A : *M.sativa* ssp. *sativa* and *M.sativa* X *varia* ; B : *M.sativa* ssp. *falcata* ; C : *M.arborea* ; D : *M.lupulina* ; E : *M.scutellata* ; F : *M. truncatula*.

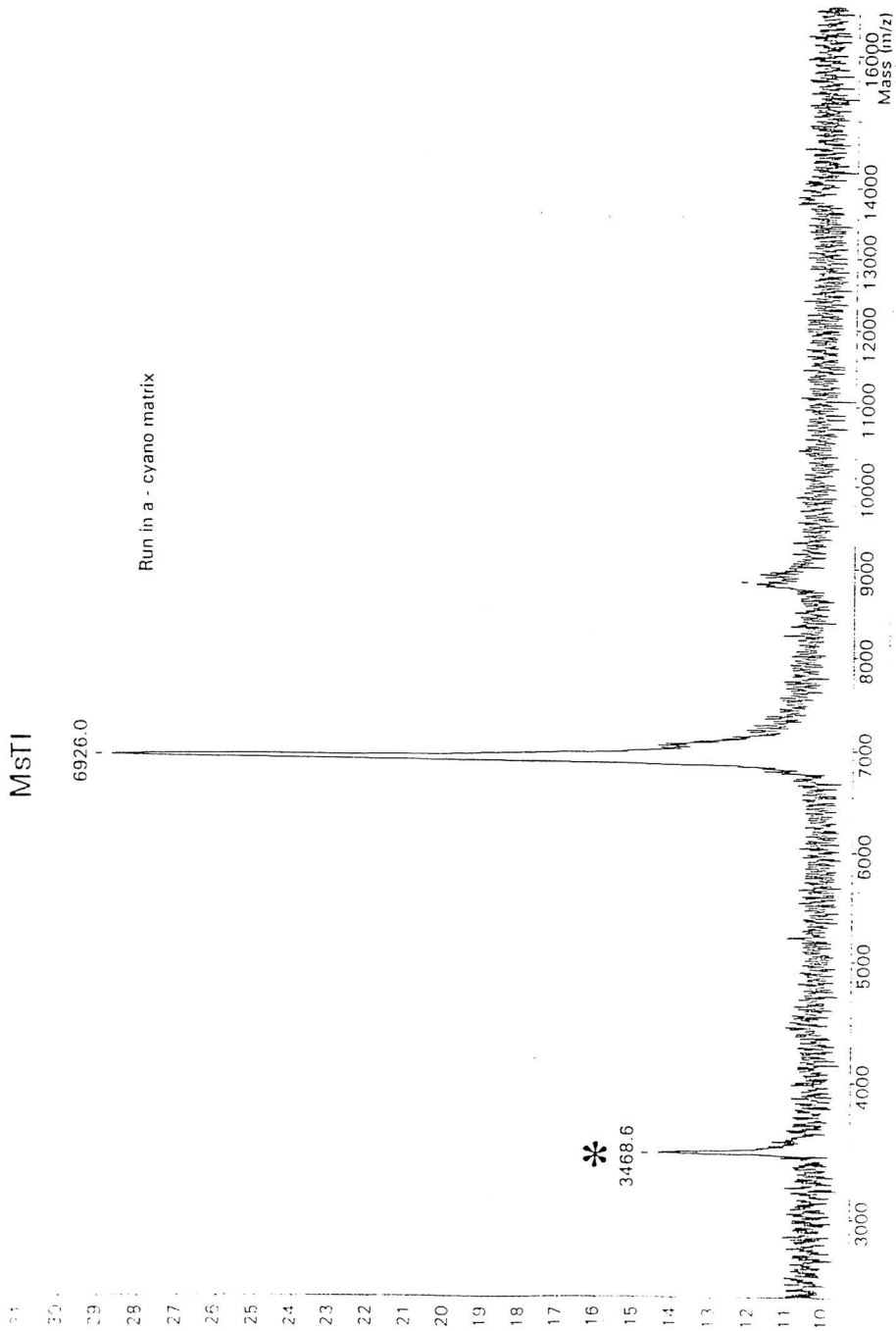


Figure 3 : Mass spectrum of MsTI after RP-HPLC. The asterisk * indicates double charge ion

IN VITRO SELECTION OF *FESTUCA PRATENSIS* (HUDS.) CALLUS ON THE METABOLITES OF *DRECHSLERA* SP. PATHOGENS.

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Leaf spot of *Festuca pratensis* are mainly caused by fungi of *Drechslera* sp. The appearance of these pathogens does not only decrease the yields but also worsens the quality of fodder. Some of these pathogens produce toxins harmful for people and animals (Shotwell and Ellis, 1976). The most common species of *Drechslera* sp. are : *Bipolaris sorokiniana* (Sacc.) Shoem. and *Drechslera dictyoides* (Drechs.) Shoem.

The purpose of this work was the selection of meadow fescue callus on the metabolites of *B. sorokiniana* and *D. dictyoides* and evaluation of resistance of regenerated plants to studied pathogens.

In the experiment, somaclonal variability additionally stimulated by mycotoxins as mutagenic factor was used.

Callus culture from ripe seeds of meadow fescue cultivar Skrzyszowicka was obtained on the MS (Murashige and Skoog 1962) medium supplemented with 5 mg of 2,4D (2,4-dichlorophenoxyacetic acid) per litre and 500 mg of caseine hydrolysate per litre.

Callus induction is performed in darkness and at temperature of 25°C. The obtained callus was passaged twice on the MS medium supplemented with 2 mg of 2,4D per litre.

Isolates of pathogens were selected in the Department of Fodder Plants in Cracow.

Callus selection was performed by means of double-layer culture technique described by Lepoivre *et al.* (1986). The first layer of medium was inoculated with 0,5 mm² mycelium pieces. *B. sorokiniana* cultures were incubated for two weeks while *D. dictyoides* cultures were incubated for three weeks. Both pathogens were developing in darkness and at temperature of 20°C. The second layer of medium was supplemented with Tilt fungicide of 1 ppm concentration in order to stop mycelium development.

In 48 hours weighed fescue calli were placed on the second layer of the medium. On the *B. sorokiniana* toxins 57 callus lines were tested while on the *D. dictyoides* metabolites 89 callus lines were tested.

After two week selection the studied and control calli were weighed again and placed onto regeneration medium MS with 0,2 mg of kinetin per litre. The plant regeneration took place at the temperature of 25°C with light intensity 40 µE/m²s.

Following the adaptation period the obtained plants were tested by spores suspension in order to test the resistance to the examined pathogens in the greenhouse conditions.

On the *B. sorokiniana* toxins a decline in calli fresh weight mass in relation to initial weight mass was recorded. The calli became slimy, brown and wet. However on the *D. dictyoides* metabolites a slight increase in calli fresh mass was noticed. The tested calli did not optically differ from control calli.

Twelve plants were obtained from selected on *B. sorokiniana* metabolites calli. Seven of them showed resistance to spores suspension inoculation in the greenhouse conditions.

Seven plants were regenerated from selected on *D. dictyoides* metabolites calli. All of them were insusceptible to spores suspension inoculation in the greenhouse conditions.

The selected form which did not demonstrate disease symptoms have been planted on the experimental field.

The double-layer culture technique is a simple method which enables the callus evaluation of the whole spectrum of the metabolites produced by the examined pathogen.

Lepoivre *et al.* (1986) used this method in their works on *Septoria* and *Cercospora* and Ahmed *et al.* (1991) on *Fusarium*. The results obtained in the experiment mentioned above are promising.

If the regenerated plants demonstrate a field resistance to the pathogens studied in this experiment, the double-layer technique can be considered useful in resistant grass cultivation.

Literature

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SESSION 7 : Variety development and assessment

BREEDING RED CLOVER FOR A REDUCED CONTENT OF FORMONONETIN

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Summary

Formononetin, a precursor of the estrogenic compound equol, is known to cause fertility problems in ruminants, especially sheep. Diploid red clover (*Trifolium pratense* L.) of the Swiss Mattenklees type has been selected for low formononetin content since 1983. Parent-progeny regression analysis revealed a high heritability ($h^2 = 0.70$) of the formononetin concentration which was confirmed by the realized gains from selection. Plot trials did not reveal a negative impact of selection for low formononetin content on important agronomic characters. An experiment in controlled environment showed that temperatures between 15 and 25°C and stage of growth did not change the ranking in formononetin concentration of the low and high formononetin selections as compared to check varieties. The differences were largest at high levels of formononetin, i.e. at lower temperatures and at early growth stages. The breeding programme resulted in the release of the cultivar FORMICA which was entered into the Swiss recommended list of forage cultivars in 1993.

Introduction

The association of fertility problems in sheep grazing clover-rich pastures with the presence of isoflavones with estrogenic activity in clovers was first detected in Australia in the late 1940's. Experimental evidence for both temporary and permanent negative effects on the fecundity and fertility of sheep is abundant. Barrett *et al.* (1965) in a long term study clearly demonstrated the progressive decline in fertility of a sheep flock continuously kept on a red clover pasture. The decline was due to more and more ewes becoming permanently sterile over the years. Morley *et al.* (1966) showed that grazing estrogenic clover for only 3 to 5 weeks was sufficient to seriously affect fertility of sheep if it took place in a critical period before mating. Formononetin is the most abundant isoflavone in estrogenic clovers. Shutt and Braden (1968) demonstrated that after ingestion by sheep, formononetin is readily metabolized to equol which in turn develops estrogenic activity. Hence, it was established that the level of formononetin in a clover-based diet is closely related to the risk of ruminant fertility problems. For practical purposes, a concentration of 3 g kg⁻¹ dry matter is considered a threshold level for estrogenic activity in sheep (Neil *et al.*, 1969).

The effect of estrogenic clovers on cattle is much less well documented than that on sheep. Austin *et al.* (1982) failed to prove a reduction in bovine pregnancy rate on a herd basis due to consumption of red clover silage. However, confirming other reports (Kallela *et al.*, 1984), they observed individual cases where a change from the red clover to a non-

estrogenic diet prompted conception of animals which had previously failed to conceive after repeated inseminations.

Efforts to reduce the formononetin content of red clover by plant breeding resulted in the release of 3 Australian cultivars between 1972 and 1992. Gosden and Jones (1978) developed a rapid technique to predict the formononetin content of red clover which was applied for selection within breeding populations of the Welsh Plant Breeding Station. A breeding programme with the aim of creating a low formononetin red clover variety of the Swiss Mattenkleee type - a form of red clover with outstanding persistence - was initiated at our research station in 1983. Here, we report on the progress in selection for low formononetin concentration and on associated effects on agronomic performance. We further investigated the effect of temperature on the expression of the genetic differences in formononetin content.

Materials and methods

Analytics

All samples used for subsequent analyses were freeze-dried and ground to pass a 1 mm mesh screen. The fluorescence method of Gosden and Jones (1978) was used to predict the concentration of formononetin.

Selection scheme

The base population used for selection consisted of a mixture of 8 previously unexploited local cultivars of the Swiss Mattenkleee type which had been selected out of a larger number of accessions according to their performance in field trials. Bi-directional selection took place on an individual plant basis in a spaced plant nursery. Elite individuals according to agronomic criteria (5 to 10 % of the population) were analyzed for their formononetin concentration in spring. Among these plants the 20 to 40 % individuals with the lowest (or highest) concentration were interpollinated, mostly by hand. Their progenies were again grown as spaced plants, and a number of elite individuals within each full-sib family was selected for the next cycle of analysis and selection. The same base population was subjected to a similar selection scheme without respect of the formononetin content. Candidate varieties were created as family polycrosses with progenies of open-pollinated elite individuals as components.

Evaluation in field trials

Agronomic performance of candidate varieties was evaluated in plot trials (9 m² plots) at 2 to 3 locations, each with 3 replications. The stands were cut 4 times a year to determine dry matter (DM) yield.

Growth chamber experiment

Plants were grown from seed in 3 l clay pots (5 plants per pot) in Conviron growth chambers at a photon flux density of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (16 h photoperiod) and day/night

temperatures of 15/10, 20/15, and 25/20°C. Harvests were taken when the plants had developed 8 fully expanded leaves (H_1 , 52, 42, and 35 days after sowing at 15/10, 20/15, and 25/20°C, respectively) ; when 50 % of the plants began flowering (H_2 , 82, 65, and 46 days after sowing) ; and at 28 days of regrowth after H_1 .

Results and discussion

Formononetin concentration proved to be a highly heritable character in the red clover population used for partly bi-directional selection. On average over three generations, parent-progeny regression analysis yielded an estimate of h^2 of 0.70 (Table 1) for the relative formononetin concentration (adjusted to the population mean of the respective generation). This high value was obtained with a single measurement of both the parent and the filial generation in spring of the respective first production year. Since formononetin values for the parents and for the progenies were obtained in different years and in different sets of experiments, restrictions to this type of heritability estimate outlined by Casler (1982) were minimal. A similar average value for h^2 (0.67) was estimated from the realized gain from selection measured on populations of the pooled progenies of the selected individuals.

The variety which resulted from two cycles of selection for low formononetin concentration successfully passed the variety trials for acceptance to the Swiss recommended list of forage cultivars (Mosimann *et al.*, 1993). This variety, named FORMICA, contains only 65 % as much formononetin as the average of all other cultivars recommended for Switzerland (Schubiger and Lehmann, 1994). We compared its agronomic performance to that of our breeding line (G8720) created by two cycles of selection from within the identical base population and to that of the standard diploid cultivar RÜTTINOVA. Annual dry matter yield of these three entries did not differ significantly although FORMICA tended to yield best in the first, but poorest in the second full harvest year (Table 2). This slight default, as compared to RÜTTINOVA, was offset by a markedly reduced susceptibility for powdery mildew (*Erysiphe polygoni*) and a tendency for a better resistance against *Sclerotinia* rot. When compared to the check variety selected from within the same genetic background (G8720), there was no net disadvantage in agronomic performance due to selection for low formononetin concentration. The high formononetin selection, however, yielded significantly less than both the check and the low variety in the first full harvest year (data not shown). The poor performance of the high selection was probably related to the narrow genetic basis (only 4 individuals selected in the second cycle) of this breeding line. The combined results of both the low and the high formononetin selection do not indicate a systematic relationship between formononetin concentration and agronomic characters.

Since leaves are known to contain markedly more formononetin than stems (Murray *et al.*, 1986), it might be expected that selection for either low or high concentration would result in a shift in morphologic characters relating to the plant's investment into leaves or stems. Leaf size apparently increased and stem length decreased due to selection for high formononetin concentration (Table 3) ; however, these changes were not reflected by an increase in the proportion of leaf to total above ground dry weight, nor was there an opposite trend due to selection for low concentration. Specific leaf weight of the check variety which had been selected without respect of the formononetin content, G8720, was

significantly lower than both the low and the high formononetin selection. These results show that although varieties selected for either high or low content differed significantly in morphologic characters, there were no consistent trends which would suggest a direct relationship with the formononetin concentration.

In the growth chamber experiment, neither temperature nor stage of growth affected the ranking in formononetin concentration of the low, high, and check varieties (Table 4). The low selection (FORMICA) always contained significantly less formononetin than the standard variety RÜTTINOVA. However, at 25/20°C, the difference was smaller, and G8720 did not differ significantly from FORMICA. The highest levels of formononetin were observed at the 8 leaf stage of growth; the lowest ones at the 50 % flower stage. At every stage of growth, the formononetin concentration of FORMICA was significantly lower than that of all other varieties. The range of formononetin concentrations among the varieties was smallest at the 50 % flower stage where the high selection (TP9145) did not differ significantly from RÜTTINOVA.

As expected from the literature (Murray *et al.*, 1986), the formononetin concentration in the leaf blades was markedly higher than in the stems and petioles (Table 5) for all varieties. However, the difference was clearly greater in the varieties with a high content. Apparently, genetically based differences in formononetin concentration are expressed mainly in the leaves. This explains partly why varietal differences diminish with progressive plant development when the proportion of leaves decreases in favour of the stems. Nevertheless, FORMICA contained significantly less formononetin in the stems than the check varieties except at 25/20°C where the trend was not statistically significant.

The small extent of the overall influence of temperature, stage of growth, and plant part on the differences among varieties agrees with the high values for h^2 and confirms that the formononetin concentration is a relatively stable, genetically controlled character. The differences are particularly well expressed at high levels of formononetin, namely, at lower temperatures and early stages of growth.

Conclusions

Selection for low formononetin content in red clover of the Swiss Mattenkleee type was favoured by a high heritability and by the lack of obvious correlations with important agronomic characters. Two cycles of individual selection were sufficient to create a variety (FORMICA) with a significantly reduced formononetin concentration and an agronomic performance which compares favourably to that of the currently most important cultivar of this type, RÜTTINOVA. A growth chamber experiment showed that the reduction in formononetin concentration was particularly evident under environmental conditions and at developmental stages where the content of estrogenic substances might become critical for ruminant fertility.

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Table 1 : Estimates of heritability (h^2) of the relative formononetin concentration based on either parent-progeny regression or on realized gains from selection.

parent generation	filial generation	heritability of formononetin concentration		
		parent-progeny regression ¹⁾		realized gain from selection ²⁾
		n	$h^2 \pm \text{s.e.}$	h^2
P	F ₁	24	0.69 ± 0.15	0.76
F ₁	F ₂	31	0.55 ± 0.40	0.55
F ₂	F ₃	39	0.86 ± 0.15	0.69
mean			0.70	0.67

¹⁾ $F_{\text{progeny}} = h^2 \cdot F_{\text{parent}}$ with F_{progeny} = mean formononetin concentration (relative to population mean) of the progeny from pair crosses, and F_{parent} = mean formononetin concentration (relative to population mean) of the two individuals used for the respective pair crosses, n = number of progenies

²⁾ $h^2 = R/S$ with R = realized gain from selection measured on populations of the filial generations and S = selection differential between the mean of selected parent individuals and population mean of the parent generation.

Table 2 : Agronomic characters of a red clover variety selected for a low content of formononetin, as compared to non-selected checks derived from either the same or a different genetic background.

	number of results	Variety		
		FORMICA	G8720	RÜTTINOVA
genetic background		same	same	different
selection for formononetin content		low	none	none
DM yield total of first full harvest year, t ha ⁻¹	5	15.5 a (102 %)	15.1 a (100 %)	15.2 a (100 %)
DM yield total of second full harvest year, t ha ⁻¹	2	12.8 a (96 %)	13.1 a (99 %)	13.3 a (100 %)
resistance against <i>Sclerotinia</i> score (1 = best)	6	2.1 a	2.4 a	2.4 a
resistance against <i>Erysiphe polygoni</i> score (1 = best)	7	2.8 a	2.7 a	4.5 b

values within a horizontal row followed by the same letter are not significantly (p = 0.05) different

Table 3 : Morphologic characters of red clover varieties selected for either low or high content of formononetin, as compared to non-selected checks derived from either the same or a different background

	Variety			
	FORMICA	TP9145	G8720	RÜTTINOVA
genetic background	same	same	same	different
selection for formononetin content	low	high	none	none
leaf size (cm ²)	23.6 b	26.2 a	23.7 b	22.9 b
proportion of leaf blades (% of above ground weight)	30.8 a	30.8 a	31.4 a	31.6 a
specific leaf weight (mg cm ⁻²)	3.28 a	3.27 a	3.04 b	3.18 ab
stem length (cm)	66.1 ab	63.1 bc	66.8 a	60.3 c

values within a horizontal row followed by the same letter are not significantly (p = 0.05) different

Table 4 : Influence of temperature and stage of growth on the formononetin concentration of red clover varieties selected for either low or high content.

	formononetin concentration (g kg ⁻¹ DM)			
	FORMICA	TP9145	G8720	RÜTTINOVA
genetic background	same	same	same	different
selection for formononetin content	low	high	none	none
<u>day / night temperature</u> ¹⁾				
15 / 10 °C	3.7 c	7.4 a	5.6 b	6.1 b
20 / 15 °C	4.2 d	7.6 a	5.6 c	6.7 b
25 / 20 °C	4.0 c	6.3 a	4.3 c	5.2 b
<u>stage of growth</u> ²⁾				
8 leaves	5.3 d	9.4 a	6.6 c	7.8 b
50 % flower	3.2 c	5.2 a	4.5 b	4.9 ab
28d regrowth	3.4 d	6.7 a	4.4 c	5.3 b

¹⁾ mean of three stages of growth ;

²⁾ mean of three temperature regimes. There was no significant three-way interaction of variety, temperature and stage of growth ($p = 0.76$) All main effects and two-way interactions were significant, with $p < 0.0001$ except for the variety x temperature interaction ($p = 0.02$)

values within a horizontal row followed by the same letter are not significantly ($p = 0.05$) different

Table 5 : Influence of temperature on the formononetin concentration of plant parts of red clover varieties at the 50 % flower stage.

		formononetin concentration (g kg ⁻¹ DM)			
		FORMICA	TP9145	G8720	RÜTTINOVA
genetic background		same	same	same	different
selection for formononetin content		low	high	none	none
<u>day / night</u>	plant part				
<u>temperature</u>					
15/10°C	leaf	4.2 c	9.8 a	6.8 b	7.2 b
	stem + petiole	2.5 b	3.7 a	3.9 a	3.6 a
20/15°C	leaf	4.3 b	9.1 a	7.0 a	7.6 a
	stem + petiole	2.5 b	3.6 a	3.6 a	3.7 a
25/20°C	leaf	4.6 b	7.6 a	5.4 ab	7.0 a
	stem + petiole	3.0 a	3.8 a	3.4 a	4.1 a

values within a horizontal row followed by the same letter are not significantly ($p = 0.05$) different

QUALITY AND QUANTITY PERFORMANCE OF LUCERNE MATERIALS IN RELATIONSHIP TO THEIR POSSIBLE USE FOR DEHYDRATION

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Summary

Cultivated lucerne is the most important forage legume in southern Europe, where its importance is raising as a result of the i) increase in public concern about the negative effects of monocropping and the consequent massive use of fertilizers, herbicides and pesticides ; ii) the increase in use of dehydration as a modern system of forage exploitation. The use of lucerne in modern rotations is based on the improvement of forage quality through intensive management such as the shortening of the cutting intervals. In the present study the effect of two cycles of phenotypic recurrent selection conducted on the landrace Casalina (Perugia) for tolerance to frequent harvest on dry matter yield and forage quality was assessed and compared to other landraces and commercial varieties. In a normal harvesting regime, yield and forage quality of the selected materials are not different from those of the control, while under a frequent cutting regime the selected materials seem to be characterized by an increase in dry matter yield and a forage quality not differing from the control. It is concluded that the increase of forage quality is more simply achieved by shortening the harvesting intervals. Moreover, valuable results can be obtained with breeding for tolerance to frequent cutting and with selection for post-regrowth after cutting. This last trait is of great importance in the attempt to decrease the dry matter losses as a result of the cutting at early stages.

Introduction

General overview

Cultivated lucerne (*Medicago sativa* L., $2n = 4x = 32$) is the most important forage legume in southern Europe where, during the last thirty years it has been grown in large areas despite a clear decline in the total cultivated surface area. However, its importance is starting to increase again as a result of the i) increase in public concern about the negative effects of monocropping and the consequent massive use of fertilizers, herbicides and pesticides ; ii) the increase in use of dehydration as a modern system of forage exploitation. In western Europe, lucerne dehydration started in 1951-60 with the main task of producing xanthophyll for poultry feed ; at that time the industrial product consisted of dehydrated meal. At the end of the fifties in France the I.N.R.A. started some experiments on cattle

feeding ; in the same period dehydrated meal was substituted by pellets and cubes, which are much more manageable. In the following decade considerable research was carried out on agronomic management of lucerne crops suitable for dehydrated forage production. During 1973-80 two subsequent energy crises negatively influenced the interest in dehydrated lucerne. Only in France did the production remain stable and new interest in its use in rabbit feeding started. At the end of the second energy crisis dehydrated lucerne resumed its importance with an interest which has increased up to present.

During the last period several studies were carried out, particularly on breeding for protein content, agronomic management, dairy cow feeding, production of protein concentrates and high density bales. The production and consumption in the European Union (EU) of dehydrated forages (of which lucerne represents the largest majority) is reported in Table 1. From 1976 to 1992 the production increased from 1.2 to 4 million metric tonnes (t) so that today there is no importation. The breakdown of the 1991 production of dehydrated forages is reported in Table 2. France is the most active producer, with 1,260 million t, 132 sites active in the dehydration process, and a share of almost 40 % of the total, followed by Spain, Italy and Germany. Actually, at the EU level 75,000 farmers are producing dehydrated forage on about 350,000 hectares, forage which is dehydrated in more than 350 production sites with 10,000 workers employed and about 150,000 livestock farmers using the product.

Breeding experience at the Istituto di Miglioramento Genetico Vegetale (IMGV-PG) and Istituto di Ricerche sul Miglioramento Genetico delle Piante Foraggere (IRMGPF, CNR-PG)

In Italy, IMGV-PG and IRMGPF, CNR-PG started a joint research project in the mid seventies whose main target was to have new materials adapted to a modern technology which requires lucerne stands able to give high quality forage at shortened intervals of cutting. The results of the research carried out for almost 20 years (Falcinelli *et al.*, 1978 ; Veronesi *et al.*, 1979, 1981, 1982, 1984, 1986, 1987, 1988, 1993 ; Arcioni *et al.*, 1980 ; Mariani *et al.*, in press), can be summarized as follows : i) phenotypic recurrent selection increased persistence of lucerne materials and both dry matter yield and crude protein yield within very frequent, frequent, and infrequent harvesting treatments ; ii) the reduction in dry matter yield related to the increase in cutting frequency is far less pronounced in selected than in control materials. As a consequence, the selected materials seem to be well suited for modern systems of lucerne exploitation.

With the aim of obtaining more information on this subject, two different trials were carried out in 1989 and 1990 in Perugia.

Materials and methods

Experiment 1

Sixty-seven clones from two cycles of phenotypic recurrent selection for resistance to frequent cutting (Selected, S) and 33 clones from the initial material (Control, C) were transplanted in the spring of 1989 as spaced plants in a field nursery and evaluated in 1990-92 for two harvesting time treatments : frequent, F (cut 5 times per year when plants

were 40-50 cm tall) and infrequent, I (cut 4 times per year at 1/10 bloom stage). A split plot design with 3 replications was used under rainfed conditions. The data reported regard dry matter yield (DMY) as a total from 1990 to 1992 (kg m^{-2}), percentage of crude protein (Nx6.25), meat and milk F.U. (Jarrige 1988) for the 1992 growing season.

Experiment 2

Twelve entries (7 varieties of which one was also sown with coated seed, 2 advanced materials and 2 landraces) (Table 3) were sown in the spring of 1990 at 40 kg ha^{-1} of viable germinating seeds in a randomized block design with 4 replications on 10 m^2 plots. Casalina and Ecotipo Umbro are two landraces still very common in Central Italy. The two advanced materials were derived from the landrace Casalina after 2 cycles of simple recurrent selection for seed yield (Casalina SY) and two cycles of phenotypic recurrent selection for tolerance to frequent cutting regimes (Casalina FC). Casalina FC was the same material utilized in Experiment 1.

The materials were grown under rainfed conditions and were cut when each entry reached full bloom : 2 times in 1990, 4 times in 1991 and 1992, respectively. Data reported regard total dry matter yield from 1990 to 1992, crude protein (%), meat and milk F.U. assessed at the first cut of 1992, as indicated in Experiment 1.

Results and discussion

Experiment 1

The total DMY of S materials was 54% and 32% higher than the DMY of material C under F and I harvesting treatments (Table 4). Materials S and C showed yield reductions of 23 % and 44 %, respectively from I to F harvesting treatments, while the DMY of material S under treatment F was 8 % higher than that of material C under harvesting treatment I. Materials S and C did not differ for CP, Milk and Meat F.U. within harvesting treatments, while data relative to harvesting treatment F were significantly higher than those of the treatment I.

Experiment 2

The dense stand experiment showed the presence of significant differences among entries for total DMY (Table 5). Over a period of three years coated Boreal produced 4.14 t ha^{-1} of dry matter more than Estival, the less productive entry. This corresponds to a difference of about 80 US dollars per year of net income per hectare and it clearly confirms the importance that farmers have to put in the choice of the right variety in their own environment.

The yield of the two landraces (Casalina and Ecotipo Umbro) was similar to that of coated Boreal and this explains well why Italian farmers still prefer the lucerne landraces. Furthermore, Casalina FC when cut at the full blooming stage did not differ from Casalina, confirming earlier results showing that, when the materials selected for frequent cutting tolerance are submitted to a normal system of exploitation, the differences in yield tend to disappear (Veronesi *et al.*, 1993). An experiment aimed at evaluating the behaviour of

control materials and materials selected for tolerance to frequent cutting from three cycles of phenotypic recurrent selection is in progress.

More research is also needed in order to better understand the differences in performance which could be a consequence of seed coating. The yield of coated Boreal was only 5 % higher than that of uncoated Boreal (31.82 vs. 30.20 t ha⁻¹) but it could be sufficient to look more closely at the consequence of seed coating, especially for short lived stands as those typical of lucerne grown for dehydration.

As far as the quality of the forage is concerned, the range of crude protein among entries was very narrow (from 19.88 % in Miral to 21.12 in Equipe) and no differences at all were noted for Meat F.U. (from 0.58 to 0.59) and Milk F.U. (from 0.67 to 0.68).

Conclusions

The increase of lucerne forage quality seems to be simply achieved through a modification of the exploitation system of the stand based on the shortening of cutting intervals. Moreover, valuable results can be obtained with breeding for tolerance to frequent cutting and with selection for fast-regrowth after cutting. This last trait is of great importance in the attempt to decrease the dry matter losses as a result of the cutting at early stages.

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Table 1 : EU production and consumption of dehydrated forages (million of metric tonnes) (C.I.D.E. 1992).

Year	Production	Consumption
1976	1.20	1.60
1980	1.55	2.00
1984	1.39	1.45
1988	2.60	2.61
1992	4.00	4.00

Table 2 : EU production in 1991 of dehydrated forages (metric tonnes x 1,000) and the number of sites active in the dehydration process (C.I.D.E. 1992).

Country	Production	Sites
Belgium	7	1
Denmark	330	20
France	1,260	132
Germany	400	71
Ireland	5	1
Italy	410	42
Netherlands	240	12
Spain	540	54
United Kingdom	80	21

Table 3 : Materials used in the 1990-91 dense stand evaluation trials.

Materials	Seed Companies or Research Institutes
<u>Varieties</u>	
Boreal	Pioneer Hi-Bred Int. Inc. (USA)
Boreal coated	Pioneer Hi-Bred Int. Inc.
Delta	Società Italiana Sementi s.p.a., Bologna (I)
Equipe	Ist. Sperim. Colt. Foraggiere, Lodi (I)
Estival	Pioneer Hi-Bred Int. Inc.
Miral	Pioneer Hi-Bred Int. Inc.
Robot	Ist. Sperim. Colt. Foraggiere, Lodi
Tornese	Pioneer Hi-Bred Int. Inc.
<u>Advanced materials</u>	
Casalina FC	IMGV-PG and IRMGPF, CNR-PG, Perugia (I)
Casalina SY	IMGV-PG, Perugia
<u>Landraces</u>	
Casalina	
Ecotipo Umbro	

Table 4 : Total Dry Matter Yield (kg m⁻²), average Crude Protein content (CP %), Meat and Milk F.U. (per kg of DM) in the 1990-92 seasons of selected and control materials in relation to frequent and infrequent harvesting treatments (HT).

Materials	HT	DMY	CP	Meat FU	Milk FU
Selected	Frequent	2.50 B	21.85 A	0.76 A	0.83 A
	Infrequent	3.08 A	18.84 B	0.66 B	0.74 B
Control	Frequent	1.62 C	22.23 A	0.76 A	0.81 A
	Infrequent	2.33 B	18.46 B	0.66 B	0.74 B

Means on the same column followed by different letters are different at $P < 0.05$

Table 5 : Total Dry Matter Yield (DMY, t ha⁻¹), average Crude Protein content (CP %), Meat and Milk F.U. (per kg of DM) in the 1990-92 seasons in varieties, landraces and selected materials of lucerne.

Materials	DMY	CP	Meat FU	Milk FU
Boreal coated	31.82 A	20.17	0.58	0.67
Casalina	31.39 A	20.75	0.58	0.68
Ecotipo Umbro	31.11 AB	20.54	0.58	0.67
Casalina SY	30.83 AB	20.95	0.58	0.67
Casalina FC	30.46 ABC	20.45	0.58	0.67
Tornese	30.30 ABC	20.07	0.58	0.67
Miral	30.23 ABC	19.88	0.58	0.68
Boreal	30.20 ABC	19.85	0.59	0.68
Robot	29.50 ABC	21.11	0.58	0.67
Equipe	29.49 BC	21.01	0.58	0.67
Delta	27.76 C	21.12	0.58	0.68
Estival	27.68 C	20.22	0.58	0.67

Means on the same column followed by different letters are different at $P < 0.05$

ALKALOIDS IN NORWEGIAN REED CANARYGRASS

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Introduction

Reed canarygrass (*Phalaris arundinacea* L.) has been of interest as a forage grass in Norway, especially in the coastal regions of West and North Norway. American and east european cultivars and local populations have shown to be high yielding and persistent. On soft peat soils the stolons increase the load bearing capacity and reduce soil damage by wheeling (Aase *et al.*, 1977 ; Østrem & Marum, 1989). A problem, however, is the content of alkaloids which can reduce forage quality and be harmful to ruminant animals (Simons & Marten, 1971 ; Marten *et al.*, 1976, 1981 ; Ulvund, 1985, Duynisveld & Wittenberg, 1993). In Norwegian breeding programmes the intention is to select plant material with less harmful types and lower alkaloid levels, in combination with high digestibility, yield and persistence.

Type and content of alkaloids in reed canarygrass

At least nine alkaloids have been reported in reed canarygrass, with total contents ranging from 0.01 to 2.75 per cent of dry weight (Marum *et al.*, 1979). The phenol p-hydroxyphenethyl-dimethylamine (hordenine) seems to be present in all plants in a rather high content, but has probably no harmful effects. More important are the indoles 3-dimethylaminomethyl-indole (gramine), N-methyltryptamine (NMT), N,N-dimethyltryptamine (DMT), 5-methoxy-N-methyltryptamine (5-MeO-NMT) and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), and the β -carbolins 2-methyl-1,2,3,4-tetrahydro- β -carboline (MTHC) and 2-methyl-6-methoxy-1,2,3,4-tetrahydro- β -carboline (6-MeO-THC). The pathways proposed by Marum *et al.* (1979) for the biosynthesis of indole alkaloids and their derivatives are shown in Fig. 1. Gramine which is present in most plants at various levels, is regarded as the least harmful (Marten *et al.*, 1981). For the other alkaloids, which may be harmful in low concentrations, a large variation in content appears among genotypes of reed canarygrass (Østrem, 1987).

Conditions such as moisture stress (Martens, 1973), high N fertilization, low light intensities (Frelich & Martens, 1972) and early cut (Woods & Clark 1971) may enhance alkaloid concentration. Concentration of indole alkaloids (per cent of DM) observed in first and second cut under different climatic conditions in west, north and central east Norway with cultivars and breeding lines from USA, Canada, Poland and Norway, showed a wide variation with growth conditions (Fig. 2). Highly significant differences for alkaloid content between the plant materials were found within time of harvest and locations. The

lower DM yield at the second cut contained a higher alkaloid concentration compared to the first cut. The prevailing humid coastal climate in western (Fure) and northern (Bodø) parts of the country resulted in lower concentrations compared to the drier eastern region (Løken). Moisture stress seemed to be the most important reason for the high alkaloid concentrations observed at Løken. Under such dry conditions the alkaloid concentration may reach levels that adversely affect animal health (Østrem and Marum, 1989).

Inheritance of alkaloid production

In breeding material of reed canarygrass at the University of Minnesota seven indole alkaloids were assigned to three phenotypic groups : Group **G** (gramine) ; group **T** (NMT, DMT and MTHC) ; and group **MeO** (5-MeO-NMT, 5-MeO-DMT, and 6-MeO-THC) (Marum *et al.*, 1979). Based on data obtained from progeny analysis following self-pollination and hybridization within and between alkaloid groups, a two-gene model was proposed.

A single dominant allele **T** at one locus controls synthesis of alkaloids for phenotypes in group **T** and a single dominant allele **M** at a second locus controls synthesis of alkaloids for phenotypes in group **MeO**. Phenotypes in group **G** are produced only when both recessive alleles **m** and **t** are homozygous. The **M** allele is epistatic to **T** and the two loci appear to be closely linked. Unidentified modifying factors appear to affect this interpretation in certain genetic backgrounds. Alleles **M** and **T** had frequencies of 0.21 and 0.01, respectively, in the diverse reed canarygrass source.

The relatively simple genetic control of the phenotypic groups **T** and **MeO** make it possible to eliminate these harmful alkaloids by breeding. The gramine phenotype is easily selected without progeny testing since it is produced only in the double recessive genotypes. It is difficult to eliminate gramine in reed canary grass, but the concentration can be reduced. Broad sense heritability for gramine content has been estimated to range from 0.47 to 0.70 in Norwegian populations and North-American cultivars (Østrem, 1987).

Alkaloids in Norwegian breeding materials

An investigation in western Norway on local populations concerning both alkaloid type and concentration of indole alkaloids, showed that one of seven populations was pure gramine types, whereas the rest contained two or more of the different groups of alkaloids. The total content in per cent of DM, ranged from 0.066 to 0.152 with a mean of 0.095, while the pure gramine cultivar 'MN-76' (USA) showed a value of 0.056. Corresponding values for fourteen Russian populations ranged from 0.026 to 0.340 (Østrem, 1987).

Six of the Norwegian populations together with the cultivar 'MN-78' from USA were screened to give only gramine-containing genotypes, while the Canadian cultivar 'Castor' in addition contained other alkaloids. 100 genotypes from each of these populations/cultivars were analyzed and the alkaloid concentrations in means and ranges are shown in Fig. 3. Concentrations mean for the Norwegian populations ranged from 0.036 to 0.079, while 'MN-76' showed 0.014. A striking factor is though the great phenotypic variation within populations. The cultivar 'MN-76' with the lowest mean content and range, was mainly bred for low alkaloid content, while the Canadian cultivar 'Castor' was mainly bred for high seed production.

Heritability estimates within some populations containing only the indole alkaloid gramine (Table 1), showed values of broad sense heritabilities from 0.40 to 0.57. This expressed sufficient genetic variation for selection of genotypes with low levels of gramine. However, as indicated in Fig. 3, the distribution of alkaloid concentration in genotypes within populations was very skew in the low direction. Then it will be easy to remove the genotypes with high gramine content, but more difficult to select the genotypes with the lowest concentration.

From each of four local north Norwegian populations 22 half-sib families were grown in field at Bodø in comparison with two breeding materials and two cultivars. At 1st and 2nd cut in one year samples were collected for alkaloid analysis (Larsen, in preparation). Content of hordenine, gramine, NMT and 5-MeO-DMT were determined (Kalèn, 1991). Hordenine showed the highest content with values (mg g^{-1} DM) of about 0.50, and with a low variation between population means and at the two cuts (Table 2). The content of gramine varied at 1st cut from 0.02 to 0.18, with a value for 'Vantage' from USA of 0.13. At 2nd cut the corresponding values were 0.06 to 0.42 and 0.39. The Norwegian cultivar 'Lara', the breeding material 'VåSr8401', and the cultivar 'Vantage' contained only gramine at both cut. Two of the local populations showed very low concentration of gramine and just a few families containing NMT and 5-MeO-DMT. The alkaloid 5-MeO-DMT was not identified at 1st cut, but was at the 2nd cut found in a few families of two local populations. The more immature grass at the 2nd cut showed a slight decrease in content of hordenine, but an increase in the indole alkaloids.

Conclusions

A large variation in both type and concentration of alkaloids has been observed in the Norwegian breeding material of reed canarygrass. The genetic background for alkaloid production, make it possible to select populations containing gramine as the only indole alkaloid. Great variation within populations for gramine concentration and significant heritability estimates, together with observations of very low concentration of gramine in some local populations compared to 'Vantage', shows the possibility to also reduce the content of gramine. If necessary, the content of hordenine seems to be more difficult to reduce.

The expression of gene involved in alkaloid production is highly dependent of environmental factors as temperature and water supply, and growth stage of the plant. The result in Figure 2 indicates, however, that the raking of populations should be the same in different environment. It should therefore be no need to test breeding material at several locations when selecting for low concentration of alkaloids.

The new variety 'Lara' was accepted on the Norwegian national list in 1992. 'Lara' which is based on 102 clones with only gramine type alkaloids, has a medium to low alkaloid content. Populations selected for lower concentration of gramine are now under multiplication for variety testing.

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Table 1 : Concentration of alkaloids (mg g⁻¹ DM) in north Norwegian populations of reed canarygrass compared to the two cultivars 'Lara' (Norway) and 'Vantage' (USA). Samples taken from field in one year at Bodø.

Population	Hordenine		Gramine		NMT		5-MeO DMT	
	Mean	std.d.	Mean	std.d.	Mean	std.d.	Mean	std.d.
Content at 1st cut								
Hamsund ¹⁾	0.45	0.091	0.02	0.010	0.002	0.009	0	
Grønnås ¹⁾	0.56	0.094	0.10	0.054	0.067	0.051	0	
Flakstad ¹⁾	0.54	0.069	0.04	0.027	0.000	0	0	
Ankenes	0.57	0.097	0.14	0.063	0.005	0.014	0	
VåSr8001	0.59		0.13		0.070		0	
VåSr8401	0.46		0.14		0.000		0	
'Lara'	0.53		0.18		0.000		0	
'Vantage'	0.51		0.13		0.000		0	
Content at 2nd cut								
Hamsund ¹⁾	0.39	0.101	0.06	0.044	0.000	0.000	0.009	0.016
Grønnås ¹⁾	0.50	0.116	0.20	0.109	0.170	0.111	0	
Flakstad ¹⁾	0.46	0.081	0.10	0.057	0.000	0	0.003	0.010
Ankenes	0.57	0.147	0.42	0.154	0.018	0.040	0	
VåSr8001	0.56		0.18		0.200		0	
VåSr8401	0.44		0.22		0.000		0	
'Lara'	0.53		0.34		0.000		0	
'Vantage'	0.58		0.39		0.000		0	

¹⁾ Mean of 22 half sib families.

Table 2 : Mean, range and estimates of broad sense heritability for alkaloid concentration (% DM) in population containing gramine as the only indole alkaloid (From Østrem, 1987).

Population	Mean	Range	h ₂
WIR	0.231	0.023-0.728	0.40
Skorve	0.069	0.004-0.554	0.57
Sørbøvåg	0.079	0.008-0.507	0.50
MN-76	0.014	0.001-0.102	0.50

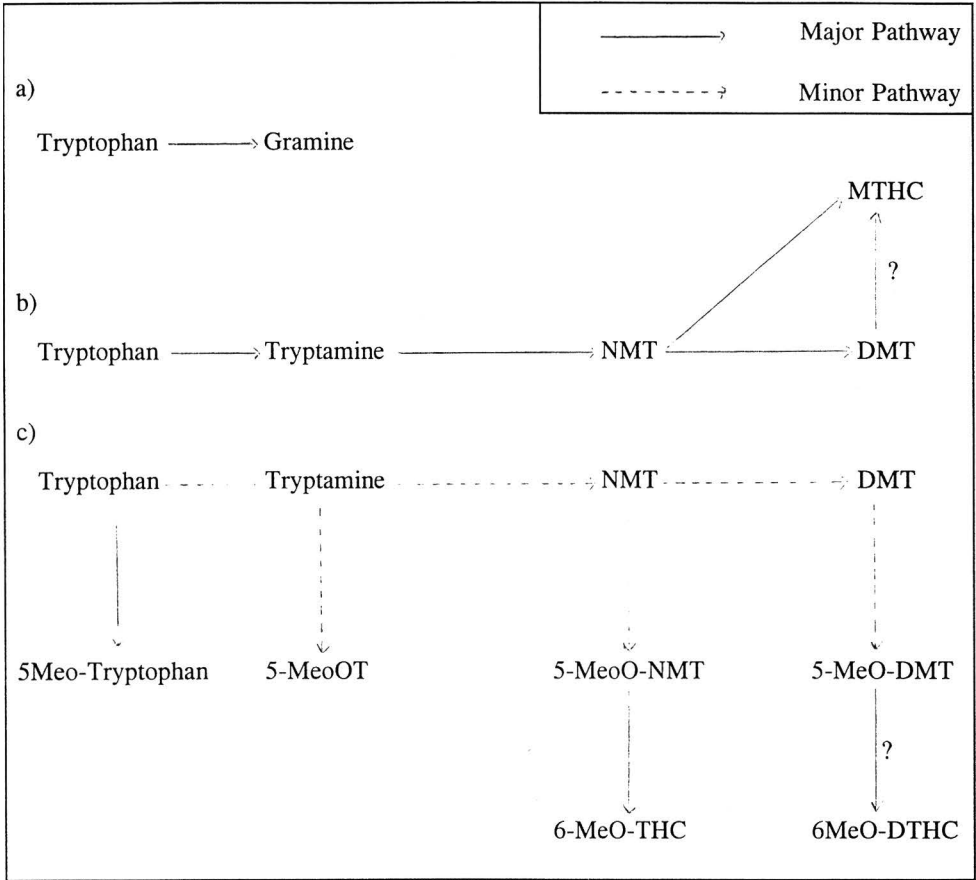


Figure 1 : Proposed pathways for the biosynthesis of indole alkaloids in reed canarygrass : a) gramine group : (*m m t t*) ; b) tryptamine-carboline group : NMT, DMT, MTHC are tryptamine-carboline derivates (*m m T -*) ; c) methoxylated tryptamine-carboline group : 5-MeO-MNT, 5-MeO-DMT, 6-MeO-THC, and possibly 6-MeO-DTHC are methoxylated tryptamine-carboline derivates (*M - T -*, or *M - t t*).

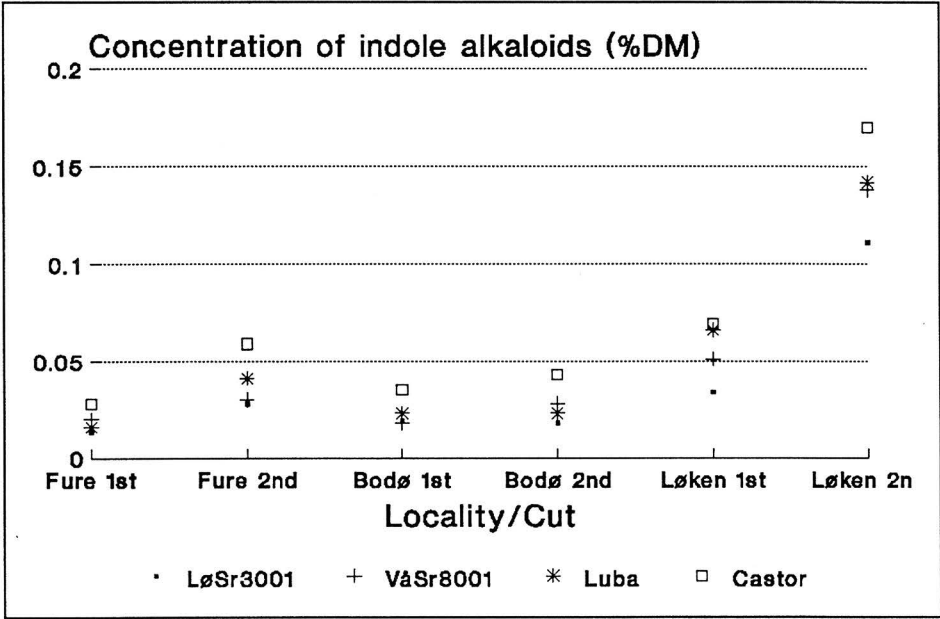


Figure 2 : Concentration of indole alkaloids at 1st and 2nd harvest at Fure (Western Norway), Bodø (Northern Norway) and Løken (Eastern Norway). The figure include the most extreme cultivars (From Østrem & Marum, 1989).

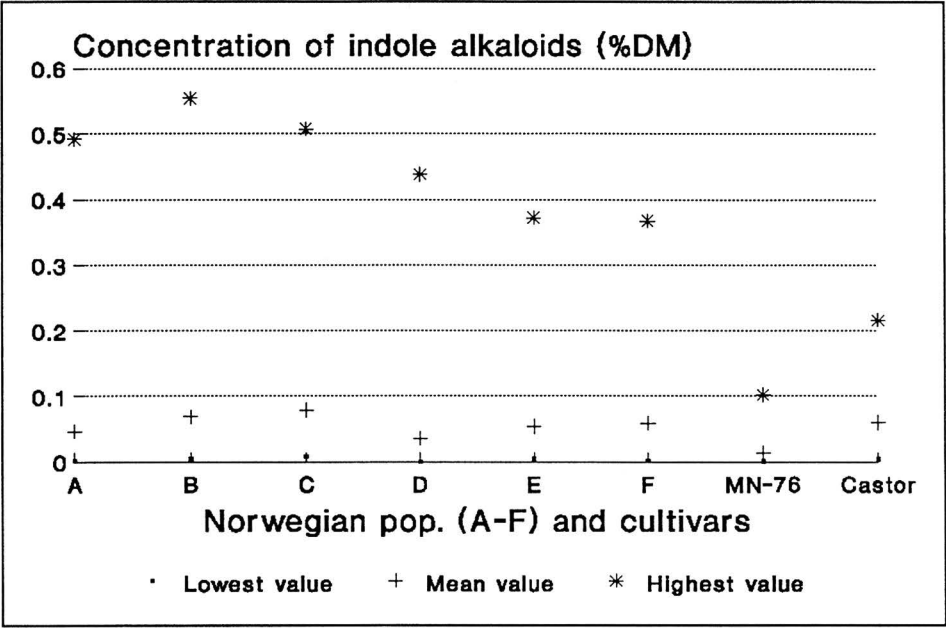


Figure 3 : Alkaloid concentration means and ranges (lowest and highest values) for six Norwegian populations and two north American cultivars (From Østrem, 1987).

BREEDING FOR IMPROVED DRY MATTER DIGESTIBILITY IN TALL FESCUE (*Festuca arundinacea*)

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Summary

A breeding programme was set up with the objective of breeding tall fescue (*Festuca arundinacea*) varieties with improved dry matter digestibility. The initial procedure was to produce a bulk intercross with plants of 25 tall fescue varieties of very diverse geographical origin.

After some preliminary selection in the F_1 , 13,000 F_2 seedlings were grown in a glasshouse and the 1,500 most vigorous, in terms of leaf extension and tiller production, were transferred to a spaced plant field nursery. Observations on important agronomic characteristics were used to identify the top 165 plants and these were subsequently multiplied vegetatively and planted into small clonal swards. Dry matter yield, sward density and other characteristics were recorded in these clonal swards during the following two year period and samples of dried herbage were retained for *in vitro* digestibility determinations.

Two new 6-parent synthetic varieties, were made by polycrossing material from selected plots : TF/D with heavy selection pressure for digestibility and TF/Y a high yield selection.

The evaluation of these varieties in plots showed that TF/D and TF/Y had similar yield and sward density to control varieties Dovey and Aberystwyth S 170 and at most cutting dates throughout the season digestibility of TF/D was superior.

Introduction

In the British Isles long-term leys for forage production are based almost entirely upon perennial ryegrass (*Lolium perenne*). Under ideal growing conditions perennial ryegrass swards can yield up to 20.0 t DM ha⁻¹ but on free draining soils in low rainfall areas production is much lower.

Production from ryegrass swards was particularly poor during the very hot, dry summers which persisted in the British Isles in the mid 1970's, resulting in severe shortages of both grazing and conserved fodder. This situation stimulated an interest among farmers in alternative forages such as maize (*Zea mays*) and also in grasses such as tall fescue which were renowned for their heat and drought tolerance.

Tall fescue is used extensively as a forage species in North America, Australia and in the drier parts of continental Europe. It is highly productive, persistent and adapted to a wide range of soil and climatic conditions but suffers from two major weaknesses : a tendency to establish poorly in the field and unpalatability to grazing stock.

A common approach to overcoming the problem of slow establishment in tall fescue has been to sow Westerwolds ryegrass (*Lolium multiflorum* var. *westerwoldicum*) as a companion grass which compensates for the low production of tall fescue during the first harvest year.

Some improvement in tall fescue palatability has been made with the breeding of soft-leaved varieties such as Barcel which do not have leaf barbs, while other breeders have produced *Festuca* hybrids with *Lolium* in order to overcome the problem.

In recent years, breeders have placed increasing emphasis upon selecting for improvement in digestibility in perennial ryegrass and some of the newer varieties, such as Portstewart which was bred at Loughgall, show a significant improvement.

In 1974 a breeding programme was set up at Loughgall with the objective of breeding tall fescue varieties with improved agronomic characteristics which were well adapted to climatic conditions in the British Isles. Initial work concentrated upon selection for improved seedling vigour as a possible means of improving rate of establishment (Faulkner *et al.*, 1982). A more extensive breeding programme was subsequently set up to see if it was possible to produce a high yielding tall fescue variety with improved digestibility.

Materials and methods

The initial procedure in this programme was to establish a field plot with 40 plants each of 25 tall fescue varieties of diverse origin including USA, Eastern Europe, Japan and the British Isles. The plants were planted in autumn 1974 at 0.5 m spacing with 4 randomised blocks of 25 rows of 10 plants of each variety. A list of the varieties used is shown below :

Alta	Datch	Hokuryo	Ludelle	Pulawska
Aronde	Demeter	Kenmont	Ludion	Roder
Backafall	Fawn	Kenwell	Manade	Rozelle
Bn 499	Festival	KY 31	Motall	S.170
Charges	Goar	Lironde	Plowdiv	Yamanami

These plants were allowed to interpollinate freely to produce a bulk F_1 harvest in 1975. A field plot, 10.0 m x 10.0 m, was subsequently established with this seed by drilling rows 10 cm apart at a rate of 10 kg/ha. Several weeks after drilling the 50 best seedlings in terms of plant height and tillering ability were identified and transferred into 15 cm diameter pots. These selected plants were grown to maturity and allowed to interpollinate freely in an isolation tent, yielding F_2 seed in July 1976.

Approximately 13,000 F₂ seeds were subsequently sown in seed trays in a soil based compost. In order to ensure uniform spacing between seedlings, 23 rows of 14 shallow holes, spaced 15 mm apart each way, were marked on the surface of the compost using a template and seeds were individually sown by hand. The trays were placed on capillary matting in a glasshouse with adequate heat, light and moisture to encourage uniform germination.

Fifteen days after sowing, the most vigorous seedlings were quite easily distinguishable and a total of 1,556 were transferred into small pots. During the autumn they were transplanted into a spaced plant nursery at 0.5 m spacing. In the following spring and summer the plants were observed at approximately monthly intervals and any small or diseased plants were killed with paraquat, leaving 165 selected plants.

In September 1980, 80 2-tiller ramets of each plant were taken and planted by hand into field plots, each measuring 0.67 m x 1.80 m to form clonal swards. Prior to planting the soil was marked with a planting template so as to ensure equal spacing between each ramet.

In 1981 and 1982, the plots were cut 5 times at regularly spaced intervals between late May (after ear emergence) and late October. Cutting was carried out at a height of 40 mm with a pedestrian operated rotary mower which had a collection bag. The fresh yield from each plot was recorded and the herbage was dried in a forced air oven to determine dry matter content. Ear-emergence date was recorded and on several occasions during both harvest years visual observations were made on sward density, leaf width and speed of recovery after cutting. Dry matter samples from cut 3 in 1981 and cuts 1 and 4 in 1982 were retained for *in vitro* pepsin-cellulase digestibility determination (D-Value), (Tilley and Terry, 1963).

At the end of the second harvest year, dry matter yields, visual observations and digestibility figures were used to identify the best clones. Two 6-parent varieties were synthesized, a high digestibility selection TF/D and a high yield selection TF/Y. Plants removed from the selected plots were allowed to interpollinate in isolation using a standard 6 x 6 polycross design, yielding Syn 1 seed.

In June 1984, seed of TF/D and TF/Y was sown in a plot trial (TF Trial I) with control varieties S170 and Dovey. The soil was a medium heavy loam with satisfactory drainage and a pH about 6.0. Plot size was 4.5 m by 1.5 m and there were three replicates in a randomised block design.

The trial was harvested with a plot harvester in 1985, 1986 and 1987 with 4 cuts per season at regularly spaced intervals between late May and late October. A compound fertiliser supplying 60 kg N, 30 kg P and 30 kg K was applied in mid March and after cuts 1, 2 and 3.

As well as the recording of yields and dry matter content, occasional series of visual scorings were made (on a 1-9 scale) of characteristics, such as sward density, when there were obvious differences between the plots. Dry matter samples were retained from each

cut for *in vitro* dry matter digestibility determination (DMD). D-values were not determined because of the extra work load involved.

A second plot trial, TF Trial II, was established in 1986 and included TF/D and two controls, S170 and Dovey. This trial was maintained in the same way as TF Trial I and was harvested in 1987, 1988 and 1989.

Results

The diverse origins of the 25 tall fescue varieties were manifested in a wide range of ear emergence. Some of the varieties were very early whereas others were quite late and despite the lay-out in a restricted polycross design of rows, all were unlikely to have contributed equally to the bulk seed of the intercross.

The subsequent examination of the F₂ plants from this cross showed quite wide differences in morphological characteristics : many plants were small or recovered very slowly after cutting while others were very susceptible to rust diseases (*Puccinia* spp.). When the 165 selected plants were eventually vegetatively multiplied and assessed in clonal plots, big differences in several important characteristics were observed. For example, sward density scores ranged from 1(very open) to 8 (very dense), spring growth from 1 (very low) to 7.5 (very high), ear emergence from 18 April to 24 May and D-value from 28.2% to 47.9% at cut 1 in 1982. Dry matter yield ranged from 664g to 1153g per plot in 1981 and from 606g to 1704g per plot in 1982.

Data recorded and observations made on the 6 selected plots (clones) used to synthesize TF/D and the 6 used to make TF/Y are presented in Table 1. Statistical analysis of these results is not possible as the trial was not replicated.

The mean sward density of both the TF/D and TF/Y groups of clones was much higher than the overall trial mean. Dry matter yield of both groups was also substantially above that of the trial mean in both years. D-value of the TF/Y group was slightly below that of the trial mean and much lower than that of the TF/D group. The TF/D group was earlier heading than TF/Y although the spring growth figures of both groups were not dissimilar.

When the new synthetic varieties TF/D and TF/Y were assessed in plots few differences were recorded in total annual dry matter yield and sward density (Tables 2 and 4), however, both TF/D and TF/Y were later heading than control varieties S170 and Dovey.

When DM samples were assessed from each cut it was found that on many occasions TF/D had superior digestibility to the control varieties.

Discussion

In recent years plant breeders have been successful in developing grass varieties which provide highly digestible, palatable herbage throughout most of the year and which are well adapted to both grazing and conservation. While much of the breeding effort has been

directed towards perennial rye-grass, improvements have also been made in less extensively used species such as cocksfoot (*Dactylis glomerata*) and meadow fescue (*Festuca pratensis*) (Frandsen and Fritsen, 1982). In the USA digestibility selection is occurring in several tall fescue breeding programmes (Sleper, 1983).

The design of the breeding programme in this project was simple and straightforward. A large gene pool was created by bringing together a relatively large number of varieties from different geographical locations, almost half known to be adapted to and to perform well in North West Europe. A wealth of genetic variation for agronomically important morphological and physiological characteristics was very evident.

Following natural intercrossing in the field and sowing in drills, representing agricultural practice, a very high selection pressure was applied only a few weeks later with respect to seedling vigour, which was taken to indicate both rapid seed germination and early tillering ability.

The very intensive selection of only 50 plants at the F_1 stage might have been expected to have resulted in a loss of some of the variation and the selection procedure to have imposed a negative selection for some of the more important characteristics such as yield and sward density but this was not borne out in the subsequent phases of the programme.

The selected F_1 material was intercrossed in isolation to produce F_2 . Some 13,000 F_2 seedlings again selected for seedling vigour and later for agronomic characteristics in a field nursery were reduced to 165 plants selected on phenotype. At this Station, staff are well experienced in the selection of perennial ryegrass genotypes which, when intercrossed, produce high yielding synthetics so for this tall fescue material similar criteria were used in visual assessments in the expectation that vigorous, large plants were likely, if used as parents, to ultimately produce high yielding swards.

The use of clonal swards is also a feature of the work of the Loughgall Station and clonal swards of these 165 genotypes were assessed over 2 years for time of emergence, for yield and for the specific characteristic digestibility in which improvement was required.

The measurement of digestibility *in vitro* using the method of Tilley and Terry (1963) is already recognised as giving good results in relation to what happens *in vivo*. But it must be recognised that digestibility changes with stage of development as affected by ear emergence. Breese and Thomas (1966) showed that small differences in development can greatly affect digestibility. In order to nullify the affect of ear emergence Van Bogaert (1980) stressed the importance of analysing several harvests of the plant material being tested in estimating digestibility.

Further work by Walters *et al.* (1967) concluded that it was necessary to measure the digestibility of material outside the influence of heading, i.e., in late summer or autumn. During the evaluation in clonal plots (see Table 1) digestibility was assessed at cut 3 during the first harvest year which would have been approximately 10 weeks after ear emergence whereas in the second harvest year digestibility was recorded at the first cut which would

have been greatly affected by ear emergence and at the fourth cut approximately 15 weeks later. Plots identified as having high digestibility during the first year at cut 3 were also found to be high at cut 1 in the following year despite the fact that they were earlier heading than the mean for the total group.

When selecting the 6 clones with which to synthesize TF/D, all three measurements of digestibility were taken into consideration with the aim that, when they were intercrossed, the new synthetic produced would have high digestibility. In a study by Nguyen *et al.* (1982) heritability estimates for digestibility were poor in the autumn but higher in spring and it was suggested that selections for higher digestibility should be made in spring. Fransden (1986) evaluated families of perennial ryegrass, cocksfoot and meadow fescue derived from crosses between parental clones selected for high and low digestibility and found that broad sense heritabilities were generally within the range 0.40 - 0.60, concluding that selection for improved digestibility should be quite effective. In this breeding programme as a synthetic based upon plants with low digestibility was not produced no conclusions regarding the heritability of digestibility are possible.

The evaluation in sward plots showed that on almost every occasion TF/D had superior digestibility to the control varieties throughout the growing season and the yield and sward density were at least as good. While it is realised that having good digestibility at any given cut is valuable, it is even more important for pastures to have adequate digestibility throughout the growing season. Thus the breeding programme achieved its required objectives.

The variety TF/D, named Heidi, was successfully added to the UK National List in 1992. However, despite the advantages of tall fescue in terms of drought and heat resistance, in recent years there has not been any increase in tall fescue seed usage in the British Isles, farmers in drier regions tending to use forage maize as an alternative to grass silage. Therefore Heidi was subsequently withdrawn in the UK in 1994 due to lack of commercial interest but testing of the variety continues in Argentina and France where the market for tall fescue is much greater.

Despite the importance of improved digestibility it is realised that acceptability, palatability and speed of digestion are equally important and the ultimate test of a variety such as Heidi will be to determine if selection for higher *in vitro* digestibility is translated into improved animal performance.

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Table 1: Performance of the clones used to synthesize varieties TF/D and TF/Y in clonal plots in 1981 and 1982

Clone No.	Sward density (1-9)		Spring growth (1-9)	D Value %			Ear emergence 1982 (1 = 1 Apr)	DM yield (g plot)	
	Aug. '81	July '82		'81 cut3	'82 cut 1	'82 cut 4		1981	1982
TF/D									
24	6.0	7.0	5.0	50.9	39.8	63.6	46	975	1325
54	6.0	7.5	4.0	44.6	41.1	60.9	46	1039	1458
110	6.0	6.0	5.5	47.0	46.7	59.7	34	940	1325
130	5.0	5.5	4.5	47.3	40.8	59.4	48	780	1435
144	6.0	7.5	6.0	47.5	42.1	59.0	46	826	1532
152	5.0	7.5	5.5	48.9	43.2	64.1	30	1100	1641
TF/D Mean	5.7	6.8	5.1	47.7	42.3	61.1	42	943	1452
TF/D									
7	7.0	7.5	7.5	43.2	33.1	56.4	46	1153	1580
15	7.0	7.0	6.5	40.2	33.2	55.5	44	1116	1489
45	8.0	6.0	5.5	46.6	32.2	58.8	42	1115	1582
52	7.0	6.5	6.5	44.5	35.9	61.8	48	1125	1516
62	8.0	8.0	3.5	44.8	38.3	59.8	50	1134	1484
138	4.0	6.0	6.0	43.2	37.6	60.3	54	1023	1704
TF/Y Mean	6.8	6.8	5.9	43.8	35.1	58.8	47	1111	1559
Overall Trial Mean (165 clones)	4.0	4.5	3.8	45.9	38.2	-	46	858	1182

Table 2 : TF Trial: Performance of four tall fescue varieties at Loughgall in 1985, 1986 and 1987

Variety	E. emergence (1 = 1 Apr. 1985)	Total DM Yield (t.ha ⁻¹)			Sward density (9 = dense)		
		1985	1986	1987	1985	1986	1987
TF/D	47	15.21	12.62	16.33	5.2	5.5	4.7
TF/Y	48	15.08	12.83	14.83	5.3	4.8	4.0
S 170	43	14.64	12.68	13.90	6.3	5.8	4.0
Doverly	41	16.02	12.06	14.87	6.3	4.7	4.3
Mean	45	15.24	12.55	14.98	5.8	5.2	4.3
SE	0.3	0.39	0.32	0.44	0.43	0.36	0.51
Sig.	***	N.S.	N.S.	*	N.S.	N.S.	N.S.
CV %	1.3	4.4	4.4	5.07	13.0	12.0	20.8

Table 3 : TF Trial I : Digestibility (DMD) Values for 3 cuts in 1985, and 4 cuts in 1986 and 1987

	DMD 1985				DMD 1986				DMD 1987			
	Cut 1	Cut 3	Cut 4		Cut 1	Cut 2	Cut 3	Cut 4	Cut 1	Cut 2	Cut 3	Cut 4
TF/D	69.3	76.2	79.7		73.3	75.4	76.0	80.1	68.2	76.7	80.1	80.9
TF/Y	69.1	75.0	78.3		72.6	72.6	74.1	79.8	72.0	72.3	76.7	78.8
S 170	65.6	70.4	76.3		71.5	71.8	69.9	77.0	69.0	71.5	73.5	76.0
Doverly	63.3	72.1	75.2		69.1	70.8	72.5	78.1	67.9	69.1	74.6	77.1
Mean	66.8	73.4	77.4		71.6	72.7	73.1	78.7	69.3	72.4	76.2	78.2
SE	0.94	0.49	0.64		1.02	0.61	0.78	0.67	1.06	1.32	0.86	1.11
Sig.	*	***	**		N.S.	**	**	N.S.	N.S.	*	**	N.S.
cv %	2.45	1.16	1.42		2.46	1.46	1.85	1.48	2.65	3.16	1.96	2.46

Table 4 : TF Trial II: Performance of three tall fescue varieties at Loughgall in 1987, 1988 and 1989

Variety	Total DM Yield (t.ha ⁻¹)			Sward density (9 = dense)	
	1987	1988	1989	Sept. 1988	Sept. 1989
TF/D	16.23	19.18	17.88	6.0	6.0
S 170	17.13	19.38	16.80	4.8	4.8
Doverly	16.77	20.32	18.45	7.5	6.3
Mean	16.71	19.63	17.72	6.1	5.7
S.E.	0.27	0.48	0.62	0.25	0.23
Sig.	N.S.	N.S.	N.S.	**	*
cv %	2.81	4.19	4.19	7.2	6.8

Table 5 : TF Trial II : Digestibility (DMD) values for four cuts per season in 1987, 1988 and 1989

	DMD 1987				DMD 1988				DMD 1989			
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 1	Cut 2	Cut 3	Cut 4	Cut 1	Cut 2	Cut 3	Cut 4
TF/D	78.1	81.0	79.6	80.8	72.1	69.8	73.3	84.9	81.9	69.9	73.9	78.1
Dover	71.8	74.1	76.0	77.9	70.6	66.7	68.0	82.5	77.4	67.6	68.0	74.9
S 170	74.1	74.9	75.7	79.7	70.1	68.3	68.0	85.2	79.1	69.7	68.4	77.8
Mean	74.7	76.6	77.1	79.5	70.9	68.3	69.7	84.2	79.4	69.1	70.1	76.9
SE	0.46	0.66	1.80	0.72	1.35	1.25	0.79	0.65	0.28	1.18	0.76	0.41
Sig.	**	**	N.S.	N.S.	N.S.	N.S.	*	N.S.	***	N.S.	**	**
cv %	1.06	1.49	4.04	1.56	3.30	3.17	1.95	1.34	0.61	2.95	1.89	0.92

BREEDING FOR IMPROVED DIGESTIBILITY IN PERENNIAL RYEGRASS

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Abstract

We have determined in 1991 and 1992 the digestibility of hundreds of clones of perennial ryegrass in the second conservation cut both with the wet chemistry analysis and with the Near Infrared Spectroscopy (NIRS). A lot of clones were allowed to produce offspring in polycross designs. We have compared the two systems of analysing and to some extent have compared analyses over the two years. The repeatability over the years was very poor and hence the heritability in narrow sense calculated from parent-offspring relationships was very low. The value of the system we used is discussed together with the feasibility of breeding for improved digestibility in ryegrasses.

Introduction

Digestibility of forage grasses is believed to have a large effect on animal output (Frandsen and Fritsen, 1982, UK Recommended List 1993/94, Vellinga and Van Loo, 1994). RvP has a long tradition in measuring digestibility in ryegrasses. Polycross progenies continue to be analysed for more than 25 years. Potential components of synthetics were always selected according to their agricultural value including digestibility. Quite often components with a superior Dry Matter Yield (DMY) were replaced by components with a better Digestible Organic Matter Yield (DOMY). Nevertheless RvP has no outstanding digestible varieties today.

Analysing polycross progenies might be not the ideal strategy to follow since at this stage of a breeding programme a lot of genetic variation has been lost. More genetic variation is available in earlier stages. Therefore we aimed at a new strategy in analysing lots of individuals during the selection phase well before the recombination phase. Such a strategy demands a quick analysing method. Since analyses by wet chemistry are very labour and time consuming we have compared the pepsine-cellulase method (Jones and Hayward, 1975) with the fast indirect Near Infrared Spectroscopy (NIRS) analysis. It is hardly possible to have analysed all cuts of large numbers of individuals although many authors, including ourselves (Reheul and Ghesquiere, 1994) are convinced that analysing all cuts provides the most reliable results. The UK Recommended list of grasses and forage legumes (1993/94 and earlier) gives it for its opinion that "the D-value of the second conservation cut is a reflection of the relative digestibility of varieties from late June onwards." We have chosen this second conservation cut in our research as a means to anticipate the UK testing system.

Material and methods

Over 1200 clones of perennial ryegrass with a wide genetic background (varieties, breeding populations, indigenous and foreign ecotypes) have been analysed in their second conservation cut. About 450 clones have been analysed in 1991 and about 750 in 1992. 183 of them have been analysed in both years. A clonal row consisted of 5-7 individuals. Two of these plants have been cut manually with a sickle at 5 cm above the ground. The first time in the third week of May (the first conservation cut, 17 May in 1991, 18 May in 1992) and a second time (the second conservation cut) around 20 June, 24 June in 1991 and 18 June in 1992. All clones were cut on the same day to eliminate differences owing to weather conditions. The quantity of ears and their development were scored at the moment of the second cut. The first characteristic was scored in a scale of 1 to 5, 5 meaning very much ears, the development of the ears was scored as given by Bommer (1959). Additional remarks were written down and we marked the plants with a wealth of leaves close to the ground as opposed to the plants with a few leaves only. Heading dates were taken on the non cut plants. All the cut material was dried at 75°C and ground over a Wiley Brabender Mill with a sieve aperture of 1 mm. The 1991 material was analysed with the NIRS equipment of Nickerson Seeds Ltd, UK (dr. Turner). The 1992 material was analysed according to the pepsine-cellulase method of Jones and Hayward (1975) in our lab (dr. Carlier) and two times with a NIRS equipment, once in Belgium at the Station de la Haute Belgique (dr. Dardenne) and once at Nickersons. The Belgian NIRS analyser has a calibration line based upon a huge stock of pepsine-cellulase analyses. The UK NIRS equipment relies on NIAB data.

A lot of the analysed clones were rejected during further selection stages owing to their inferior agricultural value. The remaining clones were polycrossed to compose 15 synthetics consisting of 4-12 components. Two strategies were followed to select the components of the synthetics : 1. "our normal strategy", i.e. a paramount attention to yield, regrowth, disease resistances etc. and finally to digestibility and 2. "digestibility first", i.e. a divergent selection and hence composition of synthetics with extreme digestibility coefficients (further on also called D-values).

The polycross offspring was sown in yield trials and the forage of several cuts of these swards has been analysed with the same methods as used for analysing the parents : pepsine-cellulase and the Belgian NIRS.

A parent-offspring relationship for digestibility was computed from the linear regression of the offspring on the parents. The heritability in narrow sense was determined as $h^2 = 2b$, being the slope of the regression line (Falconer, 1983). We calculated the regression for each polycross and hence the mean regression coefficient. The correlation between the digestibility coefficient and the DMY of the offspring was calculated. Yield trials aw 93.3 and aw 93.4 both sown in the spring of 1993 represent the offspring of the parents analysed both in 1991 and in 1992. Results of the first 3 cuts (2 reps/cut) taken in 1994 are given. Trial aw 94.1 sown in the spring of 1994 represents the offspring of parents analysed in 1992. Results of the first cut (2 reps) of 1994 are given.

Results

Figure 1 shows the correlation between the results of the pepsine-cellulase and the Belgian NIRS analysis. We found a high correlation coefficient (0.89^{***}) over a wide range of D-values (50-80). The correlation coefficient between the results of the pepsine-cellulase and the UK NIRS analysis was 0.74^{***} , and it was 0.82^{***} between the results of the two NIRS analyses.

Table 1 gives a more detailed idea of the variation found among comparable clones. Clones are grouped according to their heading date and within these groups according to the quantity and the development of ears present at cutting time. A surprising range of 25 units D-value was found in this set of clones. In this set the digestibility generally improves the less ears are present and the less developed these ears are, which is no news. However some exceptions occurred.

The set of 183 clones analysed both in 1991 and in 1992 (UK NIRS analysis) allowed us to compare the results between the two years. As Figure 2 shows we found no relationship at all in this group of clones. The mean D-value in 1991 was 68.9, standard deviation 3.4; in 1992 the figures were 73.9 and 2.3. In 1991 the second cut was taken on 24 June, in 1992 on 18 June which makes a difference of 6 days. However 1992 had a warm spring and heading dates of the same clones were on average 10 days earlier in 1992 compared to the data of 1991. Differences in stem : leaf ratios owing to differences in development are by this somewhat reduced.

The parent-offspring relationships of all the polycrosses together are shown in Figures 3 and 4. The mean heritability in narrow sense was 0.04 (range -0.07 to 0.17) for the offspring present in trials aw 93.3 and aw 93.4 (6 polycrosses; 39 clones involved). For the offspring present in trial a 94.1 (9 polycrosses ; 59 clones involved) it was 0.00 (range -0.75 to 0.40). The heritability was very close to zero. This holds true both for the divergent selection as for the normal selection.

The range in D-values of the parents ranked over about 25 units. The range in the progenies was narrowed into 5 units in trials aw 93.3 and aw 93.4. In trial a 94.1 about 10 units were left.

Figure 5 shows the correlation between the digestibility coefficient of the progenies and their DMY. This correlation was close to zero, indicating there was no particular relationship between the two parameters in this material.

We calculated the correlation between heading date (expressed as days after the 1st of May) and the digestibility coefficient in 658 clones heading between 7 May and 5 June 1992. The resulting coefficient was 0.011 indicating no relationship between earliness and digestibility in this material.

Discussion

If one has a good calibration line the NIRS method seems accurate enough to indicate the individuals with the best digestibility coefficient. Up to and including the grinding, the preparation of the sample is equally labour-intensive as for the wet chemistry analysis. It took us on average 13 minutes to have one sample prepared (i.e. cutting, labelling, drying, grinding) provided that one needs a shift of at least three people for some parts of the job. The pepsine-cellulase analysis took about 15 minutes per sample. The NIRS analysis about six times less. Furthermore the wet chemistry takes several days since it consists of several stages with different devices involved, each of them having their particular capacities and limits. The NIRS analysis is a one-step procedure resulting in a much better labour flexibility and apart from this the NIRS analysis offers the opportunity to measure other parameters simultaneously.

We found a large variation in D-value in this material. Figure 1 represents 706 clones with a range of about 30 units from 50 to 80. Not all this variation was useful during the breeding programme : we lost much of it by rejecting phenotypes with poor agronomical characteristics.

Frandsen and Fritsen (1982) reported a variation range of about 12 units in a set of 50 clones of perennial ryegrass and they cite Bugge (1976) who found a range of 24 units in 120 ecotypes of italian ryegrass. We analysed much more phenotypes.

Some more phenotypical information. Quite a lot of clones with very high D-values lacked persistence. In general the less ears on the clones at cutting date the higher the D-value. The very late heading group (heading dates later than 5 June in 1992) contained quite a lot of very high digestible phenotypes : mean D-value of 48 clones 67.3 versus 65.9 for the earlier ones.

The question raises if one can "see" good digestibility simply by viewing the plants. The answer is negative. We compared some very leafy individuals with "normal" ones within a totally comparable group of clones (same very small span of heading date -3 days-, same score for quantity of ears and same development of ears at cutting date). Leafy means here a lot of basal leaves (not leaves on the stems). We presumed these plants would score better for digestibility, but the analyses showed no difference at all.

The parent-offspring regression was disappointing and truncated the initial enthusiasm viewing the wide variation. It is important to emphasise the large numbers of parent-offspring couples involved : $n = 39$ in trials aw 93.3 and aw 93.4 and 59 in trial a 94.1. The variation in D-value in the offspring was narrowed as was to be expected but we found a heritability in narrow sense close to zero. Frandsen (1986) found a range in heritabilities in narrow sense of 0.16-0.57 in perennial ryegrass. These data were derived from the regression of F1 and F2 progeny on midparental values ($n = 50$). Furthermore he found higher values in the less digestible cocksfoot and lower values in meadow fescue. Surprenant et al (1990a) worked with timothy and calculated a narrow sense heritability of 0.60 by means of a parent-offspring (halfsibs) regression. They mention a series of values found by other authors in grasses : these values range from 0.02 to 1.06 according to the species and the experiments.

In our experiment parental clones were spaced plants and the offspring were swards. This might have influenced the results as suggested by Mc Elroy and Christie (1986) who found a low correlation for in vitro digestibility between timothy genotypes grown in simulated swards (100 plants/square m) and in space-planted nurseries. But the experiments of Surprenant *et al.* (1990b) with timothy showed that improved forage quality obtained by selection under spaced plants conditions can be maintained under sward conditions. And Frandsen (1986) found no divergent values when he computed the heritability in narrow sense based on the regression of 15 F2 progenies under sward conditions and midparental values obtained from spaced plants compared to a regression with only spaced plants involved.

Since we used the entire plants to analyse the parents and not a standardised plant part environmental effects may have influenced the results substantially. However all plants were harvested on the same day and our synthetics were composed with clones matching very closely in heading dates. Effects of temperature as reported by Ames *et al.* (1993) or differences in leaf:stem ratio as determined by heading dates (Cooke and Morgan, 1987) were minimised.

Surprenant *et al.* (1993) reported large cultivar by management interactions on quality evaluation in timothy. A one year on one location study might be insufficient to offer reliable results. This was also demonstrated in this research where the UK NIRS analysis of 1991 was compared to the UK NIRS analysis of 1992 for 183 clones. The relationship was so scattered that one must question by all means the feasibility and significance of this research. In this set of clones the repeatability of our system was non existent and hence all differences found were no reflection of the genetic background. No surprise heritabilities were low.

As a consequence analysing clones in one cut is inadequate. One needs several analyses/year over several years but this will increase the workload a lot.

Analysing hundreds of clones and not using them for the composition of the synthetics later on because their presumed inferior agricultural value seems a waste of energy. On the other hand it is the only way to discover superior individuals for the desired trait early enough in the breeding programme. This superiority is useless in a lot of cases due to negative correlations with other important traits. The alternative is acting as stated in the introduction : postponing the analysing until the end of the recombination phase. But this system didn't bring us any further in the past. As a consequence it seems inevitable to conduct a research gathering data over several years and most probably over several locations. Essentially this is not different from any other breeding activity but the analytical work involved in this kind of research makes it very hard to implement such a strategy in a practical breeding programme.

We found no relationship between DMY and the digestibility coefficient in this material. According to Frandsen (1986) one might have expected a negative relationship. And our own results with varieties and different breeding material (Reheul and Ghesquiere, 1994) point to the same direction.

We conclude from this work that the system we used gives little scope to improve digestibility in perennial ryegrass.

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Table 1 : Range of digestibility coefficient (pepsine-cellulase) in comparable material; second conservation cut, June 1992

Ear emerge	E	Q	N	Relative frequency (%) of the digestibility coefficient (pepsine-cellulase)							
				50-58.9	59-61.9	62-64.9	65-67.9	68-70.9	71-73.9	74-76.9	> = 77
25-29/05/92	7a	1-3	46	2	4	13	22	24	26	7	2
	7b	1-3	42	0	10	14	31	21	17	5	2
		> 3	29	10	10	24	28	28	0	0	0
	8	1-3	37	5	8	14	19	19	11	16	8
02-05/06/92	7a	> 3	39	31	36	15	13	3	3	0	0
		1-3	63	3	6	22	35	22	8	3	0
	7b	1-3	30	3	13	23	17	23	17	3	0
> 05/06/92	7a	1-3	28	0	7	14	36	39	4	0	0

E : Ear development (Bommer, 1959)

Q : Quantity of ears 1-5 ; 5 = very much

N : Number of clones

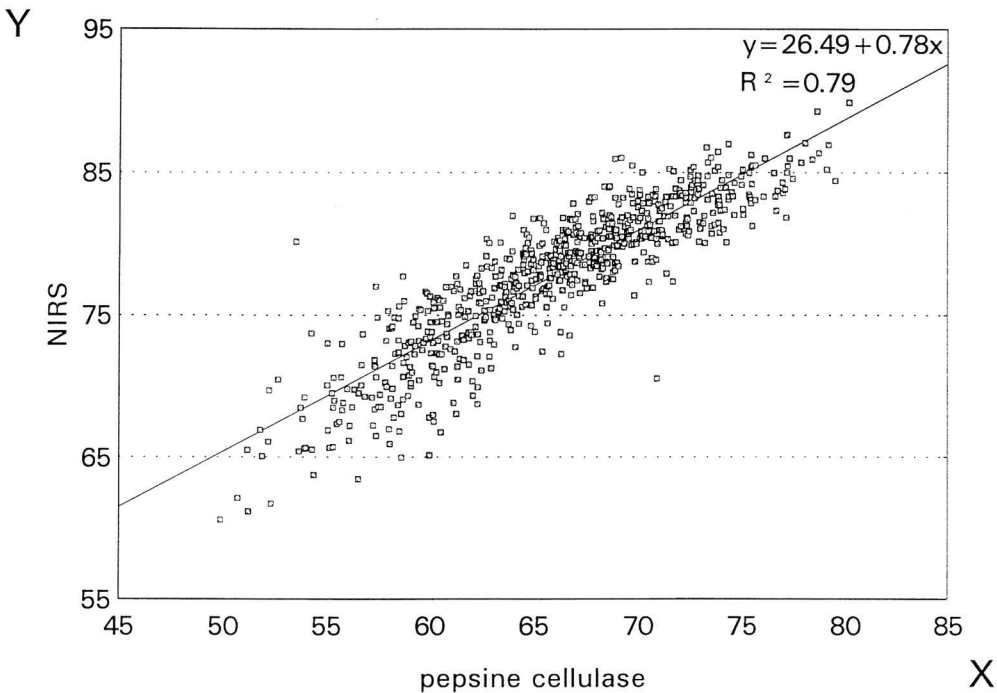


Figure 1 : Digestibility coefficients : correlation between NIRS and pepsine-cellulase (706 clones involved); second conservation cut, June 1992

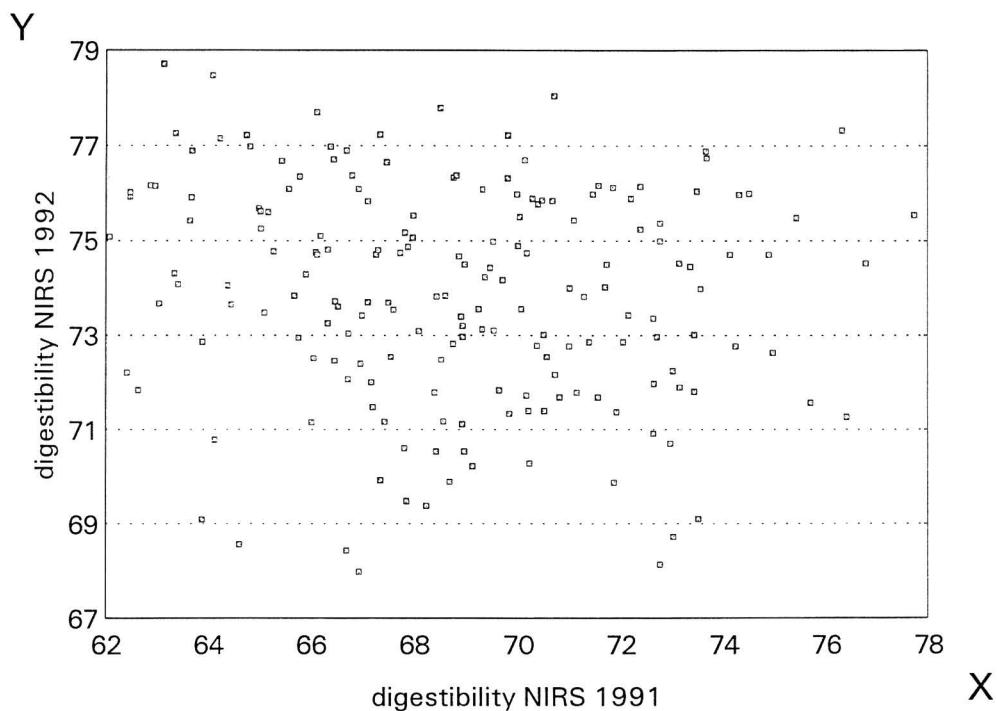


Figure 2 : Relationship between the results of a NIRS analysis of 183 clones analysed both in 1991 and 1992

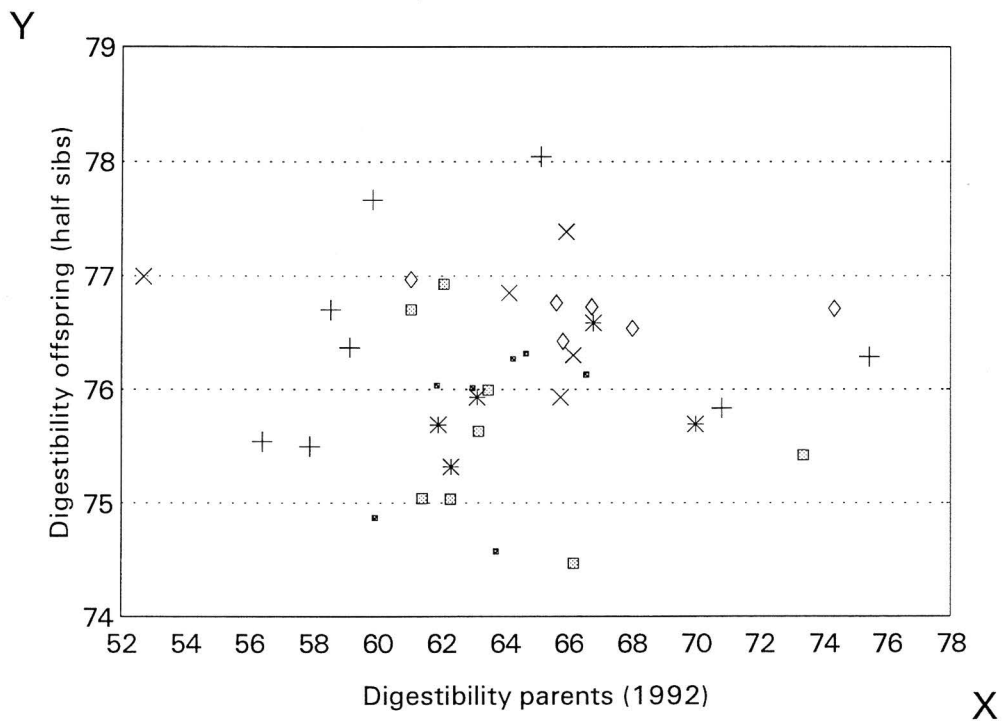


Figure 3 : Parent-offspring relationship for digestibility coefficient (pepsine-cellulase); trials aw 93.3 and aw 93.4, sown spring 1993; offspring presented by the mean of the 3 first cuts harvested in 1994; identical symbols refer to a particular polycross offspring

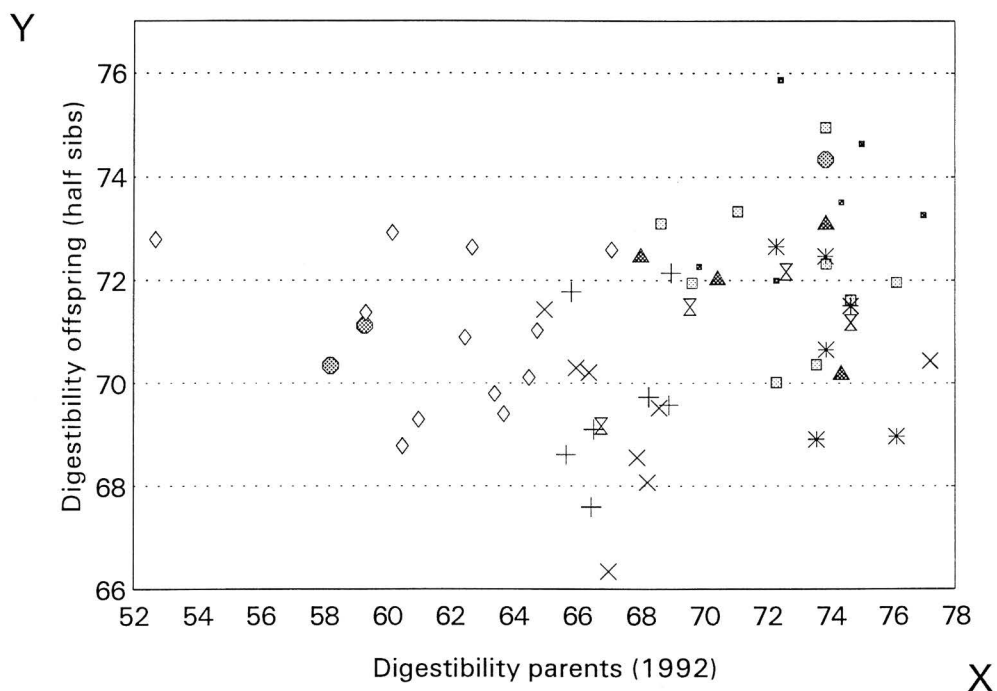


Figure 4 : Parent-offspring relationship for digestibility coefficient (pepsine-cellulase); trial a 94.1, sown spring 1994; offspring presented by the first cut in 1994; identical symbols refer to a particular polycross offspring

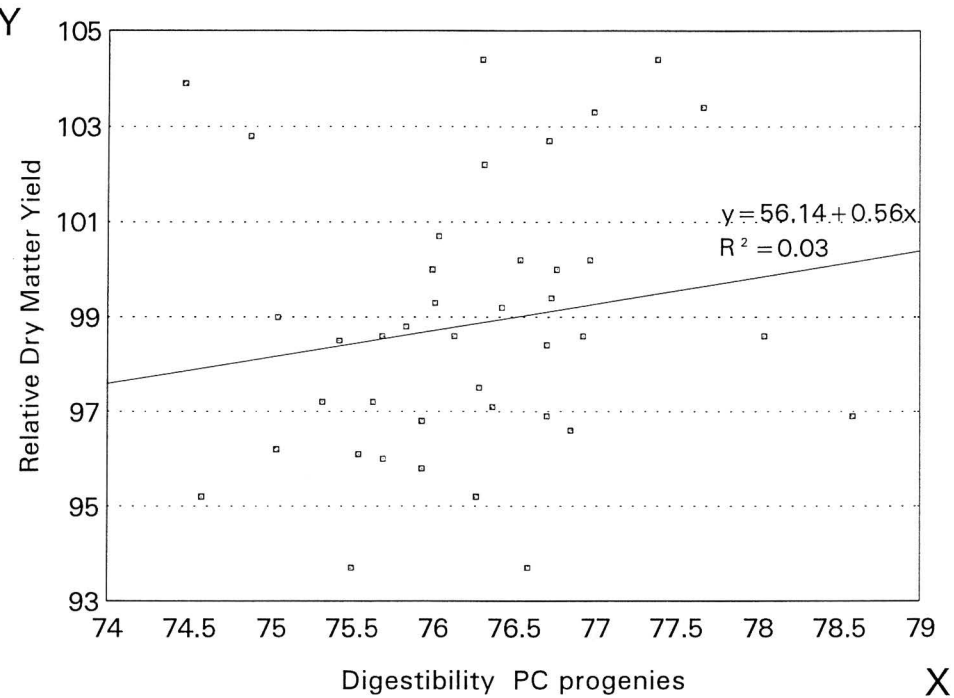


Figure 5 : Correlation between the digestibility coefficient and the DMY of polycross progenies

BREEDING FOR IMPROVED DIGESTIBILITY IN PERENNIAL RYEGRASS

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Summary

Breeding for better forage quality could play an important role in increasing efficiency of animal production with less environmental problems. Between existing varieties of Perennial ryegrass, not selected for high digestibility, differences of 2-4 % in relative digestibility exist. Differences between varieties in digestibility have been more consistent than yield differences.

An experiment is reported with selection for high and low flag leaf digestibility in a heterogeneous population of Perennial ryegrass. Two ways to estimate the heritability both gave a result of 0.7. A clear selection response was demonstrated. No unwanted correlated response was observed for other important characteristics. Synthetic varieties were obtained with an average relative digestibility up to 5 % above control varieties.

The benefits of such better digestible varieties for the cost-effectiveness of dairy farming and for a lower N-surplus on farms can only be realized when official testing authorities take digestibility into account for recommendation of varieties.

Introduction

For economic as well as environmental reasons, dairy farmers need to maximize milk production per cow, minimize the number of cows for a given milk quatum, and minimize the amount of concentrates fed.

Van Vuuren (1991) indicated that the nutrient supply of herbage commonly used in the Netherlands is insufficient for milk production over 30 kg per cow per day and results in extensive losses of nitrogen. In relation to the fermented N in the cow's rumen, there are insufficient fermented carbohydrates available. A higher proportion of better digestible cell walls should result in a decrease of N-losses by urinary excretion, a higher herbage intake and an increase in the efficiency of animal production. Higher digestibility is therefore an important breeding goal.

A.o. Korevaar *et al.*, (1988) reported that the milk quota system, together with an increased milk production per cow has led to a surplus of forage on many farms. Hence yield is a less important grass breeding objective than before. Herbage surpluses and lower levels of N-application further increase the need to breed for higher intake and digestibility.

Breeding for better forage quality could therefore play an important role in increasing efficiency of animal production with less environmental problems.

Between varieties of Perennial ryegrass (*Lolium perenne* L), small but consistent differences in digestibility exist. Visscher (1992) found differences of 2 % in average digestibility between varieties in Recommended List tests near Lelystad, the Netherlands, whereas De Vlieghe *et al.* (1994) reported maximum differences of 3.2 % in variety trials on 3 sites in Belgium.

Since 1986, digestibility tests are a standard part of the evaluation of newly bred synthetic varieties at Barenbrug Research. We found differences up to 4 % in relative digestibility between control varieties, unselected for digestibility. These differences between varieties have been more reproducible than yield differences, as illustrated by Fig. 1 and Fig. 2. The varieties Magella and Barlet have been sown as control varieties in 6 different trials in Wolfheze from 1988 until 1993. Only first harvest year digestibilities are presented, to avoid possible disturbing effects of sward density differences. On average, Barlet has a significant 2 % better digestibility than Magella. The ranking order of the 2 varieties is the same in every year. However, for yield the ranking order is quite different from year to year and the average 1 % difference was not significant.

Pilot experiments were conducted with replicated clones for measuring variation in digestibility in various plant parts, such as ears, stems, flag leaves, vegetative tillers, generative tillers as well as in completely harvested plants. In all plant parts, significant genetic variation for digestibility exists (unpublished data). This variation is greater for stems than for leaves. However, we found that correlations between the digestibility of the different plant parts was rather poor or not significant, as illustrated in Fig. 3. Selection for stem digestibility hence will hardly improve leaf digestibility. Since farmers who are interested in forage quality will tend to avoid stems anyway, we have concentrated on selecting for improved digestibility of leaves and vegetative tillers.

As could be expected, much larger heritable differences were found between individual clones or plants in these pilot experiments than between existing varieties. We concluded that there was sufficient scope for improvements beyond just 2 % better digestibility. Subsequently, selection programmes were started at Barenbrug Research in Wolfheze in 1987 to improve digestibility of Perennial ryegrass.

Material and methods

In the laboratory of Barenbrug Research in Wolfheze digestibility is assessed using an adapted form of the pepsin-cellulase method described by Jones and Hayward (1975), with 24 hour pepsin and 24 hour cellulase incubation instead of 48 hours. Standard samples were regularly sent to the IVVO laboratory in Leeuwarden for comparison. In 1987, digestibility estimates of Barenbrug Research and IVVO Leeuwarden on the same samples showed a correlation of 0.98.

A selection experiment was conducted starting from a heterogeneous recurrent selection population of intermediate heading date with a good average level for yield, persistency and rust resistance. This population was originally derived from a series of collected ecotypes in old pastures. Without selection, this base population had already shown a higher than average digestibility.

In spring 1987, 600 vernalized plants of the base population were planted in Wolfheze, the Netherlands. They proved to be fairly uniformly starting to head around June 1st. Since these plants had produced lots of new tillers after planting, heading of newer tillers of each plant continued for several weeks. Four weeks after the first heading, 25 mature flag lamina per plant were harvested of stems similar in maturity, with half or two-thirds of the ear emerged. Samples were cleaned under tap water, dried at 70°C, grinded through a 1mm screen and digested without assessment of organic matter since we had only ± 0.5 gram dry matter per sample.

The best digestible 54 plants as well as the poorest 24 plants were intercrossed in isolation in 1988 to produce seed of a positive selection and a negative selection.

The high digestibility and low digestibility selections and the original unselected population were sown in spring 1989 in 3-replicate yield trials in Wolfheze and Lelystad. Yields and various observations were taken on both sites. However, digestibility samples were only taken in Wolfheze, from every cut in both harvest years 1990 and 1991. A weighted mean digestibility for 2 years has been calculated, taking into account the yield of each individual plot at each individual cut. Per weighted mean the digestibility has been assessed of 27 samples.

The best 54 plants were also vegetatively multiplied in autumn 1987. In 1988, 5 different groups of 4-5 clones similar in DUS characteristics were assembled and isolated to produce seed. These synthetic varieties have been evaluated in the same trials as the base population and the derived positive and negative selections mentioned above. Control varieties were Barlet and Magella.

The positive and negative selections, together with the base population, were planted on a spaced plant field to look for morphological differences due to a possible indirect response to the selection on digestibility.

Results

Flag leaf digestibility of the 600 individual plants ranged from 92.3 to 105.7 relative to the population mean. The best 54 plants were on average 103.0, whereas the worst 24 averaged 95.8 for relative digestibility. The absolute level of *in vitro* digestible matter disappearance of flag leaves averaged 86.9 %.

The phenotypic variance for digestibility within the population of 600 plants was 2.84. To estimate the heritability we need an error variance, but the spaced-plant trial was unreplicated. However, we did find an error variance of 0.77 for the same character in an

earlier mentioned pilot experiment with replicated clones, also in Wolfheze in 1987. If the same error variance would apply to the trial with 600 plants, then the estimate for the wide-sense heritability of flag leaf digestibility is 0.7.

The 600 plants could be grouped into half-sib families. The variation for digestibility was larger within families with a lower average digestibility than within families with a higher average digestibility. This indicates that the inheritance of digestibility is not purely additive, but that dominance is involved in the direction of high digestibility.

In the yield trials, the weighted average digestibility during 2 harvest years of the high digestible selection was 101.0, relative to the base population, whereas the low digestible selection was 96.3. However, the response to selection varied substantially for each individual cut as can be seen in Fig. 4.

A clear selection response was obtained in the predicted directions. The phenotypic differences between the means of the 2 groups of originally selected plants was 7.2 % of the population mean. The difference in weighted mean digestibility in yield trials between the positive and negative selection was 4.7 % of the population mean (base population). So the estimate for realized heritability for selection on mean digestibility, using flag leaf digestibility selection, is 0.67.

Table 1 presents performance data for important characteristics such as persistence, winterhardiness, rust resistance, dry matter yield and weighted mean digestibility. Here, yield and digestibility data are relative to the mean of the 2 control varieties Barlet and Magella. The 2 best performing synthetics out of the 5 made are presented as well. Both are based on 4 high digestible clones of the originally selected 54.

The positive selection was significantly better in average digestibility than the base population whereas the negative selection was significantly worse. In other characteristics, there were no significant differences between these 3 populations. Also in heading date, or other morphological traits, no differences were observed as a result of selection on flag leaf digestibility.

Discussion

As mentioned in Materials and Methods, we use 24 hours instead of 48 hours as incubation time for cellulase in the digestibility tests. This is because we found larger digestibility differences and a much better discrimination between genotypes (unpublished data) by using 24 hours as compared to 48 hours, which is an advantage for breeding purposes. Also, using 24 hours is closer to the real degradation rate of perennial ryegrass in the rumen than 48 hours.

The absence of correlation between leaf and stem digestibility in our pilot study contrasts with the high correlation of 0.91 found by Tyler and Hayward (1982) in a study with 13 populations of Italian ryegrass (*Lolium multiflorum* L) in plots. However, they correlated mean leaf and stem digestibility of a composite sample per population, whereas

we correlated data per individual genotype. Also, their leaf samples included lamina and sheath, whereas our samples only included lamina.

The results of the selection experiment indicates that selection for digestibility is well possible, using simple and reproducible methods. A quite high estimate of 0.67 has been found for realized heritability, which is remarkably close to the wide-sense heritability of 0.7 for flag leaf digestibility, estimated from the variation within the 600 spaced plants.

Selection response was better for low digestibility than for high digestibility. Such asymmetry of response has been found in many two-way selection experiments according to Falconer (1960). In this case it is partly caused by the more intensive selection for lower digestibility by taking fewer, more extreme plants. Partly it may be caused by the dominance for higher digestibility, as indicated by higher digestible half-sib families showing less within-family variation.

No unwanted correlated response was observed for other characteristics such as yield, persistence, winterhardiness, rust resistance and heading date. By using only flag leaves for selection, we may have avoided a change of the leaf to stem ratio, or an unwanted selection for later heading plants.

The selection response varied for individual cuts. The highest positive response was obtained in cuts during summer. This may be caused by the fact that also the selection was done in summer.

In only one selection cycle, using variable, high-level germplasm, varieties were made with a 5 % better relative mean digestibility than currently recommended varieties. In absolute terms this would mean a 3.8 % higher digestibility.

Although yield and digestibility do not seem to be correlated, we do have a major problem combining high digestibility with superior yields. Selection effort devoted to digestibility cannot be devoted to yield improvement. Van Wijk and Reheul (1991) reported an average yield improvement of 0.5 % per year for newly listed varieties. Therefore, breeders selecting for digestibility will note that their varieties are outyielded by the newest varieties of competitors who do not select for digestibility.

Two of the 5 synthetics made out of the high-digestible selected clones were submitted for Recommended List testing in the Netherlands, one also in Belgium and one in France. Unfortunately, at present digestibility of new varieties is not taken into account in official tests in Holland, Belgium and France. Therefore it does not seem likely that these high-quality synthetics will be recommended, since they are no improvement over existing varieties in other agronomic characteristics.

The changing market environment, however, demands a shift of priorities from yield to quality of forages, as suggested in the introduction. Improving forage quality adds more value to the grass for today's farmers than improving yield.

Official testing authorities for forage grasses face a great responsibility. They can very positively influence the manure surplus, mineral balance and cost effectiveness of dairy farmers by taking digestibility into account for recommendation of varieties. Only that will lead to the desired progress in selection.

Acknowledgement

The authors would like to thank Mr. David Jonston for carefully reading the manuscript and giving valuable suggestions.

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Table 1 : Performance of selection for digestibility, the base population and 2 control cultivars combined results of 3-replicate trials in Wolfheze and Lelystad, the Netherlands, sown in spring 1989 yields of 2 harvest years 1990 and 1991. Weighted mean digestibility from Wolfheze, 2 harvest years

Response to selection on digestibility, other characters

	Persistence	Winterhardiness	Rust resistance	DM yield	% digestibility	
Pos. selection	6.1	5.2	6.5	97	104	
base population	5.6	5.2	6.7	96	103	
Neg. selection	5.4	5.3	6.5	98	100	
Candivar 1	6.8	6.4	6.2	104	102	
Candivar 2	6.6	5.3	5.4	100	105	
Barlet	5.0	3.2	8.2	101	101	
Magella	4.6	4.9	6.1	99	99	
c.v. =	5.2	6.5	4.6	1.2	0.3	
L.S.D. = ...	0.8	0.8	0.9	5	1	
Av. or 100 =	4.8	3.1	7.1	15.3 mT/ha	75.9 % ivomd	
(9 means good)						

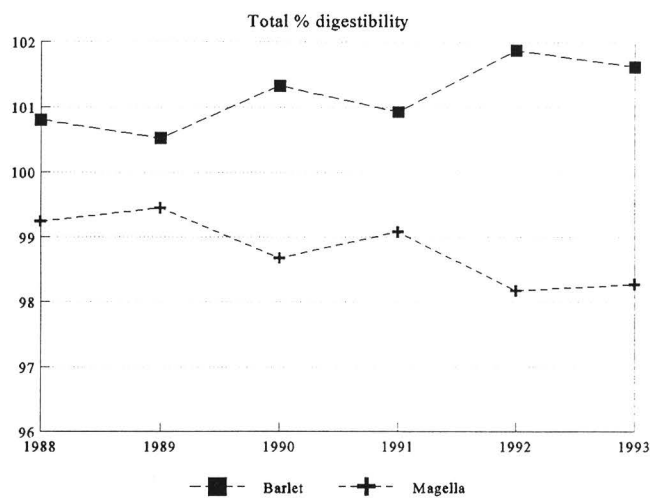


Figure 1 : Digestibility (relative) of 2 varieties of *Lolium perenne* in the first harvest years of 6 different cutting trials in Wolfheze, the Netherlands

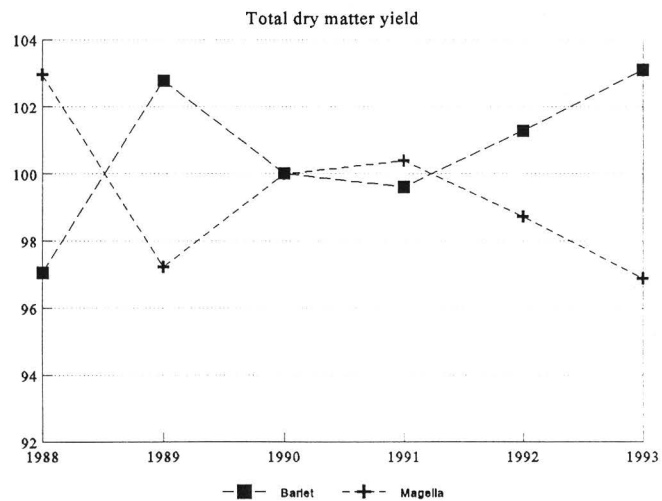


Figure 2 : Dry matter yield (relative) of 2 varieties of *Lolium perenne* in the first harvest years of 6 different cutting trials in Wolfheze, the Netherlands

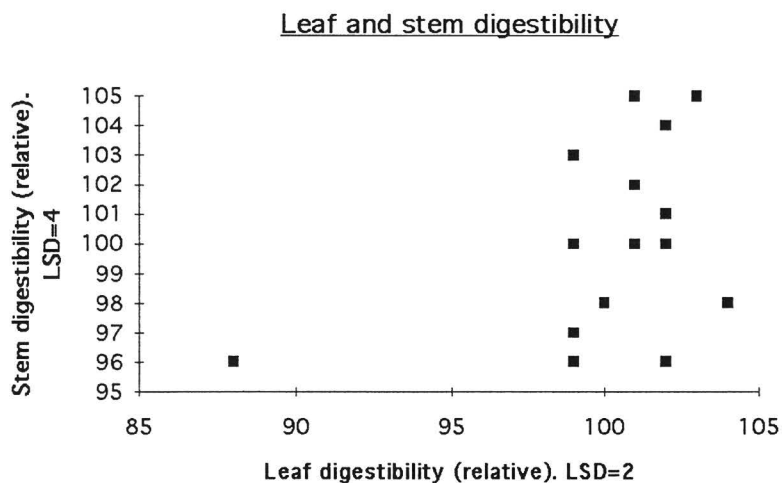


Figure 3 : Correlation between leaf and stem digestibility of 15 clones in 2 replicates
Correlation coefficient $r = 0.4$ (not significant)

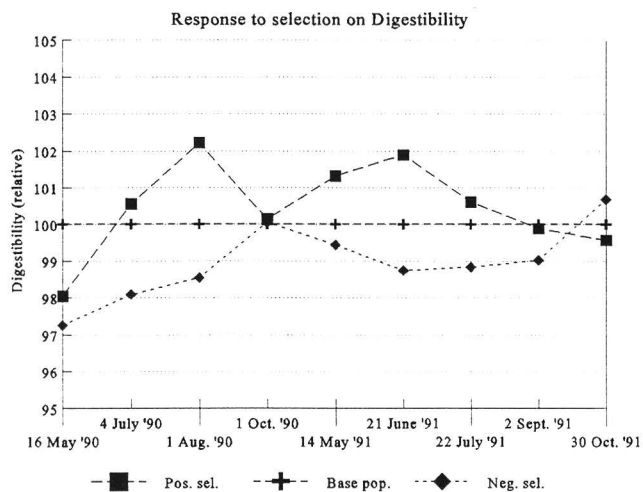


Figure 4 : Digestibility (rel.) of a base population and selections on high resp. low digestibility in a 3-replicate yield trial in Wolfheze, the Netherlands, each cut during 2 harvest years

PROGRESS IN COMBINING HIGH DRY MATTER YIELD WITH REDUCED FLOWERING INTENSITY AND IMPROVED DIGESTIBILITY IN PERENNIAL RYEGRASS

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Summary

Earlier work on perennial ryegrass has shown that it is possible to improve the mean leaf content of herbage over all harvests by reducing flowering intensity (the mean proportion of reproductive tillers during spring and summer). Since leaves are known to be more digestible and ingestible than other plant parts, this would be expected to improve herbage quality. To determine whether or not reduced flowering intensity is compatible with other important breeding objectives, a new variety (Ba11778) bred for improved herbage yield and persistency combined with low flowering intensity was compared with three control cultivars which were similar in ear emergence date to Ba11778 but were expected to range in flowering intensity (Merlinda = high, Talbot = intermediate, Magella = low). Two adjacent sets of plots were established and the sets subjected to different harvesting frequencies : five harvests (conservation management) and nine harvests (simulated grazing management) per year.

Under the conservation management, Ba11778 had a significantly lower flowering intensity than both Merlinda and Talbot and the highest mean *in vitro* organic matter digestibility during spring and summer of the first harvest year, significantly (2.5 percentage units) higher than Merlinda. Although Merlinda had higher dry matter yield (DMY) than Ba11778 at the first cut under the conservation management in both the first and second harvest years, Ba11778 gave the highest total DMY. Under the simulated grazing management, Ba11778 had the highest DMY in early spring of both years and gave significantly higher (10 % or more) total DMY than all three control cultivars in the second harvest year. In both trials, Ba11778 had much better ground cover than the other three varieties in September of the second harvest year.

Introduction

At the same stage of maturity, ryegrass leaves are more digestible and ingestible than culms and inflorescences (Hides *et al.*, 1983 ; Laredo and Minson, 1975). There is considerable genetic variation within perennial ryegrass (*Lolium perenne* L.) in the mean proportion of reproductive tillers during spring and summer (flowering intensity), which can result in significant differences among varieties in mean leaf content of herbage over successive harvests (Wilkins, 1995). Variation in flowering intensity in spring is laborious to measure directly but individuals or families with low flowering intensity tend to recover more rapidly after the first flush of reproductive growth has been harvested. Probably this is because recovery after defoliation depends not only on energy and protein reserves, but

also on the number of actively growing vegetative meristems which act as much more powerful sinks for stored assimilates than do newly developing axillary buds (Richard, 1994). In summer, when the proportion of reproductive tillers is lower, individuals and families can be ranked easily for flowering intensity by eye.

Low flowering intensity however, might have adverse effects on other important traits. Spring growth may be reduced. The onset of reproductive growth in spring is accompanied by a marked increase in leaf extension rate which, it is assumed, helps to counteract the depressing effect of low spring temperatures on growth (Parsons and Robson, 1980). Reproductive growth in spring accounts for a large proportion of total annual yield under a conservation management, although it is less important when the herbage is harvested frequently (Wilkins, 1989). Furthermore, biomass yield at the reproductive stage is an important component of seed yield in graminaceous species. It has proved to be genetically independent of harvest index to a considerable degree, and has been positively correlated with seed yield (eg. Sharma, 1993). Herbage yield in summer and autumn perhaps could be reduced as well, since culms and inflorescences can act as sinks for assimilates which might be in surplus (Lemaire *et al.*, 1988). Therefore the practical value of reduced flowering intensity as a means of improving herbage quality is open to doubt.

The present paper compares the yield and digestibility of a new variety, bred for low flowering intensity combined with high dry matter yield and persistency, with three control cultivars with similar ear emergence dates but ranging in flowering intensity.

Materials and methods

Ba11778 was produced by two generations of family selection for high dry matter yield and persistency combined with low flowering intensity within a restricted breeding population of perennial ryegrass (Wilkins, 1994). The three control cultivars (Merlinda, Talbot and Magella) have similar ear emergence dates to Ba11778. Merlinda is currently the best selling perennial ryegrass variety in the UK but has been shown to have a higher proportion of flowering tillers than Talbot (Wilkins, 1995). Talbot is the standard control cultivar used in UK National List Trials. Magella was expected to be lower in flowering intensity than Talbot because of its superior recovery after the first conservation cut and low visual rating for flowering intensity in summer during earlier trials at Aberystwyth.

Two separate plot trials were sown by hand in August 1992, each arranged in three randomised blocks. Plots were marked with herbicide, plot size being 3x1m. In 1993, one trial was harvested on five occasions (conservation management) and the other on nine occasions (simulated grazing management) with a Haldrop plot harvester and dry matter yields determined. The treatments were reversed in 1994, so that the management of one of the trials was identical to that now used in UK National List Trials. A total of 258 kg of N ha⁻¹ was applied to the conservation management and 336 kg to the simulated grazing management as split dressings. Samples of herbage dried at 80°C from the first, second and fourth harvest of the conservation management were milled through a 1mm sieve prior to chemical analysis. Organic matter digestibility (DOMD) was determined by digestion with pepsin and fungal cellulase over 48h (Jones and Hayward, 1975) and water-soluble carbohydrate content (WSC) by an automated anthrone method (Thomas, 1977). In 1994, the percentage of flowering tillers was determined shortly before the first three harvests of the conservation management, in May, June and August. Three samples of grass, each

containing more than 100 tillers, from near the centerline of each plot were cut at ground level and separated into vegetative and reproductive tillers.

Results

The four varieties varied significantly ($P = 0.05$ or less) in all the traits shown in the Tables. Varieties varied considerably in mean percentage of flowering tillers over the three sampling dates, Ba11778 and Magella being significantly lower than Talbot which in turn was significantly lower than Merlinda (Table 1). Varietal rankings in percentage of flowering tillers were not identical at the three sampling dates. In May Ba11778 was significantly higher than Magella, while in June and August it was similar to Magella. Ba11778 had the highest mean *in vitro* organic matter digestibility (DOMD) over the first three harvests of the conservation management, Merlinda had the lowest and Talbot and Magella were intermediate (Table 1). The mean water-soluble carbohydrate content (WSC) of Magella was significantly lower than that of Ba11778.

Under the conservation management, Ba11778 gave significantly higher dry matter yield (DMY) than Magella at the first harvest and the highest total DMY (Table 2). However, Merlinda gave the highest DMY at the first cut in May, significantly more than Ba11778 in the second harvest year. Under the simulated grazing management (Table 3), Ba11778 gave the highest DMY at the first harvest in April and also the highest total DMY in both harvest years. The DMY of Ba11778 was significantly (10 % or more) higher than all the other varieties in the second harvest year.

Under both managements, all the varieties maintained high ground cover until October of the first harvest year (Tables 2 and 3). By September of the second harvest year however, the ground cover of the three control cultivars had declined markedly while that of Ba11778 remained high.

Discussion

These results show that it is possible to combine low flowering intensity with improved persistency and improved DMY under a simulated grazing management. However, although Ba11778 yielded more than the low-flowering cv. Magella at the first conservation cut, perhaps because of increased tiller density, it did not yield as much as Merlinda. Since the first conservation cut can account for more than half of the total annual yield, especially when summer rainfall is low, the high yield of Merlinda at this time suggests that high-flowering types may be best for silage-based systems of animal production. But neither Merlinda nor any of the other high-flowering varieties trialed at Aberystwyth so far have proved to be sufficiently persistent to maintain high production under a conservation management in the third and subsequent harvest years.

The mean DOMD of Ba11778 under the conservation management was 1 percentage unit higher than that of Talbot and 2.5 percentage units higher than that of Merlinda, suggesting a positive effect of low flowering intensity on herbage quality. An experiment is in progress at Aberystwyth to determine the extent to which varietal differences in leaf content and digestibility are expressed under more the frequent harvesting regimes typical of grazing systems.

To ensure the commercial success of low-flowering varieties, particular attention should be given to improving seed set and seed weight to compensate for their relatively low biomass of reproductive growth. Further trials are in progress to determine if the phenotypic selection for seed yield which has been carried out during the development of Ba11778 has been effective in improving its harvest index.

Acknowledgement

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Table 1 : Flowering intensity, organic matter digestibility (DOMD) and water-soluble carbohydrate content (WSC) under the conservation management.

Variety	Percentage of flowering tillers				Mean DOMD May-September	Mean WSC May-September
	May	June	August	Mean		
Ba11778	31.9	6.9	0.8	13.2	72.55	33.62
Magella	23.6	8.2	2.3	11.4	71.04	30.58
Talbot	38.3	10.2	5.7	17.9	71.53	31.58
Merlinda	49.6	12.8	9.6	24.0	70.02	31.42
s.e.d.	3.32	1.61	1.50	1.62	0.722	1.110

Table 2 : Dry matter yield and percent ground cover under the conservation management.

Variety	Dry matter yield (t ha ⁻¹)				Percent ground cover	
	May 1993	Total 1993	May 1994	Total 1994	October 1993	September 1994
Ba11778	6.1 (121)	14.7 (116)	4.7 (108)	11.8 (107)	66	67
Magella	4.7 (93)	12.8 (100)	4.0 (91)	11.0 (100)	60	43
Talbot	5.1 (100)	12.7 (100)	4.4 (100)	11.0 (100)	63	43
Merlinda	6.7 (132)	14.1 (111)	5.3 (120)	11.5 (104)	62	27
s.e.d.	0.33	0.53	0.21	0.44	2.2	4.5

Table 3 : Dry matter yield and percent ground cover under the simulated grazing management.

Variety	Dry matter yield (t ha ⁻¹)				Percent ground cover	
	April 1993	Total 1993	April 1994	Total 1994	October 1993	September 1994
Ba11778	2.6 (117)	14.0 (112)	1.1 (210)	11.2 (113)	73	60
Magella	2.0 (90)	13.0 (104)	0.5 (110)	9.9 (101)	60	37
Talbot	2.3 (100)	12.4 (100)	0.5 (100)	9.9 (100)	58	40
Merlinda	2.5 (109)	12.7 (102)	0.9 (176)	10.2 (103)	58	20
s.e.d.	0.22	0.54	0.09	0.28	3.3	6.4

DIGESTIBILITY AS CRITERIUM IN VARIETY TESTING TRIALS

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Summary

Varieties of *Lolium perenne* were examined for digestibility in two cycles of 2 growing seasons at 3 centres. The digestibility was determined by the in vitro method of Tilley and Terry or by the NIRS-technique. The maximum difference in digestibility between varieties in the early (4 varieties), intermediate (6 varieties) and late (6 varieties) heading date groups, was respectively 3.2 units, 2.7 units and 2.6 units in the first cycle (1989-1990). In the second cycle (1991-1992) the intervals were smaller : 1.5 units, 2.2 units and 1.9 units for the early (6 varieties), intermediate (9 varieties) and late (6 varieties) groups. In the latter cycle different varieties were tested, the NIRS-technique was used and the first cut was taken earlier in the season.

The classification of the common varieties (two in each group) was the same in the two experiments, but the differences in digestibility were smaller when the first cut was taken earlier.

Within the narrow range in each group, the classification of the varieties is not always the same in every year (average 3 centres) or in every centre but some varieties seem to show consistently good or low digestibility.

If digestibility is to be taken into account for a final decision for admittance or refusal of a candidate variety to a list, differences in digestibility must be accurately determined and a lot of samples is needed for this purpose. Therefore digestibility analysis becomes very expensive. In Belgium, digestibility will not be considered for variety evaluation of *Lolium perenne* until new candidates are entered with clearly better digestibility than the current varieties.

Keywords : *Lolium perenne* - digestibility - variety testing

Introduction

In variety testing of forage crops, more and more attention is being paid to quality and feeding value because animals with a high yield potential can only produce at the most economic level when the daily intake of freshly grazed grass per animal is at maximum. In this respect, digestibility is acknowledged as a good criterion of feeding value (Walters, 1984). *Lolium perenne* is certainly the most important grass species for grassland renovation in Belgium and is the most digestible species compared to others in a common use in Western Europe.

When considering whether digestibility should be taken into account during the evaluation, of varieties of the same species, it is important to know the range in digestibility between varieties and to know the size of the genotype x environment interactions for this character.

Materials and methods

The varieties, sown in the Belgian National List tests in 1988 and 1990, were considered. They were sown in 3 locations with differing soiltypes (table 1). The varieties were divided in three groups : early, intermediate and late date of heading and the cutting dates were chosen for each group independently. So, varieties can only be compared within the groups.

In the first (1989-1990) and in the second cycle (1991-1992) respectively 16 and 22 varieties were involved. Only 2 varieties in each group were present in both cycles. At every cut, samples were taken in each replicate and dried. Then the four replicates were mixed to become one sample. Digestibility was determined by the two stage Tilley and Terry method for the first cycle. These samples were also used to compose a calibration equation for NIRS application. The digestibility of the varieties in the second cycle was predicted by NIRS.

The digestibility of a variety on annual basis was calculated as follows :

$$\text{Annual digestibility (units)} = \frac{\text{total digestible organic matter yield over all cuts}}{\text{total organic matter yield over all cuts}} \times 100$$

This calculated coefficient is used as the digestibility in the text.

Results and discussion

Range of digestibility between varieties

The difference between the highest and the lowest digestibility (on an annual basis) in each location is very variable (table 2). Differences in cutting schedule and growing conditions (soiltype, water balance, ...) may be responsible for this variation between locations and years. In the second cycle the ranges are smaller : other varieties were involved, the first cut was taken earlier in the season and the frequency of cutting was higher. This influences the digestibility in every cut and the relative importance of every cut to the total dry matter yield.

If we consider the average digestibility of the 2 growing seasons, the range is 2.6-3.2 units in the first cycle and 1.5-2.2 units in the second cycle. At this time variety trials in Belgium are managed as in the second cycle and the digestibility determination of such a quantity of samples will be done by NIRS, so the expected variation in digestibility is about 1.5-2.2 units within a group of perennial ryegrass. Visscher (1992) found differences of maximum 2 % within a group as an average of 4 years. In Germany, the range in DM-digestibility between 8 varieties with a difference in heading date of 20 days was 2.8 units (Daniel *et al.*, 1981).

Classification of varieties in different years

The behaviour of the varieties in the growing seasons 1989-1990 is already published (De Vlieghe *et al.*, 1994). Figure 1 illustrates the classification in the seasons 1991 and 1992. The conclusion for both figures is the same : in spite of a narrow range in digestibility within a group, some varieties seem to be more or less digestible than the others. Some of these less digestible varieties were also tested in the Dutch official trials and there they behaved in the same way (Visscher, personal communication).

This means that differences in digestibility between varieties exist and can be demonstrated.

Use of digestibility as a character of variety evaluation

Table 3 presents the digestibility of the varieties, listed in two cycles. Two varieties in each group were present in the two cycles as they were the standard varieties and within the group these varieties are classified in the same way. For the early and intermediate group this might be due to a difference in ploidy.

Nowadays, new varieties are compared to standard varieties of the same ploidy number so the ranges in digestibility will become smaller than the given range in each group (1.5-2.2 %).

For admittance to the Belgian National List, new varieties of *Lolium perenne* must have a positive score for an index combining : dry matter yield, persistency and resistance to crown rust. The final decision is closely connected to this index score. If digestibility is taken into account, a weighing factor must be given, which relates to its practical importance. For this purpose a tendency to be better or worse is not enough, the differences in digestibility must be more accurately defined. Analysis of well prepared samples in every cut, collected in several sites and years, is necessary to minimize the influence of cutting schedules and growing conditions on the digestibility numbers. The price of analysis with NIRS is not the insurmountable problem anymore but sample preparation costs are high. It is questionable whether digestibility differences of about 2 % on an annual basis are important enough to justify digestibility determination.

Conclusion

Now and in the immediate future varieties of *Lolium perenne* will be divided in three groups in terms of earliness of heading date and they will be compared with standard varieties of the same ploidy number. Analysis of the many samples, needed for a good digestibility prediction of grass varieties will be executed with a NIRS. In these conditions the range of digestibility on an annual basis within a group is about 2 units.

The classification of the varieties is not always the same in every year and in every site but some varieties seem less or better digestible than the others. The differences are not sufficient to justify the high costs of digestibility determination in evaluating new candidates for admission to the Belgian Catalogue. If new varieties with clearly better digestibility than the current varieties become available, this conclusion could be reconsidered.

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Table 1 : Variety trials used for digestibility determination : variety numbers, centres and husbandry

Cycle	VARIETIES			SOILTYPE (centre)	Number of cuts		N-fertilization	
	early	intermediate	late		year 1	year 2	year 1	year 2
1989- 1990 (sown in 1988)	4 { 3 diploid { 1 tetraploid	6 { 3 diploid { 3 tetraploid	6 { 6 diploid { 0 tetraploid	SAND (Geel)	4	4	360	260
				SANDY-LOAM (Merelbeke)	4	5	330	360
				CLAY (Watervliet)	4	4	420	385
1991- 1992 (sown in 1990)	6 { 1 diploid { 5 tetraploid	9 { 2 diploid { 7 tetraploid	7 { 5 diploid { 2 tetraploid	SAND (Geel)	5	4	430	350
				SANDY-LOAM (Merelbeke)	6	6	430	430
				CLAY (Watervliet)	4	4	430	380

Table 2 : Range in digestibility between varieties of *Lolium perenne* of 2 cycles : 1989-1990 and 1991-1992

Range in digestibility						
Cycle :	1989 - 1990			1991 - 1992		
Group :	Early	Interm.	Late	Early	Interm.	Late
Digestibility in an individual centre						
biggest range	6.9	4.2	6.2	3.9	3.6	3.2
smallest range	1.7	2.4	2.8	1.3	1.3	2.0
Digestibility in a centre over 2 years						
biggest range	3.9	3.3	3.6	3.4	2.6	2.8
smallest range	1.5	2.1	2.0	1.4	1.7	1.9
Average digestibility over the centres and over the whole cycle	3.2	2.7	2.6	1.5	2.2	1.9
Digestibility mean of the varieties in the group	70.8	74.8	72.5	74.8	76.0	74.5
Number of varieties	4	6	6	6	9	7

Table 3 : Relative digestibility of varieties of *Lolium perenne* (cycles 1989-1990 and 1991-1992)

T Y P E	VARIETIES	PLOIDY NUMBER	Relative digestibility	
			CYCLE 1989-1990	CYCLE 1991-1992
E A R L Y	1(*)	diploid	98.9	99.2
	2(*)	tetraploid	101.1	100.8
	3	diploid	99.1	
	4	diploid	96.6	
	5	tetraploid		101.4
	6	tetraploid		100.5
	7	tetraploid		100.8
	8	tetraploid		101.1
	(*)standard : MEAN		71.7	74.4
I N T E R M E D I A T E	9(*)	tetraploid	101.6	100.2
	10(*)	diploid	98.4	99.8
	11	diploid	98.8	
	12	diploid	98.7	
	13	diploid	98.0	
	14	diploid	99.9	
	15	diploid		99.0
	16	tetraploid		100.8
	17	tetraploid		100.5
	18	tetraploid		100.1
	19	tetraploid		101.3
	20	tetraploid		101.9
	21	tetraploid		101.3
	(*)standard : MEAN		75.4	75.6
L A T E	22(*)	diploid	101.5	100.2
	23(*)	diploid	98.5	99.8
	24	diploid	102.2	
	25	diploid	102.0	
	26	diploid	101.3	
	27	diploid	101.3	
	28	tetraploid		102.1
	29	tetraploid		102.0
	30	diploid		100.3
	31	diploid		102.3
	32	diploid		102.0
	(*)standard : MEAN		71.7	73.6

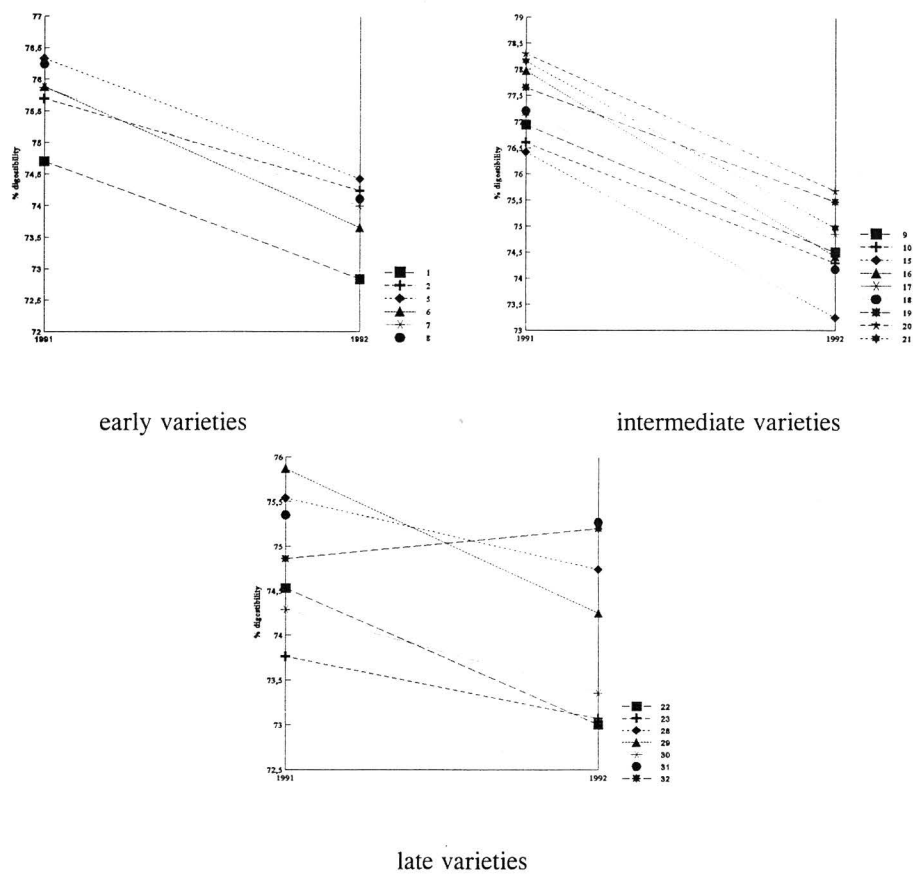


Figure 1 : Digestibility of perennial ryegrass varieties in the cycle 1991-1992

SELECTION THE LOW SAPONIN POPULATION FROM LUCERNE CV. RADIUS

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Abstract

Radius cultivar because of its valuable yield characteristics was taken to produce a low saponin population. The microhemolitical procedure presented by Jurzysta (*Acta Agrobotanica*. XXXII. 1 : 5-11) was used for evaluations of saponin content in leaf samples. The individual selection and pair crossing method was employed to produce plant stock for the synthesis of an improved population. Radius cv. contained 1 - 1.6 per cent of hemolytic saponins in leaf dry matter but variation for this character in individual plants varied from 0 to 2.5 per cent. The plants containing below 0.2 per cent of hemolytic saponins shared 0.8 - 2.2 per cent in the cultivar. In the course of 3 cycles selection all plants containing more than 0.2 per cent hemolytic saponins were discarded. Seven F₁ were received to complete low saponin plant stock for the synthesis of new population Syn. 1, which contains 0.10 - 0.32 per cent of hemolytic saponins.

Introduction

The breeding of alfalfa varieties for low saponin content is important in the improvement of forage crops for balanced feeding diet. Until the present time most of alfalfa varieties have contained considerable quantities of saponins. These toxic glycosides of complex structure and action have a particularly detrimental effect on monogastric animals, resulting in a reduction of their growth and productivity (Cheeke, 1971 ; Cheeke *et al.*, 1977). The quantity of saponins can be a factor determining the forecrop value of alfalfa in the crop rotation (Oleszek, 1986). This allelopathic effect has been observed by many authors (Mishushin and Naumova, 1955 ; Nord and Van Atta, 1960 ; Jurzysta, 1970 ; Berman, 1975 ; Pedersen, 1975 ; Musayetan, 1977 ; Oleszek, Jurzysta, 1987).

Published data which confirms the high heritability of saponin content (Jones, 1969 ; Hanson, 1972 ; Rotili, 1989) allow the possibility of effective selection for low saponin content within breeding materials. Some positive practical results of lowering saponin content by selection were achieved by Pedersen and Wang (1971), Buglos and Bocsa (1976) and Bocsa and Sarosi (1989).

The aim of our investigation was to decrease the saponin content within "Radius" variety by individual selection and pair crossing of plants containing below 0.2 % hemolytic saponins in three generations.

Material and methods

The initial material consisted of 22 plants with low hemolytic saponin content which were selected among the single spaced Radius plants in the nursery.

These plants were used to develop the improved population which consisted of 6 progenies which were received in 3 cycles of selection where plants containing below 0.2 % hemolytic were intercrossed.

Samples of leaves for saponin content evaluation were collected in the field, in the late bud formation stage, from the second cut. For quantification of hemolytic saponins in the leaves, the microhemolytic technique was applied (Jurzysta, 1979).

Results

The number of plants containing more than 0.2 % hemolytic saponins decreased rapidly in three cycles of selection (table 1). That confirmed the statements of Jones (1969), Hanson (1972), Rotili (1989) that low saponin content is a highly heritable trait. Correlation coefficient for saponin content vs. plant mass was near zero and insignificant. In a comparatively short period of time the improved population was obtained that contained much less saponins than initial variety Radius and only two times more than Szapko (Table 2, Fig.)

This population is suitable for a low saponin breeding programme.

Table 1 : Per cent of plants with saponin content below 0.2% in selection cycles

Selection cycle	Low saponin plants no.	Low saponin plants per cent of total number
I Radius cv.	22	1.6
II F ₁ plants	193	17.0
III F ₂ hybrids	515	57.0
Syn. 1	600	96.0

Table 2 : Comparisons of saponin content in Syn. 1 population and standards.

Population	Saponin content in % (mean of 3 repetitions)		
	in fresh leaf matter microhemolytic method	in dry matter of total plants	
		microhemolytic method	Trichoderma viride test
Syn. 1	0.10	0.32	0.21
Szapko	0.05	0.22	0.13
Radius	0.32	1.52	0.94
NIR - P _{0.05}	0.07	0.29	0.20

AGRONOMIC VALUE OF *FESTULOLIUM* (*FESTUCA PRATENSIS* × *LOLIUM MULTIFLORUM*) STRAINS

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Abstract

The aim of the presented study is to evaluate agronomic potential of *Festulolium* strains (*Festuca pratensis* $2n = 4x = 28$ × *Lolium multiflorum* $2n = 4x = 28$) compared to control cultivars of *L. multiflorum*, *L. perenne*, *F. pratensis* and *Festulolium*. The studied *Festulolium* strains show high fresh and dry matter yields, as well as good nutritive value - in vitro dry matter digestibility, crude protein content and water soluble carbohydrate content. Their persistency and winter hardiness is comparable to that of meadow fescue.

Introduction

The development of interspecific and intergeneric hybrids of the *Lolium-Festuca* complex aims at combining the most favourable characters of the parental species - high yield and good nutritive value of ryegrasses with the persistency, winter hardiness and drought tolerance of fescues. These hybrids offer excellent potential for producing improved grass cultivars. The tetraploid hybrids which combine the genomes of *Lolium multiflorum* and *L. perenne* have been successfully developed into commercial cultivars. Efforts to produce cultivars from hybrids combining the genomes of *Lolium* and *Festuca* species have been less successful. Within the *Lolium-Festuca* complex a special breeders' interest is directed to *F. pratensis* ($4x$) × *L. multiflorum* ($4x$) hybrids, from which several cultivars have already been obtained (Elmet, Paulita, Perun). In Poland, an extensive breeding programme on the tetraploid hybrids between meadow fescue (*F. pratensis*, $2n = 4x = 28$) and Italian ryegrass (*L. multiflorum*, $2n = 4x = 28$) has been carried out at the Szelejewo Plant Breeding Station since 1983 (Zwierzykowski *et al.*, 1993 a, b). The objective of this study is to evaluate some agronomic characters of *Festulolium* strains obtained from *F. pratensis* ($4x$) × *L. multiflorum* ($4x$) hybrids.

Materials and methods

After six generations of selection for fertility, good vegetative growth and persistency (under spaced plant conditions) five tetraploid *Festulolium* strains have been produced. Three of these strains are examined in two 3-year field trials (10 m² plots with four randomized replicates). Trials were established at the end of August 1992 and 1993, respectively ; plots were cut four times (the first cut at the beginning of heading and the next ones at 4-6 week intervals). Some agronomic characters, e.g. fresh and dry matter

yield, seed yield, crude protein content, in vitro dry matter digestibility, water soluble carbohydrate content, persistency and winter hardiness were evaluated. The cultivars of *L. multiflorum* (Lotos), *L. perenne* (Solen), *F. pratensis* (Skra) and *Festulolium* (Paulita, Perun) were used as controls (cv. Skra is diploid and the rest cultivars are tetraploid).

Results

Yield

Total annual dry matter yield of the three *Festulolium* strains (in 1993 – 1st harvest year) reached values from 20.9 to 22.3 t/ha; it was on the same level as in *L. multiflorum* cv. Lotos (23.0 t/ha) and *Festulolium* cv. Paulita (20.3 t/ha) but was significantly higher than *F. pratensis* cv. Skra (16.0 t/ha). The studied strains exhibited good regrowth after cutting and seasonal yield distribution in the first and second harvest year.

Quality

In the studied *Festulolium* strains crude protein content, water soluble carbohydrate content and in vitro dry matter digestibility were relatively high. This high quality was gained without selection. No significant differences in respect to these traits were observed between the studied strains and the control cultivars (Lotos, Solen, Skra and Paulita). It seems that selection for quality can provide *Festulolium* materials with further increased nutritive value.

Persistency and winter hardiness

All studied *Festulolium* materials were characterized by a good persistency and winter hardiness, similar as the cultivars Paulita and Skra but evidently better compared with cvs. Lotos and Perun.

Seed yield

Seed set of *Festulolium* forms obtained after six generations of selection for female fertility was above 50 % (Zwierzykowski *et al.*, 1993a). Seed production of the studied *Festulolium* strains in the field drilled plot trials – from 5.2 to 7.0 q/ha – was on the same level as *Festulolium* cv. Paulita and was slightly lower than that of *F. pratensis* cv. Skra. Further selection for the seed yield is necessary.

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THE FEEDING VALUE OF MEAL MADE OF SAPKO, A SAPONIN FREE LUCERNE VARIETY, IN THE DIET OF SOME POULTRY SPECIES AND RABBIT

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Introduction

It is not usual to evaluate new fodder crop varieties establishing animal feeding experiments. The French school of Lusignan showed the way of it (Huguet *et al.*, 1966 ; Huguet, 1967 ; Bertin *et al.*, 1971 ; Gillet *et al.*, 1983 ; Guy *et al.*, 1991 ; etc.) and in the USA the feeding value of lucerne dehydrated meal has been studied (Heywang *et al.*, 1959 ; Nakaue *et al.*, 1980) in the case of monogastric animals.

The suitability of Sapko, the saponin free lucerne variety, for feeding and replacing protein was determined in the case of the main monogastric animals up to 1990 (Bócsa *et al.*, 1986 ; Bócsa, 1991). Lucerne meal and PX were used for the experiment simultaneously. The suitability of lucerne meal for feeding was not clarified before in the case of certain poultry (layer hens, geese and ducks) and rabbit (the PX feeding was cancelled since its production has been finished in Hungary).

Materials and methods

There were 200 geese of Landes varieties (Gippert *et al.*, 1991) used for the experiments in 10 replicates and we used 120 individuals (male and female) of the local Szarvasi varieties in four replications regarding ducks. The duration of the experiment was 8 weeks in the former case and 7 weeks in the latter one. The ratio of lucerne meal was 6 % in the starter, 9 % in the raiser and 12 % - that was unusually high - in the final diet. Tetra-52 hybrids were used for layer hen experiments for 5 months. The ratio of lucerne meal in the diet was always 10 %. Besides Verko control there was another, lucerne-free control too. The ratio of lucerne meal was 40 % in the rabbit feeding experiments up to the animals were 10 weeks old.

Results

Gain of 3 weeks old geese was higher (23 % significantly) in the case of Sapko than it was in the case of Verko. However this notable difference became close to balanced (3 % non significant surplus of Sapko) to the end of the experiment (8 weeks old animals). Diet utilization was equalized perfectly too. The conclusion is that geese are sensitive to saponin only to the first 3-4 weeks of their life and this sensitivity decreases step by step. This

phenomenon was the opposite in the case of chicken and young pig that were sensitive to saponin (Bócsa, 1991).

There is a similar but not so clear tendency in the case of ducks : there was weekly weighting and the two groups became balanced on the 4.-5. week. Taking the reasons into consideration it is well known that the intestines of goose and duck are longer and their appendixes are larger - in proportion too - thus their fibre digestion is better than those of chicken. These animals are only sensitive to saponin in the youth since as grazing animals they adapted to lucerne.

Groups fed with Sapko meal produced significantly 11 eggs more in average in the case of layer hens. The difference between egg laying frequency was significant too - 78.7 %, while it was 71.9 % in the case of the control. The tendency was the following in the case of animals fed with Sapko : lower ratio of yolk and higher ratio of egg-white. The strength of egg-shell was better in this case than in the case of lucerne free control. Layer hens consumed the diet containing 10 % lucerne meal and this could replace 33 % of the soyabean protein groats content of the diet.

The metabolism experiments proved that the dry matter digestibility of Sapko was significantly better than that of Verko. In addition its fat digestibility was better while the protein and fibre digestibility were the same than those of Verko (its metabolic energy content was 7.46 MJ/kg while it was 6.88 MJ/kg in the case of Verko).

There was no significant difference between the gain of rabbits fed with 40 % Sapko or Verko meal.

The investigation of the feeding value of Sapko lucerne can be treated as a finished study in the case of the main monogastric animals.

Summary

The usual diet was applied in the experiments established with 4 varieties. However a certain part of protein was replaced with the saponin free lucerne variety Sapko and the control was Verko, a lucerne variety of high saponin content (the saponin content was equal to 0.6 and 8.5 mg/escin.).

The gain of goose was significantly higher in the first 3 weeks in the case of Sapko feeding. This difference however disappeared to the end of the 8. week. The tendency was similar in the case of duck but the equalization happened at the end of the 4.-5. week.

The biggest difference was found among layer hen groups. The egg production was 11 higher (significant) in this case. The egg-white ratio and egg-shell strength were significantly better than in the group fed with Verko or in the lucerne free control group.

Rabbit was not sensitive to saponin even fed with diet containing 70 % lucerne.

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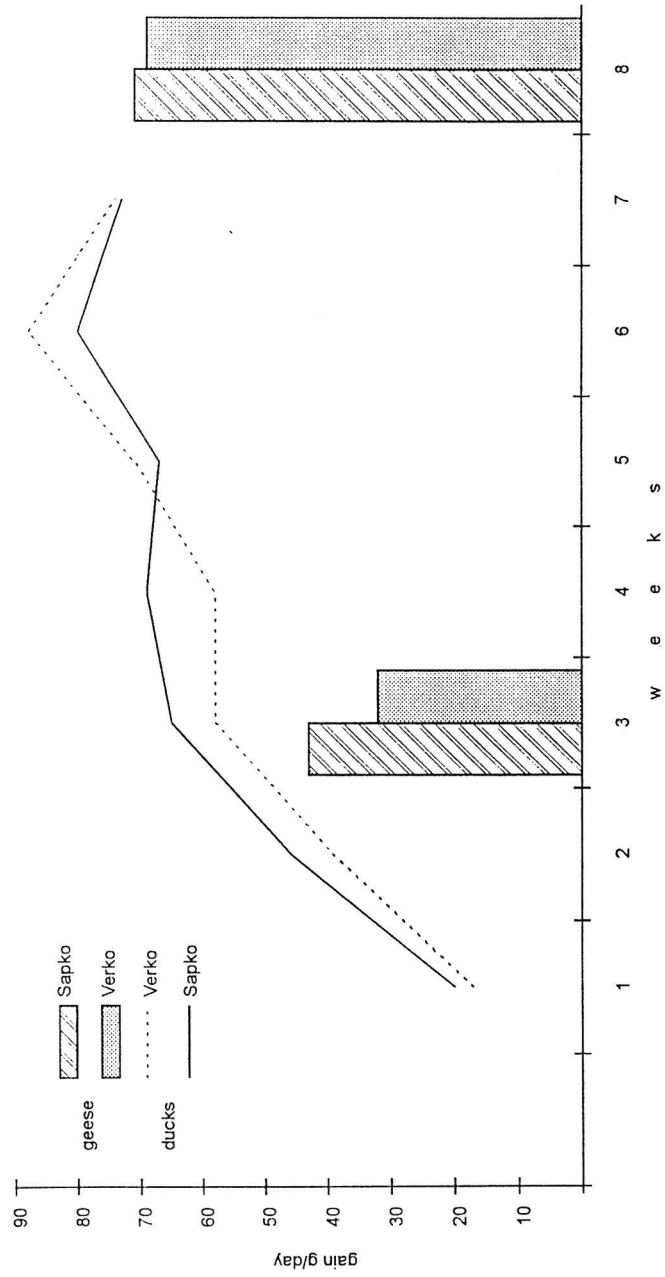


Figure 1 : Gain of ducks and geese during Sapko and Verko lucerne meal feeding experiments

SESSION 8 : Workshops

GUIDELINES FOR THE WORKSHOP DISCUSSIONS

Dirk REHEUL

Workshop topics

1. Who is calling for a better quality in forage and amenity species (farmers, the public, officials, ...)
What is the motivation ?
2. If one agrees to improve the quality, what characteristics should be improved by preference
 - in the forage species
 - in the amenity species(to help : digestibility, palatability, mineral efficiency, resistances, flexibility, anti-nutritional factors, ...)
3. Do the existing methods to measure quality aspects satisfy researchers and breeders ? Is there a need for new systems and more research ? If yes, provide details.
4. How important is the breeding research in the official institutes for the topics under 2.
5. How important is the real breeding work for better quality during the development of varieties ?
Try to quantify the weight of "quality breeding" in breeding programmes.
6. Is there, and should there be, a reward for better quality beyond the competitive advances ?
7. What quality parameters should be included by preference in the variety testing ?

REPORTS OF THE WORKSHOPS

1. DISCUSSION GROUP 1

J. INGRAM ; L. BEEREPOOT

Who wants quality and why

Agreement was rapidly reached on this topic both for amenity and forage grasses.

Consumers of forage types wished to increase their income by more efficient livestock production while amenity users wanted a more attractive product. It was recognised that the amenity grass market was highly stratified with some consumers willing to pay high prices for quality but others wanting grass that was green and grew at a minimum price.

Members of the seed trade and breeders saw the need to make sales as the ultimate objective behind improving quality, although there were individuals who were motivated by the need to achieve a goal or by the prestige of producing new varieties.

There was more than one motive for officials and this might depend on government policy. For forage species there was the improvement in competitive efficiency that resulted from quality improvement and some measure of consumer protection but environmental issues were also being considered.

Method of considering characters

The Group agreed that the way quality characters should be considered was :

- a. To assess for each character the practical value of any potential improvement.
- b. To assess the likelihood of improvement by breeding in terms of :
 - the variability available ;
 - the heritability ;
 - any negative consequences linked to the improvement.
- c. To consider how adequate the method of assessment was in both accuracy and cost terms.

In assessing whether measurement of a character was really necessary it had to be considered whether a character :

- a. needed improving ;
- b. needed maintaining at the same level ;
- c. could be allowed to decrease as selection for other traits was applied.

Both a. and b. require measurements to be made.

Quality characters in forage grasses

Dry matter content

The Group considered that improvements in DM % might be useful because :

- a. Effluent would be reduced during conservation.
- b. Intake would be improved but just how large this improvement would be was a question of debate.
- c. There was the suggestion that palatability was improved.
- d. The cost of lucerne dehydration would be substantially reduced.
- e. A more rapid drying of red clover was important.

There appeared to be adequate genetic variability which was heritable, but unfortunately it was highly negatively correlated with yield. Measurement was easy and already done.

Soluble carbohydrate

High soluble carbohydrate was useful because it :

- a. saved on additives - this might be more valuable in species low in soluble carbohydrate (i.e. legumes/timothy/cocksfoot) ;
- b. was correlated with digestibility.

Soluble carbohydrate was easy to select for but there were negative correlations :

- root growth was reduced ;
- crown rust infection might be increased.

The test costs half that of digestibility.

Minerals

Because minerals could be supplied by other means very cheaply, high mineral varieties added very little value. Experience with high magnesium varieties had shown farmers to have very little interest although it was suggested that incorporation magnesium into the herbage would reduce the risk of some animals not getting their dose of minerals.

Digestibility

This character has great value, especially on farms with a forage surplus. In the UK, farmers involved in variety recommendation rate this character among the most important characteristics of forage grasses. In Holland, farmers responded to information about digestibility by buying certain seed mixtures even when this information was not supplied by the official testing authorities. In tall fescue, the limiting factor is not digestibility but palatability. In orchard grass high digestibility is regarded as a positive attribute by the

Swiss testing authorities and it is an important characteristic for recommendation of varieties. In lucerne it is considered to be important for the dehydration use of this crop. In red clover digestibility is seen as an important characteristic.

There are ample possibilities for selecting for digestibility in perennial ryegrass and also timothy. A limiting factor to progress might be the registration of varieties, because testing officials of several countries do not take digestibility into account. Tetraploids were reported by some to be better in digestibility while others claimed diploids to be at least as good. This difference of opinion may be caused by different methods of digestibility estimation.

There are various methods for the measurement of digestibility. Some doubts were expressed about NIRS. It was concluded that it would be beneficial to have calibrations on true *in vivo* data, but that such calibrations will be hard to achieve because of the large number of points required. There were several reports that digestibility differences were smaller on clay soils than on sandy soils. Hot summer temperatures, or stress in general, increased the differences in digestibility. There may be a variety x spacing interaction and digestibility should not be measured on spaced plants but in sward situations. It was argued that it would perhaps be better to select for precise components of plants, or to do simple things such as avoiding stems or changing the leaf stem ratio instead of difficult and laborious tests. High digestibility may be correlated with low yield, although it will depend on how the selection on high digestibility is actually practised. Other correlated characters may be the amount of stems and the heading date, again depending on selection methods.

Preference, palatability

Differences were reported for perennial ryegrass varieties in France. In Holland, differences were seen, but these differences were not consistent for different cuts. Preference for tetraploids seem to be consistent. It was mentioned that cows grazed tetraploids closer to manure patches than diploids, leading to a more palatable regrowth.

Measurement of palatability differences can be done in grazing trials. The use of certain insects and rabbits has been mentioned as a possibility for small scale trials. Digestibility has been reported to correlate well with palatability so this can be used as selection measurement for palatability and vice versa. In tall fescue marked differences exist between varieties. Palatability can be measured by moving leaves between the lips but certain phenolic compounds are also reported to affect palatability. In white clover, slow growing varieties seem to be preferred.

Intake

This character was regarded by the vast majority as very important, perhaps even more important than digestibility. Differences in intake between varieties do exist, but there are no simple methods of determination suitable for small samples and many genotypes. Shear strength has been reported to have an impact on intake but so far no varieties seem to have been developed by this method. Leaf content and digestibility also have a positive correla-

tion with intake. The faster forage passes through the rumen the more forage a cow will eat per day, so speed of digestion is an important measurement of intake. The speed of digestion assessed with a shorter 24-hours digestibility test might be important in measuring intake (instead of 48 hours currently used). Ease of chewing was reported to be correlated to digestibility as well as to rate of intake. The rate of eating over short period can be valuable of intake over a day. The rate of grazing can be correlated to sward architecture, erect swards enabling a higher intake.

Protein level

In grasses protein level is usually too high nowadays. Breeders selecting for high yield will automatically select a lower protein content and it is not worthwhile breeding for lower protein content alone. In lucerne a higher protein level is still wanted, despite the already high levels of protein present. Dehydration plants prefer higher protein levels because subsidies are paid on minimum protein levels.

Quality characters in amenity grasses

Virtually all amenity grass characters can be regarded as quality characters. We only discussed those characters mentioned in posters or presentations.

Colour

This is a very valuable character. However, preference varies from light colour in northern Europe to dark colours in southern Europe. It can be selected for by visual observation as well as by machine.

Establishment

This is a very valuable character in *Poa pratensis* and in warm season grasses such as *Cynodon dactylon*. A rapid establishment is especially advantageous in southern Europe because of the erratic heavy rainfalls. It is probably not easy to improve, but a possible way is to select for heavy seed weight. Synthetic seed has been mentioned as a way to improve this character, but the costs may be prohibitive. Rapid establishment could be negatively correlated to low maintenance.

Shade tolerance

A difference exists between shade because of trees sucking the water away and letting leaves fall, and shade because of a wall. It was argued that shade tolerant varieties do not produce seed well enough so price of seed may hinder progress.

Thatch

This is a negative character. Management plays an important role in building up thatch, so breeding may not really be worthwhile.

2. DISCUSSION GROUP 2

B. BOLLER ; R. TURNER ; E. N. VAN LOO

What characteristics should be improved to improve forage quality ?

In the research and breeding work of the participants five groups of quality characteristics were identified :

1. Antinutritional factors (ANFs)

Reducing the content of ANFs is a prerequisite in all forage crops, but is especially important in crops like alfalfa in the Mediterranean area. Reduction of ANFs may have undesired effects on disease resistance.

2. Disease resistance

Often diseases cause a lower digestibility and a lower intake. Therefore, some breeders feel it is easier and more efficient to breed for better disease resistance instead of direct selection for digestibility.

Mildew resistance in legumes is important in the Mediterranean area. Crown rust resistance is important in *Lolium*.

3. Energy intake

Increasing energy intake from grasses is very important, especially for feeding highly productive dairy cattle. The energy requirements of highly productive dairy cows are so high that the energy intake from grass is not sufficient. To lower the need for imported concentrates, energy intake from grasses should be increased. The question was raised whether this was to be solved by plant breeding only and to what extent a responsibility exists for cattle breeding.

To increase energy intake by animals, digestibility should be improved, especially for the Mediterranean area (e.g. in alfalfa). However, German and Dutch breeders did not see opportunities for increasing energy intake by cattle by increasing digestibility of perennial ryegrass, because they find that methods are not reliable and too expensive, because cultivar evaluation criteria are different in different countries and because of conflicting results concerning the response to selection.

4. Dry matter distribution (leaf to stem ratio)

In alfalfa breeding for quality increasing the leaf to stem ratio helps to increase yield, palatability and digestibility, however disease resistance decreases.

5. *Yield as a quality parameter*

As was pointed out by Mr. van den Boogaert, recently in the Netherlands, yield or productivity is regarded more and more as a quality parameter. Increasing productivity creates management options for the farmer to reduce for example the nitrogen application rate without loss of production but with a reduced nitrogen surplus. Also, at lower nitrogen application rates, negative effects of longer growth periods on digestibility may be prevented using cultivars with a higher productivity. Improved productivity can be used to increase the mineral efficiency of grassland systems and can thus lead to improved quality of agricultural processes.

In general, increasing productivity gives more flexibility for the management of grassland by farmers.

Improving productivity generally increases the competitive ability of grasses. This might reduce problems with invasion of weeds and prevents changes in the botanical composition of swards that would reduce the nutritive value.

Research needs and needs for new methods for forage value evaluation

1. *NIRS*

Users of NIRS were generally quite satisfied with the method and did not have many problems with the underlying "wet chemical" methods or reference methods. It was proposed to initiate a EUCARPIA working group on standardisation of NIRS-methods in order to construct a large database of "wet chemical" data and NIRS-spectra. This initiative should expand and connect already existing networks on NIRS. Although so called local calibrations are needed for breeding purposes, nevertheless the creation of a one large database would be helpful.

2. *Need for standardized forage quality criteria for admittance of cultivars on the different national cultivar lists in Europe*

Some breeders were sceptical on the prospects of improving digestibility in ryegrasses. They were not satisfied with the existing methods, because they were too expensive for selection on characteristics for which no variation was shown among cultivars and for which selection tended to show controversial results.

Moreover, some breeders complained about the different criteria of cultivar evaluation authorities in different countries that have led to acceptance of cultivars in the one country on the basis of superior nutritive value and rejection in an other country on the basis of inferior nutritive value (Roel Vijn).

3. *Relationship between selection criteria and animal performance*

Some animal nutritionists criticise the use of only organic matter degradability as the main selection criterion for nutritive value parameter without looking at components of nutritive value like volatile fatty acids, microbial protein production and rate of degradation. These characteristics depend heavily on fermentation kinetics. Since we have only just started to look into genetic variation for fermentation kinetics, more research is needed to develop fermentation characteristics into selection criteria for breeding towards, for example, a higher energy intake of animals.

Current breeding efforts into quality characteristics

We have summarized the breeding efforts per country below, in parentheses the names of breeding companies that participated in this workshop are mentioned.

- Germany (Steinach) : no breeding efforts into quality
- Italy (ISCF-Foggia, ISCF-Lodi) :
alfalfa : breeding for less ANFs is a prerequisite
ryegrass : no efforts
- Netherlands (CEBECO-Zelder)
ryegrass : breeding for higher diseases resistance, 20-25 % of total breeding efforts
CPRO-DLO devotes about 20 % of its breeding research in perennial ryegrass on organic matter digestibility, its components and on rumen fermentation characteristics of grasses.
- Norway (Løken, Fureneset, Vagones)
ryegrass : no efforts on quality, winterhardiness is most important
Phalaris arundinacea : 50 % of breeding efforts on digestibility
- Switzerland (FAP)
No direct selection for digestibility. There is a small program on ANF. Considerable efforts (20 %) are put into improving disease resistance with the objective of improving quality.
- United Kingdom (Nickerson)
Breeding for higher quality is both necessary and important : for breeders to get their varieties commercialised ; for farmers to lower their feeding costs and to increase their relative contribution of grass in the diet.
At IGER in Aberystwyth, the main objective in forage quality research is to identify genes that may be useful in breeding programs.

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