EUCARPIA

(European Association for Research on Plant Breeding)

NEW METHODS, TECHNIQUES AND APPLICATIONS IN FODDER CROP BREEDING

Report
Meeting of the Fodder Crops Section
SVALÖV, SWEDEN
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ORGANIZING COMMITTEE

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MISCELLANEOUS NEW METHODS, TECHNIQUES AND APPLICATIONS

A FEW WORDS OF WELCOME
from S. Badoux, President of the Eucarpia Fodder Crops Section

Ladies and Gentlemen,
On behalf of the Board of the Eucarpia Fodder Crops Section, I take great pleasure in welcoming you to this conference. You have not only come from sixteen different European countries, but also from overseas, to take part in our activities and discussions. It is with particular satisfaction that I note the large number of a younger generation of grass and legume breeders amongst you and I look forward to their energetic contribution in our forthcoming debates.
Those of you who take part in the meeting in Roskilde in 1976 will remember that we paid a short visit to Sweden. This was only a foretaste, and many of you then expressed the desire to come back again. We are therefore extremely grateful to our Swedish collegues, to Dr. Sjödin and his committee in particular, for undertaking the organisation of this meeting. Sweden is a large country and here in the southern part we are only haf-way between Rome and the most northerly tip. Sweden is not only a country that is known for its size but also for its importance in the scientific field. In coming here we are retracing our footsteps to the very sources of modern plant-breeding. It is therefore with some emotion that we visit this area, where famous scientists such as Johanssen, Tedin, Nilsson-Ehle, to mention but a few, were actively engaged.
I am sure that you will hear more about plant-breeding in Sweden in the next few days. Our activities will not only be limited to the presentation and discussion of papers and posters. Tomorrow, we shall have the opportunity to visit some plant-breeding companies and to see the practical results of forage-breeding on a local farm.
I should like to conclude by expressing the hope that this will be a highly succesful meeting, and that after it, you will go your separate ways finding new enthusiams and ideas, and warmed by the satisfaction of having met old friends.

Physiological aspects of yield determination in fodder crops

by Volkmar Stoy, Svalöf AB, Svalöv, Sweden

Introduction

In this presentation the discussions are focussed on problems concerning perennial fodder crops like grasses, clovers and lucerne and only casually problems with annual crops will be mentioned although they often may be as important as those with the perennials. There are two main reasons for this decision, one being that nearly all other lectures during the conference are devoted to problems with perennial fodder crops, the other being that the subject otherwise would so broad that it would be impossible to present a reasonably comprehensive treatment during the allocated period of time.

Yield and yield formation

It may be appropriate to start this review with a definition of the concept "yield" and also to mention in what respect yield of fodder crops deviates from yield of agricultural plants in general. According to the terminology used by crop physiologists yield can be represented by the formula:

$$Y_{econ} = C_{econ} \times Y_{biol}$$

where Y_{econ} means yield, Y_{biol} total biomass production and C_{econ} the economic coefficient which is an efficiency factor. In practice very often only the production above ground is considered (except, of course, for root and tuber crops) and the term "harvest index" is frequently used instead of the term "C_{econ}".

Perennial fodder crops, together with many of the annual fodder crops, are distinguished from most other agricultural crops by the fact that nearly all, or at least a very large fraction, of the totally produced above-ground biomass can be used for consumption and therefore represents yield. In other words, harvest index often reaches values close to 1 which means that yield and total above-ground biomass production tend to be more or less equivalent.

This distinction reflects a biological difference which is of considerable importance when discussing various aspects of yield formation in fodder crops. Thus the problem of assimilate partitioning is quite different in fodder crops from that in cereals, e.g. In the latter ones a large proportion of the assimilates is transferred from the production sites (leaves, stems and ears) to special storage organs (grains) and this redistribution of organic matter represents an essential part of the yield formation process. In the perennial fodder crops, however, most of the produced dry matter is incorporated into new leaves and shoots and is consequently used both for building up new production sites and for yield formation. This fact, of course, has to be considered especially when discussing physiological aspects of yield determination in these crops. Seed production in grasses and other fodder-crops is obviously similar in principle to grain production in cereals. This particular aspect of yield will not be treated in the present review, however.

Environmental yield determinants

Since photosynthesis constitutes the basis of plant dry matter production, and hence also of yield, light is the primary limiting factor for the performance of a crop. The light climate of a particular field is determined by its geographic location, by seasonal changes and by irregular variation between years. Average values for daily global radiation can be easily recorded and an example of such determination for a site just outside Copenhagen is given in Figure 1. The data are taken from an investigation by Jensen (1979). In the same figure average values of air temperature are also presented and it is evident that in this case global radiation reaches its maximum already in early June whereas maximum air temperature is not obtained until early August. These results appear to be characteristic for the situation in many parts of northwestern Europe.

The level and time-course of incoming radiation thus sets the upper limit for potential crop production at a particular location but the actual production may in fact be much lower due to the influence of other environmental factors

such as temperature or supply of water, CO_2 and minerals.

The efficiency (ε) of the conversion of global solar radiation into chemical energy stored in dry matter (= biomass) can be deduced from the relation:

$$\varepsilon = \varepsilon_c \times \varepsilon_i \times \varepsilon_b$$ (Varlet-Granches et al. 1982)

in which ε_c = climatological efficiency, ε_i = interception efficiency and ε_b = biological efficiency

The climatological efficiency ε_c represents the photosynthetically active radiation (PAR) as a fraction of global solar radiation and has been shown to be nearly constant ($\varepsilon_c \cong 0.48$) irrespective of geographic location, solar elevation, or season of the year (Varlet-Granches et al. 1982).

In contrast the light interception efficiency ε_i varies very much during the development of a crop and many reach all possible values between 0 and 1. Leaf area index (LAI) is the most important factor determining the magnitude of ε_i (Varlet-Granches et al. 1982) but the inclination of the leaves, i.e. their degree of erectness, is also of great importance in this context. In order to achieve maximum light interception a LAI value around or above 5 is necessary in most cases. The total amount of intercepted light during a growing season is thus the resultant of the accumulated daily values of incident light and corresponding daily interception efficiency (ε_i).

The biological efficiency of the intercepted light (ε_b), i.e. the efficiency of transformation of light energy into chemical energy recovered from the plant as dry matter, also varies considerably during the course of crop development and between species and even cultivars (Varlet-Granches et al. 1982). The main causes of this variation are differences in the photosynthetic and respiratory processes but the partitioning of photosynthetic products to various plant organs (= source/sink relationships) may also be of considerable importance.

Potential production

With the aid of recorded data on incident light it is possible to calculate the potential dry matter production at a given geographic location during a selected period of time. Such an exercise has been made, e.g. by Jensen (1979), for the location Tåstrup near Copenhagen in Denmark, covering the period May-October, i.e. the main period for growth of Lolium perenne. According to these calculations there is a potential for producing 40 metric tons dry matter per hectare during a season at this location whereas the actual production at the same time site and period and with ample supply of water and mineral fertilizers only reached a value of 22 tons per hectare (Jensen 1979). There is thus a substantial gap between theoretically possible yields and those achieved in practice and an obvious task is now to find out if and how this gap can be diminished.

From what has been said in the foregoing it is evident that there are two major areas which should be looked upon especially, namely 1) light interception and 2) the efficiency of transformation of light energy into chemical energy.

Light interception

Ideally an area of land should be covered by a crop with 100 per cent light interception throughout the entire growing season. This is impossible for various reasons, one being that complete cover cannot be achieved during the initial stages of crop establishment and another relating to the fact that plants lose their light interception ability (and their potential to use the decreased amounts of intercepted light) during the phase of senescence at the end of the growing season. In the case of many fodder crops there is still another feature that has to be considered. In these crops the growing canopy is reduced more or less frequently either by cutting or by grazing which of course affects light interception quite considerably. The pattern of light interception in frequently cut crops of grass (Figure 2) and lucerne (Figure 3) has been investigated by Spiertz (1982) and it is evident from these measurements that it is very important that regrowth of the canopy after

cutting proceeds as fast as possible. The positive effect of an ample supply of nitrogen on the rate of regrowth is also clearly demonstrated in these experiments (Figure 2). An abundant supply of water is another factor which of course exerts a profound positive influence on the rate of regrowth.

In grazed swards, particularly in those which are grazed continuously, the situation may be rather complex. Regrowth of hard-grazed swards produces leaves which are photosynthetically very active (they are exposed to full sunlight to a large degree) but the production of such a sward is nevertheless substantially less than in a sward which is grazed more leniently (Parsons et al. 1983a). The reason is obvious, in the hard-grazed sward LAI is not more than around 1 whereas in the leniently grazed sward it is about 3, thereby guaranteeing a nearly complete light interception throughout the season in the latter. As a matter of fact such leniently grazed swards exhibit a gross photosynthetic production which is nearly as high as that of an infrequently cut crop, (Figure 4, Parsons et al. 1983a).

With respect to dry matter production in these two different types of swards the leniently grazed swards were also clearly superior to the hard-grazed ones since the proportions of respiration losses and transport of photosynthates to non-harvestable parts were similar in both types. However, when considering harvest figures, i.e. the amount of grass taken in by the grazing animals, the situation was reversed. Now maximum intake and hence maximum yield was achieved in swards which were characterized by a relatively low LAI and incomplete light interception (Figure 5, Parsons et al. 1983b).

The complexity of optimum canopy architecture is also discussed by Wilson (1984). He rightly points out that the most suitable model may be quite different for a sheep grazing system and a system where the harvested crop is used for conservation, and he also draws attention to the fact that e.g. an erect grass type, which as a monoculture may not be the most efficient one, tends to be highly successful when grown together with clover (see also Evans

et al. 1983). It is thus quite obvious that it is impossible to specify a plant ideotype or an ideal crop architecture which represents an optimum solution for all situations and purposes. Instead every case has to be treated individually and the best plant and canopy type has to be found for precisely that situation. A consequence of such considerations is the necessity, for example, to breed grass and clover types, which are especially disignated to grow together, instead of making chance mixtures of types originally bred for use in monoculture (Wilson et al. 1980, Rhodes 1981).

Transformation of light energy into chemical energy

Only a limited fraction of the intercepted light can actually be transferred into chemical energy and recovered as plant dry matter. The magnitude of this fraction (ε_b) varies considerably between species, cultivars and cultivation systems, thus Varlet-Granches et al. (1982) reported values between 0.057 and 0.037 for different crops of lucern and maize. There are several factors which contribute to this variation, the most important ones being the efficiency of the photosynthetic process and the different kinds of respiratory processes which consume part of the photosynthetically produced dry matter.

Photosynthetic production

The maximum rate of photosynthesis (P_{max}) has been shown to differ considerably both between and within crop species and the heritability of this trait appears to be high (Wilson and Cooper 1969, Wallace et al. 1972). Yet there is little evidence that selection for high values of P_{max} results in consistent increases in dry matter production (Wilson and Cooper 1970, Hart et al. 1978) and the prospects of improving the light harvesting efficiency via an increase of P_{max} seem to be rather limited.

There has been much speculation about the possibility to transform the C_3-pathway of photosynthesis, which is the common one in nearly all crop plants of the temperate climate, into the more efficient C_4-pathway of maize, sugarcane and other tropical or subtropical crop plants. This possibility will not be discussed further here because a) such a transformation is a

formidable task which presumably will take a very long time if it ever will succeed (or at least result in plants which can be used for practical purpose) and b) it is by no means certain that such a change actually will increase the production level of these plants when grown in the humid and cool climate of north western Europe.

A more promising attempt to increase total photosynthetic production is to select for a better adaptation of the plant material to high light intensity. It is a widespread experience that net photosynthetic production of a sward is lower during the end of the summer than during its beginning despite the fact that the amount of incident light energy is approximately the same and light interception is complete in both cases. One reason for this discrepancy is the fact that air and soil temperature have their seasonal maximum about two months later than incident light and that consequently the respiratory losses are larger in late than in early summer(Jensen 1979). Another important cause is the tendency of many grass species to adapt their later emerging leaves to the low-light conditions at the bottom of the canopy which makes them incapable to respond fully to the higher light intensities they meet when they expand into the upper layers of the canopy (Figure 6) (Robson 1973, Woledge 1973). This type of shade-adaptation is not general, however, thus it does apparently not exist in white clover for instance (Dennis and Woledge 1983).

Investigations carried out at the Welsh Plant Breeding Station have indicated shown that there exists considerable genetic variation with respect to the degree of low-light adaptation in grasses like perennial ryegrass (Figure 7) and that it is possible to use this character in a plant breeding program (Wilson 1981, Robson 1982).

Respiratory losses

Part of the intercepted light energy may be lost immediately via the process of photorespiration (which occurs simultaneously and competitively with photosynthesis) or at some later stage of plant development through so-called dark-respiration.

The possibility to achieve large gains in dry matter production (30-50%) by eliminating the process of photosrespiration has stimulated the phantasy of biochemists, crop physiologists and agronomists ever since this phenomenon was discovered and described by Decker (1955). Although the biochemistry (Tolbert 1979, Beck 1979) and physiology (Zelitsch 1979, Canvin 1979) of this process have been worked out in considerable detail there is still great uncertainty about the ultimate biological function of photo-respiration and whether or not it is possible to obtain the expected increases in dry matter production by reducing photorespiration drastically, for instance be altering the characteristics of the key-enzyme rubisco. It is therefore at the moment impossible to decide if attempts to select for decreased photo-respiration will be worthwhile or not.

With respect to dark-respiration the situation is obviously different. Dark respiration can be devided at least into two components, "growth respiration" and "maintenance respiration" (McCree 1974) and frequently, even a third component, "cyanide-resistant respiration" (Lambers 1982) is present. The exact physiological function of these various components of respiration, and particularly their relation to yield in crop plants, is far from clear (Lambers 1985). However, a number of investigations have been carried out with grasses which strongly indicate that selection for low dark respiration may be a valuable tool in the search for highly productive genotypes in these species.

Wilson (1975, 1982) used dark respiration in fully-grown leaves and roots as an approximate estimation of maintenance respiration in ryegrass and was able to select "slow" and "fast" respiration lines in F_1-populations originating from the variety S.23. The "slow" lines exhibited a respiration rate that was about 20% lower than that of the original S.23 and these lines have consistently outyielded the mother variety by 10-30% in the field (Wilson and Jones 1982).

The means by which the greater yield is achieved have been investigated at the Grassland Research Institute at Hurley by Robson and his collaborators (Robson 1983). Their research has shown that the "slow" lines use their excess carbon

to produce more tillers per unit ground area (Figure 8b) which in turn produce more leaves after a cut and hence exhibit a faster recovery of light interception and net photosynthesis per ground area (Figure 8a) (Robson 1982). When the "slow" and "fast" lines both have reached full light interception the rate of net photosynthesis per ground area is identical. The advantage of the "slow" respiration lines builds up gradually over successive regrowth periods and both shoots, stubble and roots show increased dry weights per unit area (Figure 9) (Robson 1982).

These results look, indeed, very promising and there appears to be scope for substantial yield increases by a systematic selection and plant breeding work. Yet there is a slight feeling among some workers in the field that things may look too good and that some hitherto not observed or recognized penalties may exist (Robson 1983, Lambers 1985). Such penalties could for instance be a reduced plasticity and adaptation ability of the plants (Lambers 1985) but other negative effects are also possible. It is therefore an urgent task for crop physiologists as well as plant breeders to investigate these problems in more detail in order to avoid negative surprises later on.

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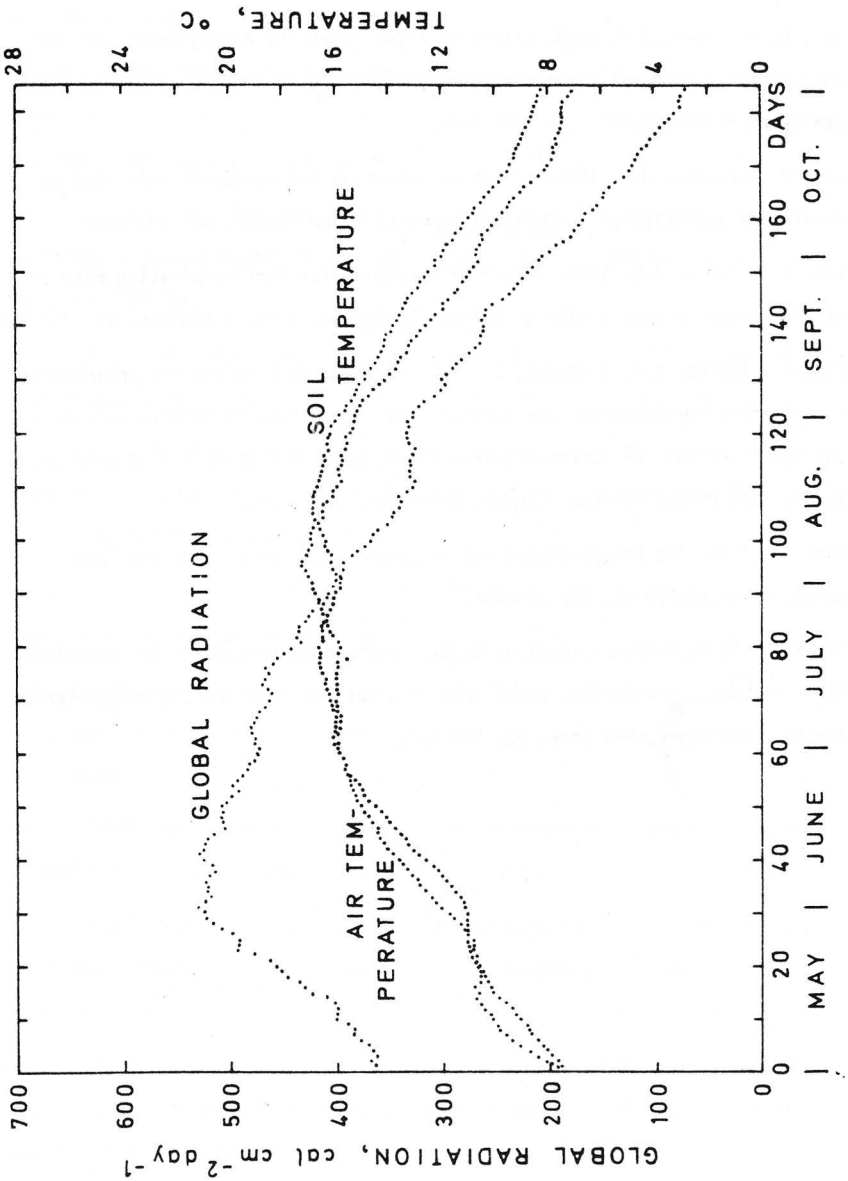
GLOBAL RADIATION, cal cm⁻² day⁻¹
TEMPERATURE, °C
GLOBAL RADIATION
SOIL TEMPERATURE
AIR TEMPERATURE
MAY | JUNE | JULY | AUG. | SEPT. | OCT.
DAYS

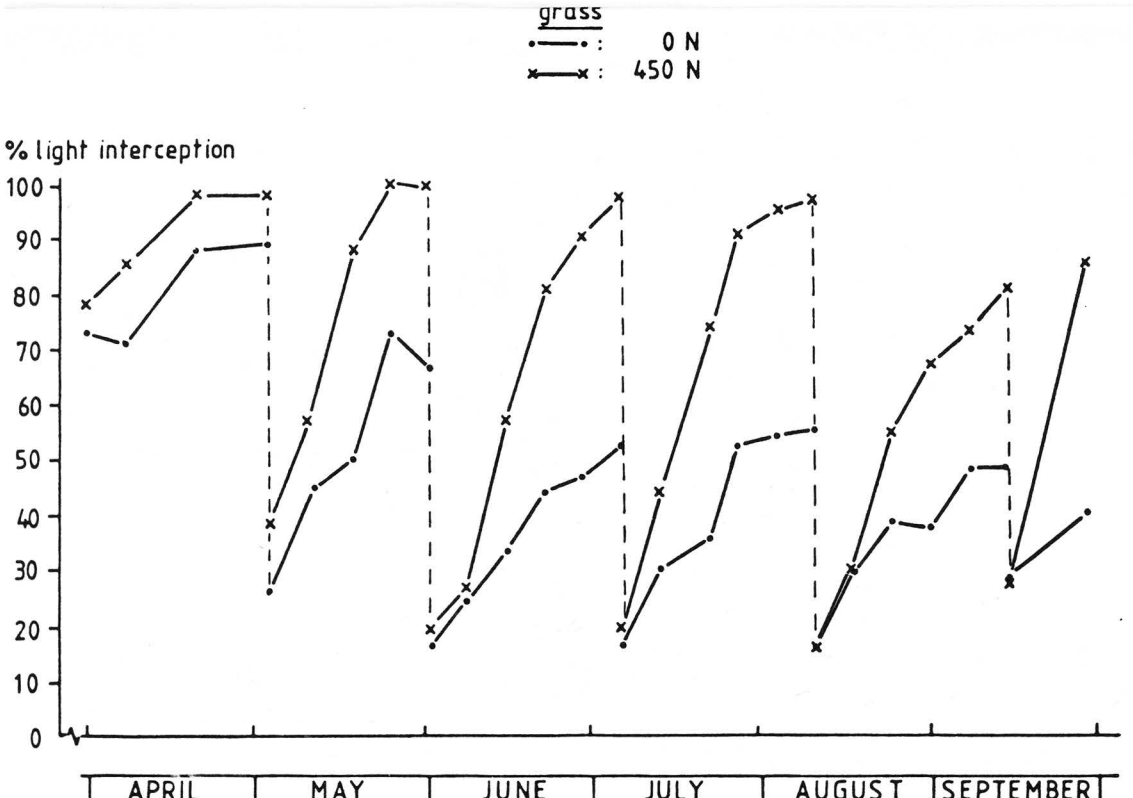

Fig. 2. Pattern of light interception throughout the growing season (1980) for grass. Location: Flevopolder, The Netherlands. (From Spiertz 1982, p. 31)

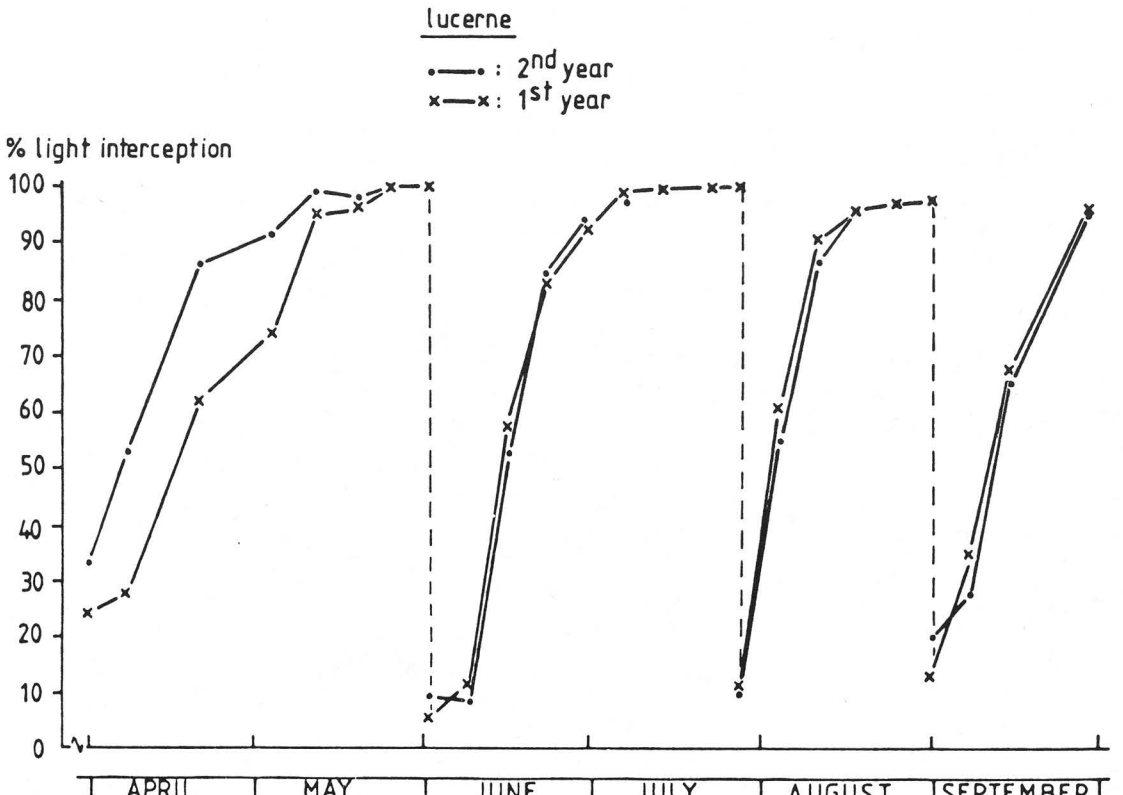

Fig. 3. Pattern of light interception throughout two consecutive growing seasons (1980 and 1981) for lucerne. Location: Flevopolder, The Netherlands. (From Spiertz 1982, p.31)

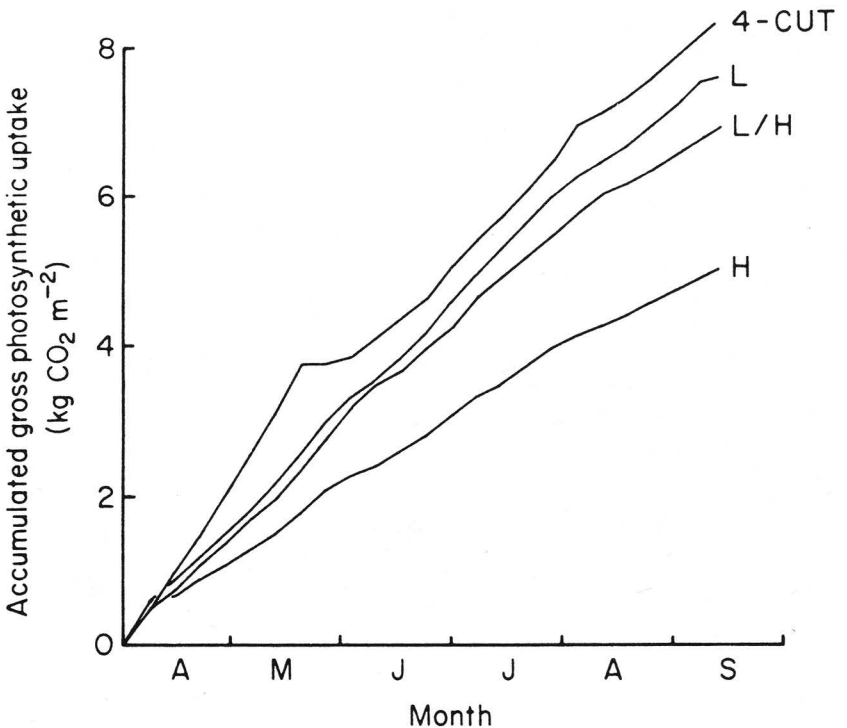

Fig. 4. The effect of management on total gross photosynthetic uptake over the grazing season, April - September. Accumulated gross photosynthetic uptake was calculated for hard (H) and lenient (L) continously-grazed swards; also a sward cut four times (4-CUT) during the grazing season; and a continously-grazed sward in wich LAI fluctuated between 1.0 and 3.0 (L/H). (From Parsons et al. 1983a, p. 124)

C balance of continuously-grazed swards

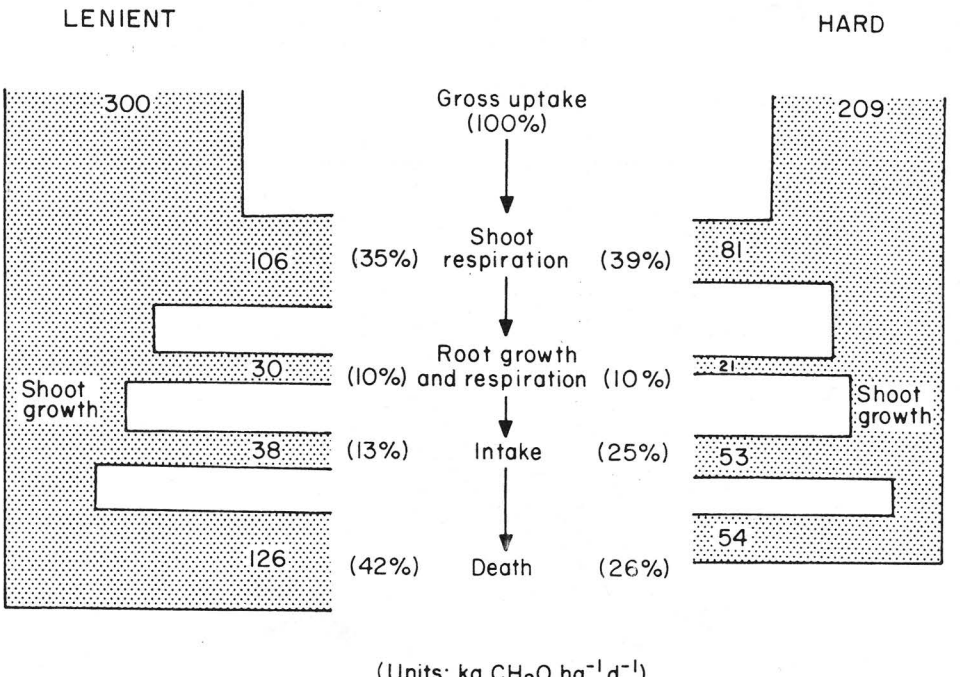

Fig. 5. The flow of matter in continously-grazed swards. The diagram summarizes the balance between gross photosynthetic uptake, animal intake, and the loss of matter in shoot respiration, the growth and respiration of root, and the death of tissues, under hard and lenient continous grazing. (From Parsons et al. 1983b, p. 134)

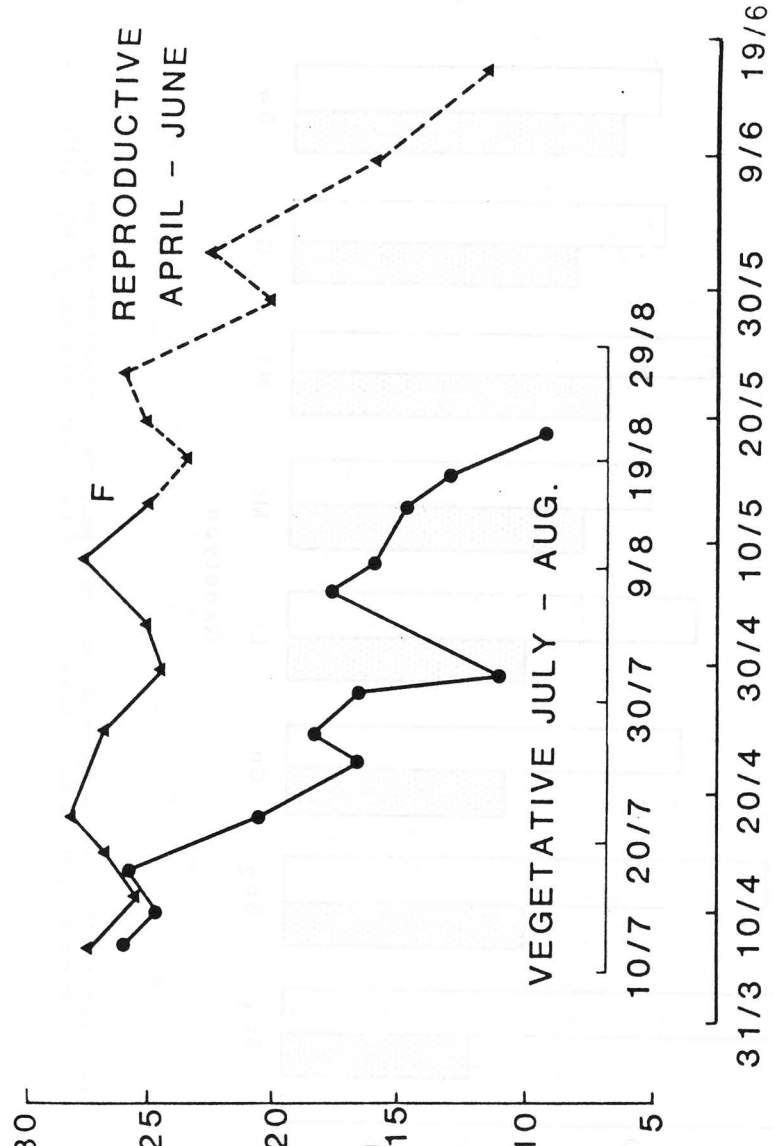

Fig. 6. Net photosynthetic at saturating light intensities of successive youngest fully expanded leaves taken from an increasingly dense (LAI rising from 0.5 to 4.5) vegetative sward of S24 perennial ryegrass during July - August (•) and from flowering tillers in a reproductive crop during April - June (▲) up to and including the flag-leaf (F) and as the matter aged (----). (From Roson 1983, p. 136)

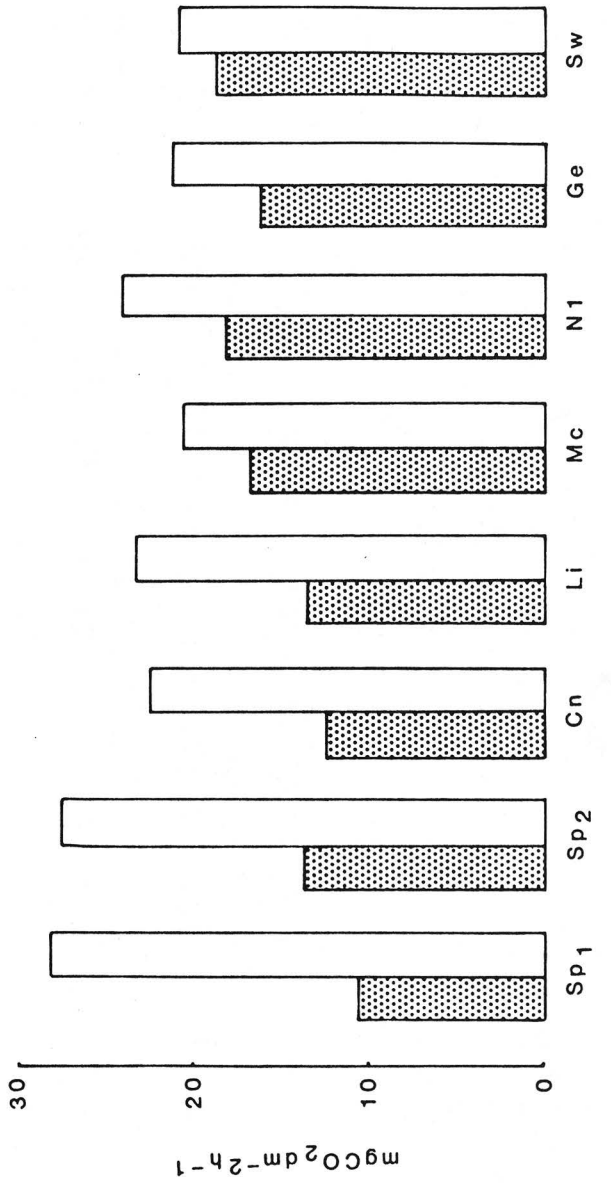

Fig. 7. Variation in light adaptation of leaves of 8 genotypes of perennial ryegrass: net photosynthesis at 104 Wm^{-2} (shaded) or 50 Wm^{-2} (unshaded). (From Robson 1983, p.137)

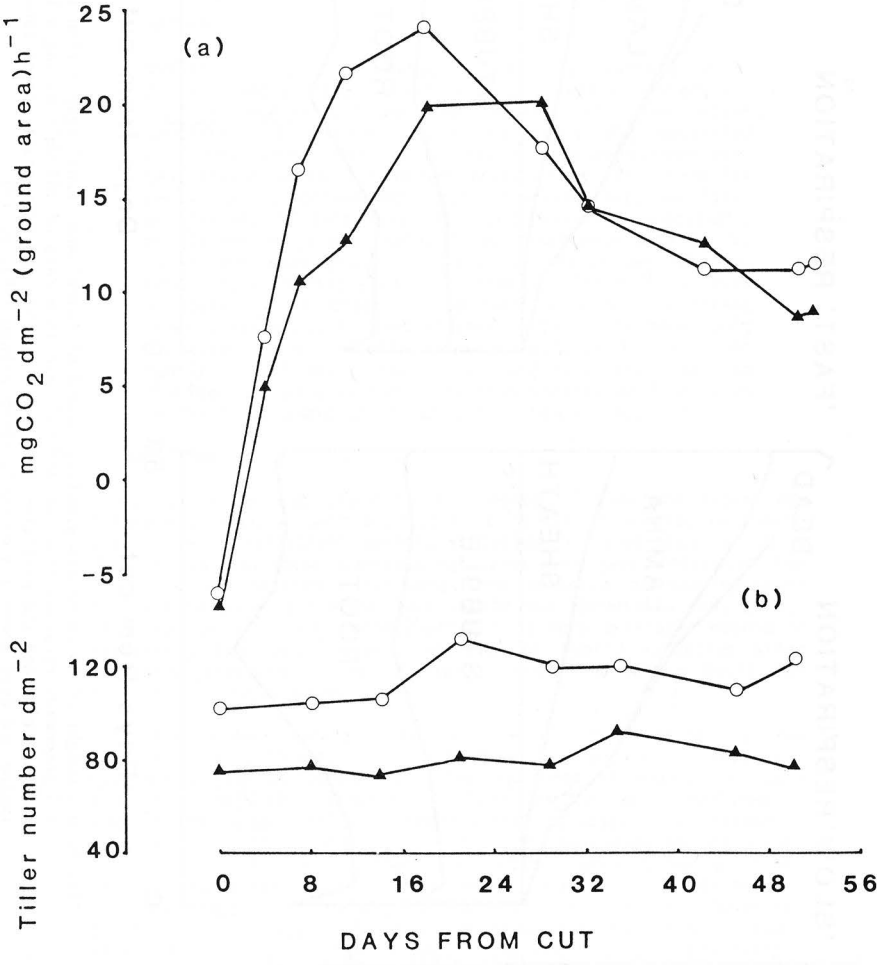

Fig. 8 (a) Canopy net photosynthesis at 20°C and 107 Wm^{-2} and (b) tiller number of simulated swards of 'slow' (○) and 'fast' (▲) respiration lines of S23 perennial ryegrass during a late regrowth period; by day 24 both swards were fully light intercepting. (From Robson 1983, p.141)

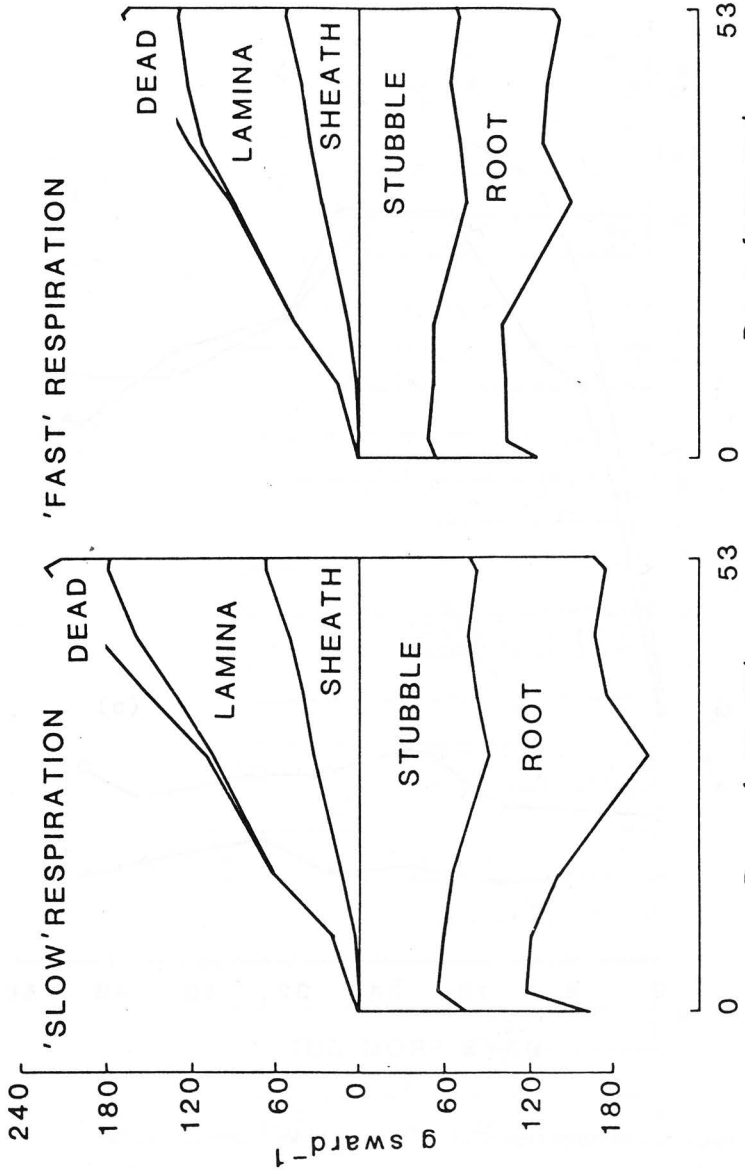

Fig. 9. Dry weight of 50 cm square simulated sward of 'slow' and 'fast' respiration lines of S23 perennial ryegrass grown at $20^{o}/15^{o}C$ and 16 h days of 107 Wm^{-2} during a late regrowth period and its partition into root, stubble and harvestable shoot (sheath, lamina and dead above a 5 cm cut). (From Robson 1983, p. 140

BREEDING FOR DRY MATTER YIELD IN PERENNIAL RYEGRASS BY WIDE HYBRIDISATION AND RECURRENT SELECTION

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ABSTRACT

Diploid F_1 hybrids between extremely early-growing, early-flowering and late-flowering perennial ryegrasses (Lolium perenne L.) had 24% higher yields and better persistency than three control cultivars when subjected to a mixed conservation/simulated grazing management over two harvest years. Recurrent spaced plant selection for early spring growth and late flowering date in later generations of this type of hybrid material produced a population which was simiar in ear emergence date to cv. Talbot but when grown as plots had better persistency, better early spring yield and slightly higher yields over all cuts. A polycross progeny test revealed significant genetic variation in overall yield within this gene pool; variation which currently is being exploited by recurrent family selection. Regression analysis was used to distinguish conservation types from general purpose types in the first yield trial but not in the second.

INTRODUCTION

Breeding for herbage yield by any method is slow and expensive. Before embarking on such a project it is therefore wise to ensure that there is sufficient genetic variation. A good means of doing this is to hybridise contrasting types and seek heterosis for yield. Such heterosis indicates the presence of complementary dominant genes at different loci in the two parents (Jinks, 1983). If it can be achieved, recombination is a more desirable method of harnessing such genes than production of hybrid varieties since varieties produced by recombination provide a better basis for further crop improvement.

This paper reports the performance of hybrids between extremely early-flowering and late-flowering persistent perennial ryegrasses (Lolium perenne L.) and progress in exploiting such hybrids by recurrent selection. There are two major problems in identifying high yielding recombinant genotypes of perennial ryegrass. Firstly, total annual yield of individual spaced plants is a poor indicator of their performance when grown as plots (Hayward and Vivero, 1984). This necessitates selection either for possible components of yield, or for plot yield itself. Secondly, genotypes which excel under frequent close cutting and high levels of fertility tend to give disappointing yields under conservation managements and vice versa. Although undoubtedly there are circumstances in which specialist cultivars are useful, British farmers generally prefer cultivars which can be used either for conservation or grazing depending on the needs of their animals in any particular year.

MATERIALS AND METHODS

Plant material. From 75 survivor plants selected from four-year-old plots of extremely early-flowering ecotypes collected from the uplands of Northern Italy, three were selected which were winter hardy and highly persistent when grown as clonal plots. There was one from each of three populations (Welsh Plant Breeding Station accession numbers, Ba 8596, Ba 8622 and Ba 8590). These were hybridized with late-flowering plants from the cultivars, Perma, Vigor and S.23. Flowering was co-ordinated by manipulating daylength and temperature. Hybridization was by the technique of automatic cross-pollination without emasculation. Those plants arising from self-pollination were recognised easily by their early or late-flowering date. The proportion of such plants in the three F_1 hybrids evaluated in experiment 1 was below 1%. F_1 hybrids involving all three early-flowering clones and five late-flowering clones (two from cv. Vigor and three from cv. S.23) were polycrossed in a pollen-proof glasshouse compartment. The later flowering F_2 plants (about half the population) were polycrossed again. Approximately 3,500 F_3 were grown as spaced plants, and 30 or more plants which combined good growth in March (assessed visually) with late flowering date were polycrossed. This selection was repeated for two further generations. The 36 F_5 parents at the final polycross were each split into four clonal replicates and plants fully randomised within each of four islands. Seed was collected from the individual mother clones and used for experiment 2.

Experiment 1. In August 1981, 240 plants of each of the three F_1 hybrids and three control cultivars (S.24, Lidura and Frances) were grown from seed in a glasshouse in shallow boxes (50 x 36 x 8 cm) filled with John Innes No. 3 compost, 60 per box. Boxes were fully randomised in each of four replicate blocks. They were transferred to the field in October. In February 1982, when roots had bound the compost thoroughly, turfs were removed from the boxes and placed in two rows back-to-back in a shallow trench. Guard plots were placed at the end of the rows. The surrounding gaps were filled with soil, the plots were rolled thoroughly and labels placed at the outside corner of each plot to mark the borders. In 1982, plots were cut seven times (in March, May, June, July, August, October and November) and in 1983 five times (in March, May, June, July and October). There was very little growth during late summer 1983 due to lack of water. Except for cut 1 of each year when plots were mowed, the plots were cut individually with sheep shears and the herbage dried and weighed. Plots were cut at a height of 5 cm in May and at 3 cm on other occasions. Compound fertilizer in the form of 20:10:10 N:P:K was applied in February and immediately after each cut. The amounts of N applied at each cut in kg/ha were as follows: cut 0 = 75, cut 1 = 100, cuts 2-6 = 50, cut 7 = 25.

Experiment 2. In August 1983, seed of the 36 half-sib families, together with the control cultivars Talbot and RvP (*Lolium multiflorum* Lam.) were sown by hand as 1 x 1 m plots, 3 g per plot. Plots of each genotype were fully randomized in each of four replicate blocks. To minimize border effects, plots were arranged in rows, back-to-back, and guard plots sown on the end of each

row. Plot borders were marked with creosote. In 1984, plots were cut at a height of 3 cm in February and April, 5 cm in June and July and 3 cm in August, September and October. In 1985 plots were cut in February, April, June and July. Dry matter yields were determined at the last five harvests in 1984 and the last three in 1985. Ear emergence dates were recorded in June 1984, and visual estimates of ground cover and damage by Rhynchosporium made in October 1984 and May 1985 respectively.

RESULTS

Experiment 1, performance of F_1 hybrids compared with control cultivars. All the cultivars and hybrids had similar dates of ear emergence (Table 1). Over all cuts, there were highly significant differences in dry matter yield. When analysed separately, there were no significant differences in yield among the hybrids nor among the control cultivars. The mean yield of the hybrids was 124% of that of the control cultivars.

TABLE 1. Performance of three F_1 hybrids and three control cultivars when grown as mini-plots over two harvest years

Genotype	Dry matter yield per cut (g)	Mean emergence date	Mean ground cover (1-10)
S.24	53.3	44	4.5
Lidura	53.4	46	6.0
Frances	54.3	45	6.3
Ba 8596.1 x Perma.1	70.2	43	8.3
Ba 8596.1 x Perma.2	66.8	45	7.0
Ba 8596.1 x Perma.3	63.1	42	8.3
LSD (5%)	3.9	3	1.4
P	0.001	0.01	0.001

Over all hybrids and cultivars mean dry matter yield varied significantly (P = 0.001) between cuts; from 145 g at cut 2 in the first year to 11 g at the final cut of the second year. There was a highly significant (P = 0.001) genotype x cuts interaction, which was also significant when the hybrids and control cultivars were analysed separately (P = 0.05 and 0.03 respectively). These genotype/cut interactions were examined further by means of the regression technique proposed by Finlay and Wilkinson (1963) for the analysis of genotype/environment interactions. When the mean yield of each genotype was regressed on the mean yield of all genotypes at each of the ten cuts, there was significant (P = 0.001) heterogeneity among the individual genotype regressions. When analysed seprately, there was significant heterogeneity among the regressions of both the control cultivars and the hybrids (P = 0.05 in both cases). Ba 8596.1 x Perma.1 (b = 1.26 ± 0.08) was superior to the control cultivars only at the heavier cuts while Ba 8596.1 x Perma.2 (b = 1.05 ± 0.02) performed well at all cuts and thus appeared likely to be the best general purpose genotype. The hybrids as a group differed significantly (P = 0.01) from the control cultivars as a group (b = 1.13 ± 0.03 and 0.87 ± 0.03 respectively). Among the control cultivars, cvs S.24 and Lidura were best at the larger cuts (b = 0.90 ± 0.03 and

0.94 ± 0.07 respectively) and cv. Frances was best at the smaller cuts (b = 0.76 ± 0.03).

Experiment 2, polycross progeny test on F_5 plants. Families varied significanty in mean yield over the eight cuts (P = 0.05 when tested against families x cuts). The mean yield of the families was slightly, but not significantly higher than the perennial ryegrass control cv. Talbot (Table 2). Seven families

TABLE 2. Performance of 36 half-sib (polycross) families and two control cultivars as sown plots

	Mean yield per cut (t/ha)	Mean yield in April 1985 (t/ha)	Mean ground cover (1-10)	Mean ear emergence date	Rhynchosporium susceptibility (1-5)
Mean yield of 36 families	2.88	1.09	7.8	51	2.9
Lowest family	2.73	0.90	7.0	46	1.5
Highest family	3.11	1.33	8.5	54	4.0
Talbot	2.75	0.77	6.8	54	1.0
RvP Italian	3.41	0.73	3.5	45	2.3
LSD (5%)	0.20	0.15	0.7	2	0.8
P	0.01	0.001	0.001	0.001	0.001

significantly outyielded cv. Talbot but none yielded as well as the Italian ryegrass control cv. RvP. Cuts varied significantly (P = 0.001) from 5.99 to 1.09 kg/ha and there was a significant (P = 0.001) family x cuts interaction. But on this occasion when family yield was regressed on the mean yield at each cut, there was no significant heterogeneity among the family regressions. in April of the second harvest year, all but two families significantly outyielded both control cultivars. Cv. RvP performed worse than usual at this time of year as it suffered considerable damage during the preceeding winter.

The families also varied significantly in ground cover in autumn, mean ear emergence date, and susceptibility to Rhychosporium spp. This latter trait was derived from the early-flowering parents. Mean family ground cover was better than that of both control cultivars, but mean family susceptibility to Rhynchosporium was significantly worse than cv. Talbot. Simple correlations between family means (Table 3) suggest that selection for yield alone would result in no change in ground cover but may cause an improvement in resistance to Rhynchosporium and a shift towards early flowering.

TABLE 3. Simple correlations of family means for four traits

	Yield	Ground cover	Emergence date
Yield	-		
Ground cover	0.001	-	
Emergence date	-0.433**	0.061	-
Rhynchosporium	-0.341*	-0.300	0.048

**, P = 0.01; *P = 0.05

DISCUSSION

Humphreys (1985) reported heterosis for yield in hybrids between an early-flowering ecotype from the Swiss uplands (Ba 9436) and the late-flowering cv. Vigor (Melle Pasture), but the degree of the heterosis was considerably smaller than occurred in the present experiment 1. Although the two results are not strictly comparable since Humphreys used a simulated grazing management throughout whereas two conservation cuts were included here, these results do suggest that this Italian material is a potentially more useful parent in breeding for yield than Ba 9436. The Italian material is even earlier flowering than Ba 9436 and has better spring growth at Aberystwyth. It is however, less winter hardy. The Italian material is very susceptible to Rhynchosporium but the Swiss material is very susceptible to crown rust (Puccinia coronata), so in using either ecotype attention has to be given to improving resistance to one or more pathogens.

In the first experiment, regression analysis revealed differences between genotypes in the way in which yield was distributed over cuts. One hybrid in particular showed a marked tendency to concentrate yield at the heaviest cuts, even though its mean yield per cut was not significantly higher than the other two hybrids. Among the control cultivars S.24 and Lidura showed the greater tendency to concentrate yield at the heavier cuts. Both management (cutting height and interval, N level) and seasonal growth patterns combined to produce the large differences between cuts. Thus it would not be possible to use such regression analyses to predict genotyope behaviour under different managements. For this, a better understanding of the physiological or morphological origins of genotype/management interactions would be required.

The extreme earliness of flowering of the Italian parent posed a major problem in using it to improve yield. Even the F_1 hybrids were too early flowering for a general purpose ryegrass and there was marked segregation for inflorescence emergence date in succeeding generations. Multi-trait selection from the F_2 onwards would have involved discarding most high yielding genotypes because of inappropriate flowering date. Since determining productivity under plot conditions is much more difficult than recording emergence date, intensive recurrent spaced plant selection was carried out for two easily measured traits, late emergence date and early spring growth. Because each was a polygenic character possessed by one or other set of parents, it was hoped that most of

the genes conditioning yield would be retained in the gene pool and that further breeding could concentrate primarily on yield. The results from experiment 2 indicate that this approach has been at least partly successful. Early spring growth of the F_6 as a whole was significantly better than that of cv. Talbot and emergence dates were only slightly earlier. Furthermore, polycross families varied significantly in yield over all cuts. But several further generations of selection for yield, late emergence date, persistency and resistance to Rhynchosporium will be necessary to exploit the genetic variation within this gene pool to the full.

ACKNOWLEDGEMENTS

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Yield and persistency of Lolium perenne under different levels of competition - Preliminary results.

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1. Introduction

The growth and production of grasses takes place under high levels of competition whereas the selection of individual plants in breeding programmes is often carried out among plants subjected to little or no competition. Information on the effect of competition on yield and persistency is consequently of importance in determining selection strategies. The preliminary results presented here describe the behaviour of Lolium perenne genotypes under different levels of competition.

2. Materials and methods

48 plants of each of four clones A, B, C and D were planted in various mixture at *a high level of competition* with a distance of 12 cm between the rows and 2 cm between the plants. In order to be able to separate plants within the rows, thin plastic markers (14 mm x 80 mm) were placed between plants. As the markers were only approx. 1 cm below and a few mm above the soil surface, normal competition among adjacent plants occurred both at the top zone and at the root zone. If the neighbouring plant had died or was weak, vigorous plants could obtain more space by displacing the plastic markers. Where full plant number was maintained some of the most vigorous plants were forced to spread out into the rows. 24 plants of each of the above clones were also planted at *a low level of competition* with a plant spacing of 12 cm x 12 cm. In some treatments the clones were *mixed* in such a way that each plant had different neighbours in each replication. Other treatments comprised only single genotypes. Irrigation was used when required, and optimal amounts of K and P were supplied once each year. Nitrogen was supplied at a rate of 93 kg N in spring and a further 47 kg after each cut. Dry matter yields per plant were determined in 5 cuts each year over 4 years. The first cut was taken at early ear emergence, and the following cuts at intervals of 5 weeks. In all cases a cutting height of 3-4 cm was employed.

3. Persistency

3.1 Low level of competition (12 cm x 12 cm) - Figure 1

At the low level of competition the total number of plants in both single genotype plots and in genotype mixtures was reduced only by about 14% during the 4 years. *Subjected to no or only to a low level of competition, these genotypes were thus able to survive for at least 4 years even when 5 cuts were harvested each year.*

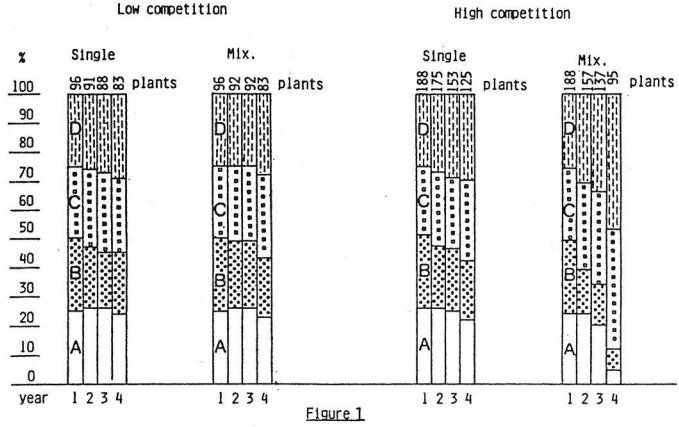

Figure 1

Persistency of 4 clones of Lolium perenne
Total numbers and percentage of living plants by end of each year

Each clone maintained between 20% - 30% of the total numbers after four years both as individual genotypes and mixtures. Clone B was the weakest with only 20% survival in the fourth year.

3.2 High level of competiton (2 cm x 12 cm) - Figure 1

At the end of the first harvest year the number of plants was only slightly reduced, and was the same for single genotype plots and for mixtures. In single genotype plots the high level of competition had some effect in the third harvest year and a pronounced effect in the fourth year where the accumulated loss was 24% compared to 14% at the low level of competition. Under these conditions of intragenotypic competition clones A and B each accounted for 20% of the total number of plants surviving after the fourth harvest year. In genotype mixtures, however, the pattern of survival was completely different. In the first year each clone accounted for 25% of the mixture. In the fourth year about 50% of the total number had died due to high mortality rates in clone A and B. Nearly all plants of

the strong competitors C and D survived even after 4 years. These results indicate that *some genotypes may have a low persistency at high levels of competition even if they have a fairly high persistency at low levels of competition or under intragenotypic competition. Other genotypes are able to persist both at low and high levels of competition.*

4. Total yield per unit area - Figure 2

Dry matter yield in figure 2 applies to unit area irrespective of number of dead plants. At high competition (2 cm x 12 cm) the total plot size was 0.46 m^2 with initially 192 plants. At low competition (12 cm x 12 cm) the plot area was 1.38 m^2 with initially 96 plants.

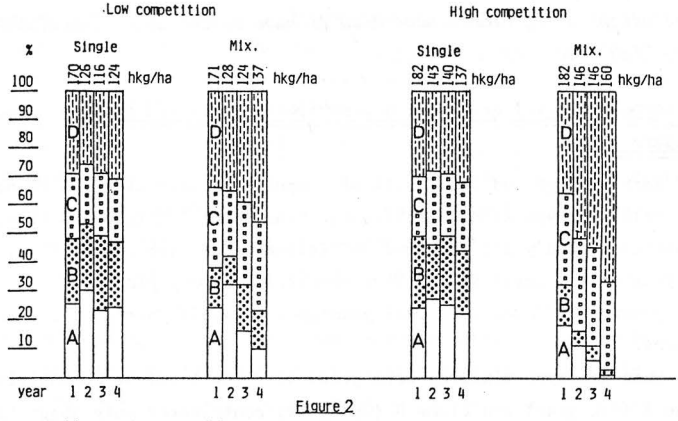

Figure 2

Yield per unit area and distribution of yield between 4 clones of Lolium perenne

The total yield in hkg dry matter per ha was high both under low competition (170 hkg/ha in the 1st year, 127 hkg/ha in the 2nd year) and at high level of competition (182 hkg/ha and 145 hkg/ha, respectively). In the Danish official variety tests the yield levels for Lolium perenne are about 130 hkg/ha in the 1st harvest year and 100 hkg/ha in the 2nd year.

The high yields obtained in these experiments are probably due partly to the fact that clones C and D were selected for persistency and high yielding capacity, and to the fact that uniform plant spacings were used. Thus each plant was given an opportunity to yield more than the average plant in a normal drilled sward where distances between plants are often very irregular, most plants having only

a few mm to competitors on both sides while other plants are neighbours to fairly large gaps in the rows.

The total yields were high at the 12 x 12 cm spacing both for single genotypes and in mixtures. The genotypes were thus able to utilize the fairly large space available even in the first harvest year.

This observation is supported by relating fig. 1 to fig. 2. At the high level of competition the numbers of plants in genotype mixtures are reduced during 2nd, 3rd and 4th year (from 157 to 95, i.e. 40% lost, fig. 1). At the same time the yields per unit area are rather constant or even slightly increased in the 4th year (from 146 to 160 hkg/ha, fig. 2). Furthermore the yield of genotype mixtures was higher than that of single genotype stands except in one case where the yield was the same. *These observations show that certain genotypes are able to utilize the extra space available where neighbouring plants have died or have become weak, especially under high levels of competition.*

5. Competition influencing the distribution of yield between clones. - Figure 2

Both at high and low levels of competition each of the 4 clones in single genotype stands contributed from about 20% (clone B at high competition, 2nd year) to about 30% (clone D, at high competition, 4th year) of the total yield. *Thus competition among plants of the same genotype allowed even weak genotypes to yield fairly well over 4 years.*

When genotype mixtures were grown at a low level of competition, clone A (4th year) and clone B (2nd year) contributed only about 10% to the total yield even though these two genotypes accounted for 20% of the total number of plants, (fig. 1). *This demonstrates that competition between different genotypes even at low levels can have an effect on the yield of each plant.*

At the high level of competition clone A and B were almost eliminated and clone C maintained a fairly constant contribution of about 30%. Clone D increased its contribution to total yield from 36% in the first year to 67% in the fourth. *Thus high levels of competition can have a drastic effect on the dry matter productivity of individual clones, some genotypes are almost eliminated and give very low yields, whilst others persist and show increased productivity.*

I thank Miss Else Granberg for handling thousands of single plants from planting through harvesting and dry matter analyses to final calculations. I also thank Dr. Brian Dennis for revising the English text.

Shortening the breeding cycle of perennial grasses under artificial conditions.

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Abstract

Experiments were conducted in growth chambers and in the field to determine the flowering requirements of cultivars and local ecotypes of Dactylis glomerata, Festuca pratensis and F. arundinacea. All three species have to pass through the juvenile, inductive and realization stages. There is a considerable variation between and inside populations in respect to their inductive conditions. F. pratensis can be induced at an earlier stage then F. arundinacea, and D. glomerata, but all three species have very high vernalization requirements, so that it is difficult to get several generations within a year. As seed production in growth chambers is expensive, it is proposed to combine natural and artificial conditions to shorten the breeding cycle.

Under field conditions, most temperate perennial grasses require two years to complete a multiplication cycle. Species such as Dactylis glomerata, Festuca pratensis and F. arundinacea are biennal tillers (BOMMER,1961; COOPER and CALDER, 1964). Before flowering, the plants have to pass through three successive developmental stages: the juvenile, inductive and postinductive or realization stages (CALDER, 1963). Only on completion of the juvenile stage can they be induced to flower by exposure to low temperature and short days. This inductive or vernalization stage may last from a few weeks to several months. However as perennial grasses behave as long-day plants, a postinductive or realization period has to take place before heading, flowering and seed setting.

Forage breeders may be interested in the accelerated production of successive generations for several reasons: genetic studies, regeneration of seed collections, synchronization of flowering in relation with crossing or production of advanced generations. In all cases, it is

important that the plants due to be cross-fertilized will flower abundantly and at the same time.

Numerous and extensive investigations have been conducted to determine when "the ripe to flower stage" is attained: on D. glomerata (CALDER, 1963; BLONDON and CHOUARD, 1965; WILSON and THOMAS, 1971; KOZUMPLIK and CHRISTIE, 1972), on F. pratensis (BEAN, 1970; KLOSS, 1973; GILLET, 1976) and F. arundinacea (TEMPLETON and al., 1961; BEAN, 1970).

Unfortunately, due to differences in genetic composition and environmental conditions, the results do not always concur. The purpose of this study is to collect further information about the induction requirements of our breeding material and to compare results obtained in growth chambers, greenhouses and field conditions.

Material and methods

Due to the limited capacity of growth chambers, nine different experiments were successively conducted from 1979-1985. Young plantlets or tillers of European ecotypes and cultivars were transplanted in 15 cm pots and maintained at about 20/16°C day/night temperature. At the end of the juvenile stage vernalization took place in growth chambers at a 6/4°C regime. A daily 10 hours illumination was provided by fluorescent tubes, irradiance being 0.6-0,8 mW/cm^2 at the plant level. On completion of the vernalization period, temperature, light intensity and day length were progressively increased so as to avoid devernalization. At heading time, temperature was 25/18°C, day length 16 hours and irradiance at the plant level 5,0-5,5 mW/cm^2. In some experiments plants were induced to flower during winter in an unheated greenhouse and transferred to the field in April.

Results

Only a few indicative results will be presented here. On several occasions (BEAN, 1970; KOZUMPLIK and CHRISTIE, 1972), wide divergences have been observed in the induction requirements between species, between varieties and inside of each population or ecotype; similar differences appear in our material as shown on table 1.

Table 1. Inter- and intraspecific differences in induction requirements expressed by the percentage of heading plants and the number of fertile tillers after 10 weeks vernalization.

Cultivar	Days before heading	Percentage headed plants	Mean number inflorescences per plant
Cocksfoot Lara	66	44	3,6
Cocksfoot Motterwitzer	63	100	6,3
Meadow fescue Predix	67	63	9,9
Meadow fescue Bundy	71	75	15,8
Tall fescue Barcel	66	44	6,3
Tall fescue Manade	51	88	13,9

No clear relation was found between the induction requirements and the earliness of a cultivar as expressed by the number of days before heading in the realization stage. It is not always easy to determine when a plant is ready for induction. Several authors have tried to relate the completion of the juvenile stage to the morphology of the plant, to the bud development (GILLET, 1976), to the number of developed leaves or to the leaf area. KOZUMPLIK and CHRISTIE (1972) working on cocksfoot found that the juvenile stage is completed with the emergence of the eight leaf on the main tiller. In growth chambers, this is the case 7-8 weeks after sowing.

Table 2. Influence of the juvenile stage duration on the mean number of inflorescences and the seed yield (g/plant) after 10 weeks vernalization.

Cultivar	Duration of the juvenile stage					
	4 weeks		7 weeks		10 weeks	
	heads	/ yield	heads	/ yield	heads	/yield
Meadow fescue Predix	11,4	1,1	36,4	1,0	31,0	3,2
Meadow fescue Tetraploid	10,4	1,6	22,0	2,4	7,2	1,7
Cocksfoot Predix	0	0	10,6	0,9	16,8	1,5
Cocksfoot Lara	0	0	10,6	4,4	20,0	2,6
Number of weeks germination harvest		24		27		30

As shown on table 2, meadow fescue may be partly induced already after 4 weeks juvenile stage on the contrary to cocksfoot. In this connection, it could be mentioned that GILLET (1976) found no absolute juvenile stage requirement in meadow fescue.

Long-day perennial grasses are reported to have extreme cold and short-day requirements (COOPER and CALDER, 1964). However reports are contradictory concerning the minimum duration of the inductive period. In some cases, (KOZUMPLIK and CHRISTIE, 1972; FEDOROV, 1973; KLOSS, 1973) only 4-6 weeks were necessary, in other cases up to several months. In our experiments, better results were obtained with a 10 weeks induction period than with one of 6 or 8 weeks (table 3).

Table 3. Influence of the duration of the primary induction period on the mean number of inflorescences and on seed yield (g/plant).

Cultivar	Plants were exposed for 6 weeks heads	6 weeks yield	8 weeks heads	8 weeks yield	10 weeks heads	10 weeks yield
Meadow fescue Predix	4,9	0,4	5,7	1,4	11,2	3,7
Meadow fescue tetraploid	0,2	0	4,8	0,3	20,3	3,3
Cocksfoot Predac	7,0	1,5	7,9	1,5	19,7	2,5
Cocksfoot Lara	10,3	0,1	20,6	1,9	40,8	6,8

If the induction requirements are fulfilled, the number of heads and the seed yield is then largely influenced by the previous growth conditions. Plantlets of Prefest meadow fescue which were 4 weeks old when vernalized produced half as many seeds as those of 17 weeks old well-tillered plants or ones grown in a dense sward for 30 weeks (table 4). Plants which had just produced seeds could not be induced again immediately without a rest period.

Table 4. Tillering and heading according to the previous growth of Prefest meadow fescue plants.

	4 weeks old plantlets	17 weeks	30 weeks dense sward
Tiller number at the beginning of the realization stage	4,0	13,0	8,3
./. plants with inflorescences	100	100	100
Number of heads per plant	17,2	40,8	39,8

Discussion

The foregoing experiment suggests that the three successive stages which culminate in the flowering of a perennial grass are not independent, but correspond to the natural succession of the seasons, so as to avoid the illtimed development of inflorescences. The abrupt changes of temperature, illumination and nutritional conditions, which are sometimes imposed on the plants in growth chamber experiments may explain their failure to flower. The danger of devernalization of induced plants after exposure to high temperatures is well known. The development of inflorescence may be reduced when juvenile plants are abruptly transferred to cold conditions. The conclusion of our experiments is that seed production in artificial conditions is rather poorer than in the field.

Growth chambers are very useful to determine the induction requirements of the plants but they are rather expensive and the available space is limited. In the field, summer sowing may shorten the breeding cycle if the weather conditions are favorable. Cocksfoot and tall fescue are sown before the end of July but meadow fescue may be established at the beginning of August.

The combination of greenhouse and field growing may be an interesting alternative to get seeds rapidly. Plants can be sown late in the autumn in a heated greenhouse prior to vernalization and transfer to the field.

Seeds of meadow and tall fescue which were sown in December produced plants which produced sufficient seed in July. However incomplete heading was observed on some cocksfoot ecotypes.

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COMPARISON OF THE FIRST AND SECOND GENERATION SYNTHETICS OF TALL FESCUE WITH DIFFERENT NUMBER OF CONSTITUENTS (*)

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Introduction

Our tall fescue breeding work is aimed to develop synthetic varieties suitable for the irriguous plains of our country, able to give high yield of harvestable biomass also in summer season.

These varieties should be utilized either for monoculture meadow structures or in binary grass-legume structures (tall fescue + lucerne, white clover + tall fescue etc.).

For this reason, persistency and stability of forage yield through the cuttings are considered as the most important traits.

Material and methods

The whole program includes two different parental populations. The first derives from polycross of plants selected in competitive conditions within the cultivar Manade (Manade x Manade); the second one derives from polycross of plants selected within different cultivars (Manade x Kentucky 31 x Lodigiana x Syn 2). Only the first population (Manade x Manade) is concerned in this paper.

On the basis of progeny test results, of the degree of fertility and of the synchronism of flowering , twenty mother plants have been chosen, sufficiently similar for the vigour.

The following Syn 1 generation synthetics have been formed (Table 1): four 5-clone Syn 1, three 10-clone Syn 1, two 15-clone Syn 1 and one 20-clone Syn 1.

The polycrosses were made in greenhouse by artificial wind.

The second generation was obtained by intermating 15 plants for each constituent of the respective first generation synthetic that is:

75 plants for each 5-clone Syn 1
150 plants for each 10-clone Syn 1
225 plants for each 15-clone Syn 1
300 plants for the 20-clone Syn 1.

(*) This work was supported by funds from Ministry of Agriculture (Piano Foraggero Zootecnico).

The first and second generation synthetics were tested in greenhouse, in concrete boxes/plot 150 cm long, 25 cm wide and 45 cm high, at a density of 300 plants per square metre (distance between the plants on the row 2.8 cm).

The Syn 1 seeds were obtained two years before the Syn 2 seeds So, we have previously measured the germinability in Petri dishes and the vigour of seedlings 20 days after germination. Samples of 20 seedlings for each constituent of each synthetic variety have been collected; dry matter yield of seedlings (leaves + roots) has been registered. Seedlings were transplanted in the concrete boxes in February, according to a randomized block scheme with 12 replicates (640 plants per synthetic variety). Nitrogen was supplied at the trasplanting time (50 Kg/ha) and after each cut (40 Kg/ha).

The results of the first 6 cuttings concerning dry matter yield per plot are discussed in this paper; the interval between the cuttings was 25-28 days.

Results and discussion

In Table 2 there are the data concerning germinability and dry matter yield of seedlings after twenty days.

The germinability was measured after 8 days, according to the international testing; the Syn 2 were better germinating than the Syn 1 only in 4 synthetic varieties out of 10. By contrast, the dry matter yield of seedlings after 20 days was higher in Syn 2 when compared with Syn 1 in 8 synthetic varieties out of 10. These results confirm that the age of seeds affects the vigour of seedlings.

Table 3 shows the data concerning dry matter yield per plot. We have to underline the great homogeneity of the data, not only within each generation but between Syn 1 and Syn 2 generations as well.Within each generation, such homogeneity can be explained as a consequence of an intentional choice of parental clones sufficiently similar for vigour. It is more difficult to explain the results concerning the ratio between Syn 2 and the corresponding Syn 1. This datum shows that there is no inbreeding effect in synthetic varieties, whatever the number of constituent may be.

It is known that a limited number of parents in Syn 1 generation produces an inbreeding depression in Syn 2 and subsequent generations (Corkill 1956, Busbice 1970, Gallais 1981, Wright A.J. 1981).

The formula generally admitted is that of Sewall Wright:

$$F_2 - F_1 = \frac{F_1 - \bar{P}}{n}$$

by which it results that the difference between the first generation (Syn 1) and the second generation (Syn 2) is as much lower as much greater the vigour of parents. This formula explains as well why when the number of parental clones increases the difference we observe between the two generations becomes lower.

Conclusions

How to explain our experimental data? We underline, at first, that the analysis of the data of each synthetic variety, made throughthe cuttings does not seem to assign an important role to the vigour of the seedlings Syn 2, because the average of the last three cuttings is not different from that of the first three ones. After such statement, and referring to the parental populations we utilized, two hypothesis can be made:

1. Five clones can be considered as an optimal base number. It would be sufficient to avoid a significant inbreeding effect. In perennial rye grass Breese and Lewins (1960) recommended four clones as optimal base number.

2. The clones we utilized in this experiment are perhaps resistant to the negative effect of inbreeding, as it occurs in many cases concerning Dactylis glomerata (Rotili et al. 1984).

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TABLE 1. Syn 1 and Syn 2 parents

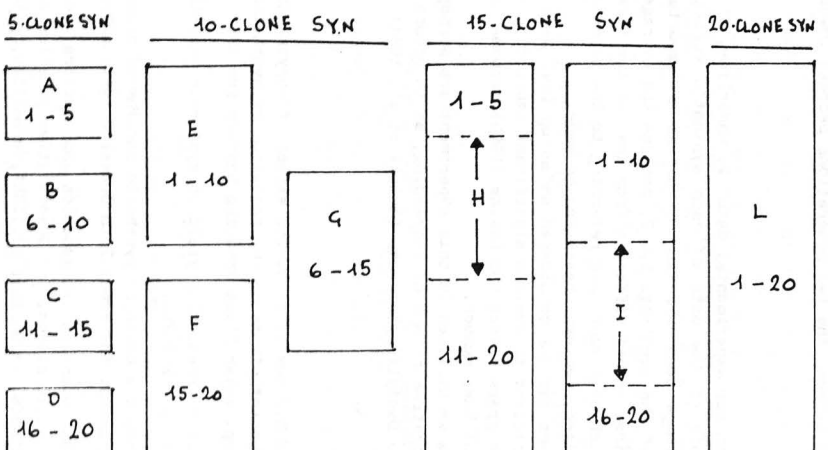

Table 2. Comparison (Syn 2/Syn 1) x 100 for germinability and seedling dry matter weight at 20 days after germination.

Synthetic varieties		Per cent germinability	Dry matter weight (g) Samples of 20 seedlings
5 - clone Syn	A	102	142
	B	104	117
	C	121	109
	D	100	100
10 - clone Syn	E	89	95
	F	89	142
	G	101	125
15 - clone Syn	H	110	115
	I	112	145
20 - clone Syn	L	109	142

Table 3. Dry matter yield per plot. Averages of 6 cuttings.

Synthetic varieties		Syn 1	Syn 2	(Syn 2/Syn 1)x100
5 - clone Syn	A	35.69	35.94	101
	B	33.78	35.38	102
	C	33.17	34.72	102
	D	34.47	36.17	103
10 - clone Syn	E	34.77	34.07	98
	F	35.49	35.00	99
	G	34.32	34.06	99
15 - clone Syn	H	36.18	34.73	96
	I	35.02	35.32	101
20 - clone Syn	L	34.50	35.42	103

The genetical control of apomixis in Poa pratensis.

by Gösta Julén

Apomixis in relation to plant breeding can be looked upon from two different points of view. On one hand a plant with apomictic seed formation will give completely uniform offspring and a cultivar based on such a plant will maintain this uniformity generation after generation without any special work made by the breeder. One the other hand, species with normally apomictic seed formation have the disadvantage that it create problems in effectionating genetic recombinations for development of new improved cultivars. This type of species is represented among Swedish agricultural crops by smoth stalked meadow grass, Poa pratensis. The breeding of this species in Sweden, as well as elsewhere, has mainly consisted of the collection of existing apomictic types under different ecological conditions, multiplying them and testing them for there agronomic value. All known varieties now on the market have been obtained in this way. Apomixis in Poa pratensis if of the aposporic type. Normal embryosacs, with the reduced chromosome number are developed but besides them also aposporic embryosacs which are organized in exactly the same way as the normal ones but with unreduced chromosome number. Normally the haploid embryosac deteriorates and disappears and the new seed is developed from the aposporic embryosac without any fertilisation of the eggcell. In this way the seed will have exactly the same genetical set up as the mother plant. The apomixis in Poa pratensis is, however, not obligate. Sometimes the normal embryosac will survive the competition from the aposporic one and will after fertilisation develop into the seed. In cases when this occur there will be an offspring plant aberrating from the mother plant. The degree of the apomixis is different in different apomictic clones and it can also be influenced of environmental conditions. Most commercial cultivars have a high degree of apomixis. For instance the wellknown Swedish cultivar Fylking produces only four per cent of the seed in the sexual way.

In several cases efforts have been made to increase the variation in apomictic Poa pratensis by crossing it to related sexual Poa species. In all reported cases of such work the F_1 and sometimes also the F_2 plants become completely sexual. Almgård (1966) made embryological studies on the hybrids between Poa pratensis and Poa longifolia. He found in the F_1 plants only sexual and no aposporic embryosacs. In later generations the apomixis is restored in some of the plants which will give uniform progeny.

In the early fifties when I was working on breeding of Poa pratensis I tried to increase the variation by using x-ray treatment of the seed. A large number of morfological mutants where obtained. Of greater interest was, however, that observations of the offspring generation showed, that a fairly high number of treated plants had produced seed in the sexual way resulting in a very variable offspring (Julén 1958, 1960). In following generations apomixis was restored in some cases. In that way new apomictic plants with other characters than those of the original mother plant were developed. Thus it seems to be possible to induce sexual seed formation for one or two generations when crosses can be made and in later generations have a segregation between sexual and more or less completely apomictic offspring plants.

The varying degree of apomixis in different apomictic clones and the influence of the environmental conditions have made that it has been extremely difficult to give a satisfactory description of the genetical background to the apomixis. Extensive investigations on Poa pratensis carried out in Sweden and elsewhere seems to verify the findings of Müntzing already in 1940 that the apomixis of this seemingly allopolyploid species is multifactoral and that "concept of apomixis in this species is due to a rather delicated genetic balance". A comparison of the results from various investigations seems, however, to give at least an indication of how this genetic balance is constructed (Julén and Åkerberg, in press). The results from these investigations, on interspecific crosses between Poa pratensis and related sexual species as well as results on the mutation material studied at Svalöv, indicate that it is likely that the genetic balance is controlled by two independent

genetical systems. The first of these systems, which is the basic one, consists of a set of genes controlling the ability to develop aposporic embryosacs. The other system consist of genes controlling various characters influencing the competitions between the aposporic embryosacs and the sexual one in those individuals which are genetically able to produce aposporic embryosacs.

If the first system is disturbed either by crossing with sexual types or by mutations the first generation offspring will be completely sexual. That has been the case in all interspecific crosses. Almgård in his embryological studies on hybrids between Poa pratensis and Poa longifolia never found any aposporic embryosacs in the F_1 plants. In embryological studies by Grazi et al, (1961) in the mutation material at Svalöf on plants which on basis of the behaviour of the offspring were classified as completely sexual. It was found that these plants did not develop any aposporic embryosacs. After selfing or backcrossing to apomictic types the genetic system might again be restored and in following generations there will be a segregation between sexual types and types with various degree of apomictic seed formation. The degree of apomixis will now depend on various genes controlling the competition between the two types of embryosacs. The phenotypic expression of these genes might be influenced by environmental conditions. Thus, while genetically sexual plants are completely sexual, the degree of apomixis in the genetically apomictic plants can vary from allmost complete apomixis to very high frequency of sexual seed formation. The degree depend on the genetical set up also on the influence of environmental conditions. Further investigations on Poa pratensis and of other aposporic polyploids are desirable in order to reach more complete knowledge of this intricate system and make possible a more systematic breeding work of these normally apomictic species.

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REFLECTIONS ON THE POSSIBILITIES OF IMPROVING SELECTION TECHNIQUES FOR FORAGE QUALITY

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INTRODUCTION

In 1978,at Eucarpia meeting,Dr WILHELM LAMPETER said "for most European farmers it is more advantageous to improve the digestibility of the basic diet by 2 % than to heighten the yield by one ton/ha". Seven years later this opinion is still of interest and breeding for quality remains an essential concern of forage plant breeders.

Forage quality is estimated through its influence on animal production. This production depends on feed consumption, digestibility, relative contents in energy and protein.

The genetic component of each of these parameters interacts with :

- the animal consuming the forage, the nature (milk or meat) and level of the production,
- the type of food (green forage, silage, hay and so on ...),
- the quality of farming practices,
- the plant stage at harvest.

Moreover concentrates allow

- to correct an eventual desequilibrium in the chemical composition of the basic diet,
- to complement the diet for high producing animals.

This suggests how many sided the concept of forage quality may be.

To what extent can the plant breeder act on quality ? What are the relations between criteria measurable at farm level and laboratory criteria, the only ones the plant breeder can take into account ?

VOLUNTARY INTAKE

During 3 years, at Lusignan, P. GUY has been comparing the voluntary intake by sheep in metabolism cages, of six lucerne cultivars preserved either as silage or hay. The results are summarized in table 1.

Table 1. Voluntary intake of lucerne varieties by sheep in metabolism cages grammes DM/Kg metabolic weight*

	1982 Silage	1983 Silage	1984 Hay	Average
Lutèce	100,4 1	74,0 1	69,2 1	81,2
Magali	90,3 3	71,9 2	67,7 2	76,6
Résis	94,0 2	57,4 5	66,0 4	72,5
Belfeuil	89,0 4	63,0 3	64,3 6	72,1
Europe	83,4 6	59,0 4	65,1 5	69,2
Milfeuil	83,5 5	46,7 6	67,2 3	69,8
Average	91,4	62,6	66,9	

Lutèce - Magali - Résis - Belfeuil - Europe - Milfeuil

* metabolic weight = live weight^{0,75}

The dry matter content of silages had an influence on the voluntary intake and this was taken into account. But no correlation could be shown up between voluntary intake and some measurements made simultaneously : in vivo and in vitro digestibility, fiber content, total nitrogen content, stem thickness and so on ...

Apparently, these observations are inconsistent with many others : several authors make mention of positive correlations between voluntary intake and digestibility but it must be noted that, in this case, the voluntary intake was measured with dairy cows (CONRAD 1964). A number of others, among them SCEHOVIC (1979), showed, for grasses, relations between voluntary intake measured with sheep in metabolism cages and chemical composition of forage (fiber and lignin content ...).

The discrepancies may be only apparent. In a series of experiments in which the plant breeding stations of Changins and Lusignan were partners, and which we will consider again later, M. GILLET took the observations illustrated in figure 1.

In 1980 at the 1st cycle, five grass varieties (one Italian rye grass cultivar and four tall fescue cultivars) have been compared during four weeks with sheep in metabolism cages. Every day, voluntary intake and digestibility were recorded. Then the daily results were brought together for each week. Taken as a whole, the data actually show a positive correlation between voluntary intake and digestibility. But, if we consider the results week by week, it becomes clear that the observed relation does not

express a cause and result situation but shows the effect of a common factor (the age) acting on each variable.

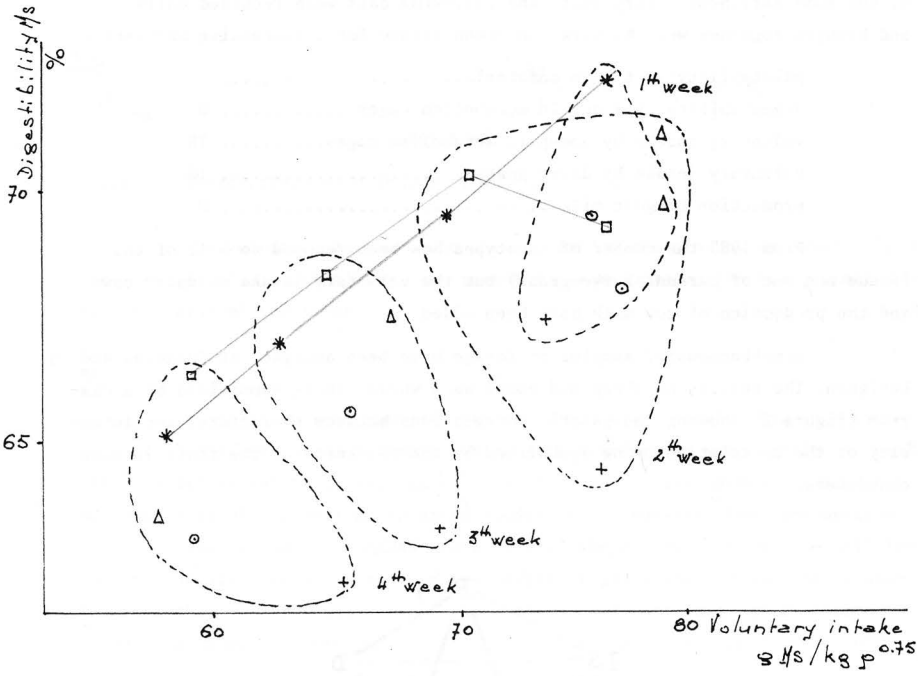

Figure 1. Relations between digestibility and voluntary intake of 5 cultivars during four weeks (from the mid April to mid May).

Getting rid of time enables to compare genotypes and to establish the absence of genotypic correlation between voluntary intake and digestibility. Thus the observed correlation is of no use for the plant breeder. It happens in this experiment that regression lines for each genotype are parallel. But certainly there are instances when the slopes of the regression lines differ, the differences beeing of genetic nature.

The voluntary intake of a forage is an essential criterion of quality, but one may wonder whether its evaluation with non producing animals is an accurate one. Besides,measures with sheep in metabolism cages require relatively large quantities of forage, then large quantities of seed, which does not fit the demands of plant breeding. In cooperation with J. SCEHOVIC, M. GILLET has carried out,since 1979,comparisons between producing (dairy

goats, dairy cows) and non producing animals (sheep in metabolism cages). These animals were fed 5 genotypes of tall fescue, and one Italian rye-grass of the same earliness. Every year, the following data were recorded daily and brought together week by week, on green forage for 2 successive harvests :

palatability in trough cafeteria..... P
digestibility by sheep in metabolism cages ..... D
voluntary intake by sheep in metabolism cages..... IS
voluntary intake by dairy goats ..... IG
production of goat milk ..... M

From 1983 the number of genotypes has been reduced to 3 (2 of tall fescue and one of perennial rye-grass) but the voluntary intake by dairy cows and the production of cow milk have been added.

Simultaneously, samples of forage have been analyzed at Changins and Lusignan. The results on sheep and goats as a whole can be summarized by a diagram (figure 2) showing the genetic correlations between characters, the intensity of the correlation being symbolized by the thickness of the trait joining characters.

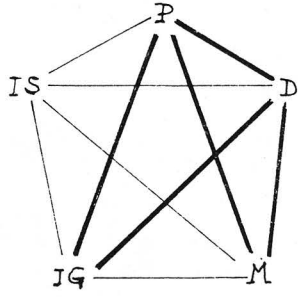

Figure 2. Correlations between characters P - D - IS - IG - M.

In this study, voluntary intake by sheep in metabolism cages proved not very precise and correlations including this character are not very significant. On the contrary, the voluntary intake by producing dairy goats as well as the milk production are highly correlated to palatability and digestibility.

One can then consider, at least as a starting point of study, the following cause-effect relations between characters (figure 3).

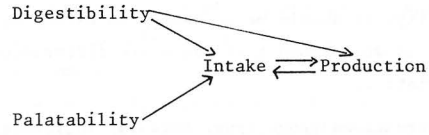

Figure 3. Cause-effect relations between characters.

The voluntary intake would then depend on the production level, digestibility and also palatability, the potential animal production being limited by filling value of the forage.

PALATABILITY

At least in some species, this trait is worth considering in a breeding programme. It can be estimated by in situ grazing, or more easely (M. GILLET 1983) in trough cafeteria experiments. In this case, measurements require only small amounts of forage (one kg green fodder for a measure), they are precise, sensitive and reliable. J. JADAS-HECART (1982) showed the existence in tall fescue of a negative relation between the height of grass when grazed and palatability : this relation still exists with the same forage in a trough cafeteria trial, and must be taken into account in evaluating the genetic variability for palatability.

As quoted by S. BADOUX (1978) the palatability of tall fescue is more or less strictly related to leaf flexibility which allows a first choice in segregating populations.

Recently, in trough cafeteria experiments with sheep, at Lusignan, we noted that spraying rye-grass with tall fescue juice makes it unpalatable. No reciprocal effect is observed when tall fescue had been sprayed with rye-grass juice. Following this observation, J. SCEHOVIC (1985) showed that tall fescue and rye-grass juices differ significantly as to the content in substances thought to be repulsive (volatile bases, ammociac, sulphur components), or attractive (aldehydes, ketones). In like manner, he noted that an increase in sulphur components goes with rust infection in Italian rye-grass, which reinforces the interest that must be devoted to breeding for resistance (C. MOUSSET and A. GALLAIS 1974).

In no case, during this study as in a number of previous ones, has J.SCEHOVIC found a relation between palatability and soluble sugar or nitrogen content.

These observations were made on tall fescue, a species reputed to loose rapidly its palatability. It should be necessary to extend these findings to other species : even now it is well known that there are differences in palatability between rye-grass varieties.

If these observations prove true, this may influence the way of selecting in the offspring of festucololium hybrids. In 1984, J. JADAS-HECART, during grazing experiments, noted that the cultivar Kenhy, selected by BUCKNER in the offpring of fescue x rye-grass hybrids, was very palatable in spite of an evident lack of flexibility. In the same way, the offspring of double-cross between tall fescue of european and mediterranean origine are, at every season, more palatable than their parents.

DIGESTIBILITY

The evolution of the digestibility of grass during the reproductive phase justifies the energies devoted to increasing the ease of management. At that time we can observe an inverse relation between digestibility and fiber and lignin content (SCEHOVIC 1979).

It is possible, at least in some species, to put forward differencesof in vivo digestibility between genotypes having equivalent ease of management and earliness : we have quoted such an example at the bigining of this paper. But the discrimination by means of sheep in metabolism cages is not easy : it requires a sufficient number of animals and a regular supplying of green matter and can be made only in the latest stages of the breeding.

A relation between genotypic differences of digestibility and differences of physicochemical characters seldom occurs (G. JULEN, 1978). We can consider two experiments as examples.

J. JADAS-HECART (1985) found in an inbred line of tall fescue from the South West of France, some plants with brittle leaves, very palatable and very digestible ; the analysis of fiber components revealed the following differences :

	Normal plants	Brittle Plants
Crude fiber	20,9	16,8
A D F	24,5	18,2

In fact this trait is but a curiosity. Its genetic control (it is a 3 recessive character) makes it very difficult to use and its very nature brings about

severe growth troubles.

Another instance is the bm gene in maize whose bm3 allele lowers the lignin content of the stem by 50 % (3,5 % instead of 7 %) without modifying the crude fiber content. In a programme devised to breed maize especially fit to forage production Y. BARRIERE, A. GALLAIS et al. (1985) converted 7 commercial hybrids to bm3. Then they compared the feeding value as a silage of four of them, to their normal counter parts. In 10 experiments with sheep including 68 comparisons, the organic matter digestibility of bm maize was higher by 2.4 points on an average (73.8 vs 71.4) than in normal maize, and voluntary intake was raised by 5.3 % (50.0 vs 47.5 g DM/kg metabolic weight = live weight $^{0.75}$). bm and normal maize were compared in 6 experiments corresponding to a total of 46 pairs of bulls (complementation in both instance being 1 kg soy-bean oil cake DM/day).

Bulls fed bm maize

- consumed 7.95 kg silage DM/day vs 7.38 kg for normal maize
- showed an average daily weight increase of 1463 g vs 1265 g
- gave the same dead body weight (337 kg net weight) as the bulls fed normal maize but in 155 days vs. 170 days
- had a feeding efficiency (average daily weight increase g/kg DM voluntary intake) of 163 g vs. 150.

In an experiment where dairy cows were fed silage maize ad libitum, the supplement (barley + soya-bean oil cake) for cows fed normal maize was 3.5 kg DM/cow/day, that of cows fed bm maize being 2.2 kg.

Dairy cows fed bm maize

- consumed 11 % more silage than those fed normal maize (2.5 vs 2.2 kg DM / 100 kg live weight)
- produced 5 % more milk (23.9 kg vs 22.7/day at 4 % fat.

Nevertheless these advantages go with some draw backs : the silage yield/ha for bm maize is lower by 0 to 20 % than the normal counter part depending on genotypes, this yield decrease concerning mainly the cob yield. Moreover there is an increased susceptibility to lodging, variable also according to genotypes.

Taking all these characters together led us to enter upon a specific programme of genetic improvement using bm1 and bm3 genes.

These instances are extreme and it is a great deal to do to be able to use indirect physicochimial criteria as an aid for breeding purpose.

Do other solutions exist ?

For the last 3 years M. GILLET et al. have been comparing with dairy cows, fed green forage in trough, the feeding value of a perennial rye-grass Réveille, 2 tall fescue cultivars, Clarine (normal type) and Lubrette (selected for palatability). The results summarized in table II show an improvement not only in voluntary intake by dairy cows and in milk production but also in digestibility.

Table 2. Comparison of feeding values with dairy cows fed green forage in trough

	Réveille	Lubrette	Clarine
	1st cycle - 3 weeks		
Vol. Int. ($g/kg^{0.75}$)	126.0	124.9	108.9
Digestibility	78.9	71.6	69.3
Milk prod.	27.9	27.3	25.9
	2nd cycle		
Vol. Int.	124.3	119.7	107.2
Digestibility	70.7	67,5	67.4
Milk prod.	21.7	20.3	19.8

But it must be said that if Lubrette was selected on purpose for palatability it was quite unconsciously selected for digestibility : unconsciously but was it only mere chance ?

At last, the reliability of our means of evaluation has been appreciably improving during the last years with the commercial availability of constant quality enzymes allowing us to perform routine tests of digestibility with amylase, pepsine, cellulase (M. LILA et al. 1985). The A.P.C. method is more precise and reliable than the rumen juice method and it enables us to get satisfying calibration curves on the Near Infra Red Spectrophotometre.

One care of some plant breeders is to raise the soluble carbohydrates content. Perhaps it should be possible with this indirect approach to improve lucerne digestibility or to make easier orchard-grass ensiling, for example.

In spite of techniques improvement, it remains still difficult to analyze in this respect a segregating population. Since the first attempts, the day and hour amplitude of variation is well known. The following example illustra-

tes that, in addition to these troubles, an uncertainty arises regarding the choice and the preparation of portions to be analyzed.

In 1979 and 1980, J. JADAS-HECART compared 8 tall fescue cultivars of European origin to 21 offsprings from a polycross of amphidiploïd-lines (these amphidiploïd come from colchicine treatment of sterile hybrids between tall fescue of European and Mediterranean origin). At every harvest soluble sugars were systematically determined. For 2 consecutive years there has been a significant correlation between the sugar contents at all harvests of a year. The average results for 2 years show that soluble sugar content is significantly higher in amphiploïds than in hexaploïd fescues.

	Yield DM t/ha	Soluble sugar % DM
European	9.8	15.8
Amphiploïds	11.9	17.9

Comparing a broader range of genotypes, SCEHOVIC analyzed several extracts and got the following results :

Table 3. Soluble sugar content of Festuca arundinacea and of its hybrids (ranking between brackets)

		g/kg in dry matter	g/kg in green matter	g/kg in juice
Fescue	Clarine	86.6 (7)	23.2 (2)	12.3 (2)
	Lubrette	98.1 (3)	20.2 (3)	11.7 (3)
Amphiploïd	E x M	95.6 (4)	18.0 (5)	9.9 (6)
Festulolium	Hazel	114.9 (2)	18.7 (4)	11.1 (4)
	Kenhy	95.4 (5)	28.5 (1)	13.1 (1)
	179.8	119.9 (1)	15.9 (7)	10.6 (5)
	540	89.5 (6)	16.9 (6)	7.0 (7)

Due to the fact that the analyses are made on dry or green matter, the rankings differ greatly. Supposing a successful selection, what should be the consequence on yield of an increase of soluble sugar content ? The information in our possession comesfrom too little a number of years to answer this question.

NITROGEN CONTENT

Nitrogen content of grasses used in a proper way, cannot be considered a limiting factor (G. BUGRE, 1978) but, for various reasons, attempts have been made to improve it.

One also knows that an important part of organic nitrogen is found as amino acids and short bonds polypeptids. The utilization of soluble nitrogeen implies that it goes to the mating of rumen micro organisms and implies also sufficient available energy. Another question is the possibility to increase by breeding the proportion of non soluble nitrogen.

Studying the growth of forage grasses in relation to climate and nitrogen fertilization, J. SALETTE and G. LEMAIRE observed, among other things, the evolution of protein content relatively to dry matter evolution. The attained conclusions are briefly as follows :

- this evolution may be represented (figure 4) by a set of dilution curves whose general equation is $N\ \% = \alpha\ (DM)^{-\beta}$ with $0 < \beta < 1$ both α and β increasing with nitrogen fertilization level (J. SALETTE and G. LEMAIRE 1981).

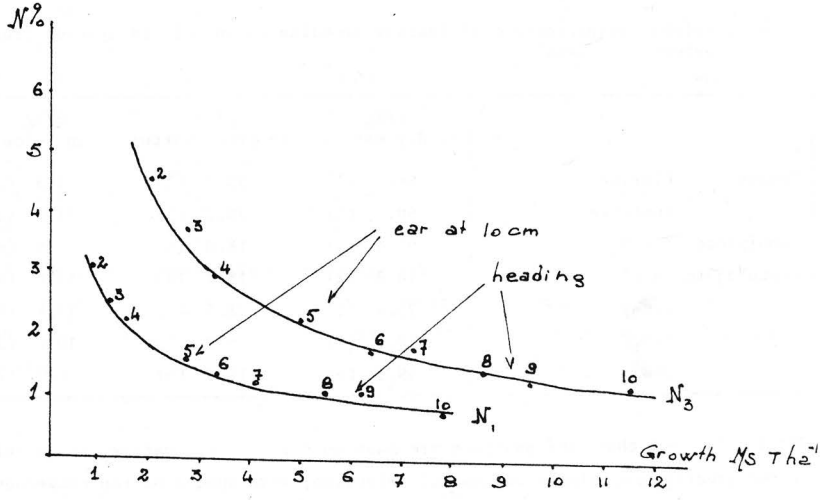

Figure 4. Relation between nitrogen content and spring growth for the Ludelle cultivar with 2 levels of nitrogen fertilization
N_1 = 60 kg/ha in autumn
N_3 = 60 " "
+ 120 kg/ha at the end of the winter.
1 to 10 : dates of cutting from March 10 to June 8.

- there is a limit curve corresponding to the fulfilment of plant nitrogen requirements, beyond which the nitrogen content of the plant increases without an increase in dry matter production.

- J. SALETTE and G. LEMAIRE (1984) think that when the environmental nitrogen offer is not limiting, the nitrogen taken by the plant comes in addition to the amount necessary for the achievement of the growth potential determined by climatic conditions. When the nitrogen offer is limited, the growth rate would adapt itself to nitrogen availability by limiting the density of grass stand.

According to the observations of these authors there is no difference between limit curves for different genotypes of the same species and even for different temperate grass species. In return, when nitrogen fertilization is lower than that corresponding to the limit curve, the α and β coefficients vary according to species and probably to cultivars. Then, the relations of nitrogen dilution depends on cultivar and environmental conditions (J. SALETTE and G. LEMAIRE, 1984).

In conclusion, when the amount of nitrogen available for growth is not a limiting factor, the only difference between genotypes is the run speed on the limit dilution curve. When the amount of nitrogen available for growth is the limiting factor, the only perceptible differences between genotypes are related to their different aptitudes to extract nitrogen from the soil. In the present state of knowledge it must be admitted that the increase in nitrogen absorption rate can only bring about passing from one dilution curve corresponding to N_1 available nitrogen level, to another one corresponding to level N_2 higher than N_1. All the same, on this may be superposed a genetic variation of another nature concerning a change in the run speed on the curve.

Concerning the ratio of soluble organic nitrogen to total nitrogen, J. SALETTE et al. 1984 have studied during two successive years, the growth of 6 Italian rye-grass cultivars, of which 4 diploïds and 2 tetraploïds at 3 levels of nitrogen fertilization (50, 100, 150 kg/ha). For each nitrogen level and for 4 harvest dates, it was possible to estimate the evolution of total, organic, soluble, nitric and insoluble nitrogen content.

- The tested nitrogen levels raise in the same proportion the soluble and insoluble nitrogen.

- The soluble to total nitrogen ratio decreases during the growth from 0.45 to 0.30 but does not depend on the amount of nitrogen applied.

- No significant difference between varieties was shown as well regar-

ding the soluble and total nitrogen percentages as the evolution of their proportion during growth.

CONCLUSION - SUMMARY

Feeding value is a combination of voluntary intake and digestibility. Numerous factors act on the variability of this character and their control is far from being known. But we are aware now that a part of the variation is of genetic origin.

As to the voluntary intake, revealing the genetic variance requires considerable means for it needs producing animals. On the other hand, breeding work calls for criteria that must be simple, cheap, adapted to a large mass of plants. Chemical criteria used up to now proved disappointing. For some species or groups of species it is possible to use palatability but it is of no use for others. Moreover it can be used only at advanced stages of a breeding programme.

Sheep in metabolism cages allow us to get animal references for digestibility but the search for criteria fit to selection purposes and genetically correlated to these references is as imperative a need as before. The "in vitro" digestibility with commercial enzymes represents, at least for a number of forage plants, a sensible and reliable tool of measure. The indirect criteria used till now often deceived the plant breeders'hopes but others are now under investigation.

At last, it seems that breeding can have but a limited hold on nitrogen content of forage grasses. On the other hand, selection is likely to increase the efficiency of nitrogen fertilizers.

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LEAF TYPE IN RELATION TO CHEMICAL COMPOSITION, NUTRITIVE VALUE AND SEASONAL GROWTH OF PERENNIAL RYEGRASS

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SUMMARY

Perennial ryegrass populations, derived from one variety (Ruanui) and displaying inherent differences in leaf length, mesophyll thickness and epidermal ridging, were grown as small field plots and cut either frequently (6-7 cuts) or infrequently (4-5 cuts) over two years. Determinations were made of cellulose, water soluble carbohydrate (WSC), total N and the minerals P, K, Ca, Na and Mg, in samples from several harvests.

The short-leaved population had less cellulose (1-4% units) and WSC (2-5% units) and more total N (0.2-1.2% units) than the original population. However its growth was poor and it lacked winter hardiness. The population selected for thick mesophyll also had less cellulose (1-4% units) was higher in WSC (2-4% units) and had significantly ($P<0.05$) more Ca and K than Ruanui. The selection for shallow epidermal ridging had cellulose levels 2-5% units less than Ruanui overall and significantly ($P<0.05$) more Na and Mg than the deep ridge selection. As in earlier studies it also achieved faster growth than Ruanui at times of high potential soil water deficit. Predicted DMD measured in May, was correlated ($P<0.001$, $r = -0.60$) with cellulose. One percent difference in cellulose was associated with 2.5% difference in digestibility.

INTRODUCTION

Genetic variation in grass leaf morphology and anatomy is often associated with variation in growth and in response to environment (Wilson, 1981). For example, intra-varietal selection for long leaves can markedly improve dry matter yielding capacity under infrequent cutting systems (Rhodes, 1973). Conversely, short leaved selections are often more suited to frequent cutting. Similarly the extent of epidermal ridging can influence drought tolerance and water-use efficiency in Lolium (Wilson, 1981) and Festuca (Silcock and Wilson, 1981). At the cellular level, the size of mesophyll cells and thickness of mesophyll tissue can determine rate of photosynthesis per unit leaf area (Wilson and Cooper, 1967).

However, plant tissues differ in their nutritional value to the animal so that variability in morphology and anatomy is likely also to be associated with variation in herbage quality. For example, mesophyll and phloem are easily digested whereas vascular bundles, lignified cells and some epidermal cell walls may not be digested at all (Hanna, Monson and Burton, 1974; Selim, Wilson and Jones, 1975). Although the water soluble carbohydrates are digested faster it is often the level and digestibility of the more complex polysaccharides such as cellulose, a major portion of all cell walls, that has a major effect on rate of feed intake (Jones and Bailey, 1974).

Aspects of the relative feeding value of a grass variety can therefore be described in terms of its chemical composition, and the effects of selection for traits likely to influence quality can be judged by comparing selected populations with the original. The present paper describes chemical composition of herbage from simulated swards of Lolium perenne populations selected for contrasting expression of several leaf characteristics. Agronomic traits over the first year of growth are also described.

MATERIAL AND METHODS

Populations

The populations were 1) Lolium perenne cv. Grasslands Ruanui, and five others selected from that cultivar for one of the following leaf characteristics; 2) long leaves; 3) short leaves; 4) thick mesophyll; 5) deep epidermal ridging and 6) shallow epidermal ridging. These were obtained after two generations of selection as described by Wilson (1971).

Procedure

Simulated swards (0.5 m^2) of each of the six populations were established and grown in the field from May in year 1 to October in year 2. There were eight plots of each population, arranged with four blocks each split into two cutting treatments, each of which randomly incorporated eight plots, one of each population. Fertilizer (3.5 g, 20:10:10 NPK) was applied to each plot at 4-weekly intervals during the growing season. Plots were harvested (5 cm cutting height) either infrequently (4 times in year 1, 5 times in year 2) or frequently (7 times in year 1, 6 times in year 2). Herbage, excluding a 5 cm border row, was dried at 85°C overnight, weighed and prepared for chemical analysis by grinding through a 1 mm sieve. Total shoot growth between harvests was calculated as crop growth rate (CGR) in g dry matter m^{-2} day^{-1}. Winter survival was recorded in April of year 2.

To supplement previous anatomical and morphological data (Wilson, 1971) ten young, fully expanded leaves of each population were taken at random from the infrequently cut plots at two of the first year harvests. These leaves were used to measure the various traits for which the populations had been selected (Wilson, 1971).

Analytical

Cellulose was determined by a technique essentially as described by Bailey (1967). Water soluble carbohydrates were extracted by shaking in cold water and colorometric estimation of carbohydrates by an automated anthrone technique. Total nitrogen was determined by an automated alkaline phenol-hypochloride technique following Kjeldahl digestion. Samples were dry ashed and minerals determined on the acid extract by conventional procedures. The predicted dry matter digestibility of the dried ground samples taken from each infrequently cut plot on 3 May was determined using the pepsin cellulase digestion technique of Jones and Hayward (1973).

RESULTS

Leaf anatomy and morphology

Table 1 shows that relative differences in leaf characteristics for which the populations had been selected (Wilson, 1971) were maintained under the present simulated sward conditions. Among all populations, long leaves tended to be relatively wide. The

TABLE 1. Leaf characteristics of the populations - August

Population	Leaf length (mm)	Mesophyll thickness (mm)	Ridge angle (degrees)
Base variety	230	0.150	50
Thick mesophyll	230	0.177	41
Long leaf	300	0.168	43
Short leaf	139	0.152	43
Steep ridges	204	0.163	33
Shallow ridges	221	0.196	61
LSD 5%	32	0.020	7.0

population selected for thick mesophyll (mean depth of mesophyll tissue) also had relatively large external leaf thickness. Similarly thick leaves were exhibited by the populations selected for deep ridges but there was no necessary relationships between external thickness and mesophyll thickness. The thickest layer of mesophyll was found in leaves of the 'shallow ridge' population. Epidermal ridge angle was not consistently associated with any of the other traits.

Chemical composition

Tables 2 and 3 show the cellulose, water soluble carbohydrate (WSC) and nitrogen contents of the populations at three harvests in year 1 of the frequently (Table 2) and infrequently (Table 3) cut

TABLE 2. Mean percentage cellulose, water soluble carbohydrate (WSC) and total nitrogen (N) in leaves sampled at 1973 (year 1) harvests of frequently cut plots of Lolium perenne cv. Grasslands Ruanui selection lines

	Cellulose			WSC			N		
Selection line	19 July	16 Aug.	27 Sept.	19 July	16 Aug.	27 Sept.	19 July	16 Aug.	27 Sept.
Ruanui	20.9	22.8	21.5	7.2	7.7	8.5	4.6	3.3	3.8
Thick mesophyll	19.6	20.0	20.3	8.0	10.4	10.0	4.7	3.4	4.0
Long leaf	21.1	20.6	21.4	7.1	8.1	8.2	4.7	3.2	3.8
Short leaf	18.7	19.1	19.8	6.1	6.5	8.0	5.3	4.0	4.0
Steep ridges	20.0	21.3	21.6	6.5	8.3	7.6	4.9	4.0	4.0
Shallow ridges	18.7	20.0	18.7	7.3	7.6	11.0	4.8	3.8	3.9
LSD 5%	1.4	1.7	0.8	1.3	1.6	1.0	0.15	0.18	0.13

TABLE 3. Mean percentage cellulose, water soluble carbohydrate (WSC) and total nitrogen (N) in leaves sampled at 1973 harvests of infrequently cut plots of Lolium perenne cv. Grasslands Ruanui selection lines

	Cellulose			WSC			N		
Selection line	19 July	16 Aug.	27 Sept.	19 July	16 Aug.	27 Sept.	19 July	16 Aug.	27 Sept.
Ruanui	20.5	26.0	22.8	6.3	11.2	9.8	3.4	2.6	3.3
Thick mesophyll	18.7	22.2	22.1	5.5	11.7	16.5	3.9	2.7	3.4
Long leaf	20.2	22.5	23.0	5.7	11.1	8.8	3.6	2.6	3.2
Short leaf	19.5	22.0	21.6	3.1	8.3	7.0	4.3	3.1	3.5
Steep ridges	20.8	24.4	22.3	4.8	10.3	9.3	4.0	3.3	3.5
Shallow ridges	19.7	21.1	21.1	4.6	9.6	9.5	3.8	3.2	3.5
LSD 5%	1.4	1.9	1.1	0.9	1.9	2.2	0.30	0.51	0.30

plots. In general, three populations had less cellulose than the original variety, those selected for thick mesophyll, short leaves and shallow epidermal ridges. The extent of this difference varied between cuts but was greatest at the August harvest. Differences in WSC were less marked although the thick mesophyll selection tended to have more than the original variety under frequent cutting and the short leaf selection less under both cutting regimes. The latter was also higher in nitrogen than Ruanui. The same constituents were measured in the harvest of 3 May in year 2 under infrequent cutting. At that time rankings among the populations were similar to the previous year (not shown).

There were also significant ($P<0.05$) differences between the populations in mineral content (not shown). The thick mesophyll selection had more K and Ca than the original variety, the short leaf line more P, and the shallow ridge selection more Na and Mg (+25%).

There was a highly significant ($P<0.001$) negative correlation ($r = -0.56$) between percentage cellulose (range 15.0-19.5%) and digestibility (78.8-85.4%) at the May cut, with a 1% difference in cellulose associated with 2.5% units of digestibility (not shown).

Growth and winter survival

There were also large and significant differences in growth of the populations (not shown). Under both cutting regimes mean annual CGR of the short leaf selection was only 3.8 g m^{-2} day^{-1}, compared with a range of 8.1-11.2 g m^{-2} day^{-1} in the others. Under infrequent cutting, the two ridging selections were approximately 10% more productive than Ruanui annually but their seasonal patterns of growth were very different. In spring, the steep ridge selection was most productive ($P<0.05$) whereas in late summer and autumn the shallow ridge line grew faster ($P<0.05$). The long leaf type displayed similar productivity to Ruanui. Under frequent

cutting, again the shallow ridge selection was particularly productive in late summer/autumn. Under both regimes the thick mesophyll selection was about 10% less productive than Ruanui. The short leaf selection was significantly ($P<0.05$) less winter hardy than all the other populations, approximately 63% of plants surviving under infrequent and 78% under frequent cutting (not shown).

DISCUSSION

Present results show that significant differences in ryegrass chemical composition, important in animal nutrition, can arise from differences between populations in leaf morphology and anatomy. They also show that in the case of cellulose, in particular, differences can be caused by different anatomical variations. For example relatively low cellulose was apparent in populations selected for shorter leaves for thick mesophyll and for shallow leaf ridging (Tables 2 and 3). The agronomic behaviour of these particular populations was however very different from one another (above). The implication therefore is that while selecting for low cellulose directly can be successful mechanistically (Wilson, 1965) correlated changes in leaf anatomy and morphology can have unpredictable agronomic consequences. In the present experiments, low cellulose with shallow ridging clearly provided, agronomically and nutritionally the most desirable combination of traits. The role of shallow ridging in aiding drought tolerance in ryegrass (Wilson, 1976) provides a further benefit from that characteristic which also seemed to be associated with enhanced magnesium. The latter could well be important in combating hypomagnesaemia in cattle but magnesium levels were generally low in the present experiments so that this would require further examination.

Despite its low cellulose content the present short-leaved selection was clearly undesirable in several respects (low WSC, poor growth, lack of winter hardiness). Although growth of the thick mesophyll population was no better, and sometimes worse, than that of Ruanui its apparent advantages in low cellulose, high WSC and high calcium and potassium levels may make it nutritionally valuable.

Despite the significant ($P<0.001$) negative correlation between cellulose and digestibility each differed by 3-4 units at the same level of the other (not shown). Therefore, although it appears that selection for low cellulose will result in increases in digestibility there also appears to be scope for selecting for cellulose at the same digestibility level. Ulyatt (1969) has shown that relatively small differences in cellulose (3 units) can lead to substantial differences in feed intake (5%) and animal liveweight gain (20%).

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IN VITRO RUMEN SELECTION CRITERIA FOR NUTRITIVE VALUE OF SMOOTH BROMEGRASS (BROMUS INERMIS LEYSS.)

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Since the inception of the small-sample in vitro digestion procedure for analytical research (Tilley and Terry, 1963), many modified techniques have been proposed and utilized for various aspects of forage-animal research. In particular, forage breeders have relied mainly on in vitro procedures to estimate the digestibility (or disappearance, as many authors prefer) of herbage dry matter or organic matter. With the wide acceptance of the detergent system of cell wall analysis (Robertson and Van Soest, 1981) in the U.S., in vitro techniques may be expanded to estimate the digestibility of individual cell wall constituents or the total cell wall. The objectives of this research were to quantify genotypic variation for in vitro digestibility of cell wall constituents and to correlate concentration with digestibility of these constituents in smooth bromegrass (Bromus inermis Leyss.). A secondary objective was to initiate a preliminary investigation of the genotypic variability for the rate of in vitro cell wall digestion and the lag time for initiation of digestion in smooth bromegrass.

Materials and Methods

Eighteen smooth bromegrass clones were selected from the B8 experimental population for divergent in vitro dry matter digestibility (nine were classified as high IVDMD and nine were classified as low IVDMD) (Ehlke and Casler, 1985). The clones were established as spaced plants at Arlington, WI in 1981 and again in 1983 in a field 0.8 km from the first field. Each planting was designed as three randomized complete blocks. Plant spacings were 0.6 m for the 1981 planting and 1.2 m for the 1983 planting.

Both plantings were fertilized with 83 kg K/ha, 14 kg P/ha, and 100 kg N/ha in the spring of the first year after establishment. A random 0.3-kg herbage sample was clipped from each plant in each planting when all plants had just reached the heads-emerged maturity stage in the first year after establishment. Since maturity did not vary among clones, herbage was sampled on a single date within each year. Clipping height was 5-cm. Herbage samples were dried at 55°C in a forced-air dryer. Dried samples were ground in a

hammermill to pass a 1.0-mm screen and reground through a cyclone mill to pass a 1.0-mm screen. The second grinding was done to obtain more a uniform particle size distribution within samples.

Herbage samples were analyzed for IVDMD using the two-stage direct acidification procedure of Marten and Barnes (1980) with 0.25-g duplicate samples. Cell wall constituent (neutral detergent fiber, acid detergent fiber, acid detergent lignin, cellulose, and hemicellulose) concentrations were estimated by the sequential procedures of Robertson and Van Soest (1981). In vitro digestibility of cell wall constituents was estimated by performing similar analyses on residues from duplicate samples from the first stage of the IVDMD procedure (48-hr digestion with rumen microorganisms). In this manner, the in vitro digestibility of cellulose, for example, could be computed from knowledge of the concentrations of total cellulose and indigestible cellulose in each herbage sample.

The same herbage samples were also analyzed for the rate of in vitro cell wall digestion and lag time for initiation of digestion by the procedures of Smith et al. (1971) and Mertens (1977). Briefly, this involved estimating cell wall digestibility on duplicate samples incubated with rumen microorganisms for 0, 6, 12, 24, 48, and 72 hours. Rate and lag were computed, assuming first-order kinetics of digestion, from the log-linear regression of the amount of digestibile cell wall remaining at a specific time vs. incubation time. Estimated rate and lag constants for each herbage sample were then analyzed by traditional statistical methods.

Results and Discussion

Genotypic variability was significant for the concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose, and hemicellulose and for in vitro digestibility of dry matter, NDF, ADF, cellulose, and hemicellulose. Broad sense heritability estimates were 0.60 or above for all characters, except in vitro lignin digestibility (IVLD) (Table 1). The low heritability for IVLD suggested poor repeatability of clonal performance for this character. This was due to extreme variability among replicates and duplicates; because of the low mean value (3.9%), almost half of the observed values of IVLD were negative.

The clone x year interaction (CxY) was significant for concentration and IVD of NDF and ADF and for IVD of cellulose (Table 1). The significance of CxY for NDF and ADF concentration was contrary to a previous study in which replication over years was facilitated by sampling herbage from consecutive years of the same

Table 1. Broad sense heritability estimates and significance levels for the clone x year interaction for concentration and in vitro digestibility of dry matter and cell wall constituents.

	Dry matter	NDF	ADF	Lignin	Cellulose	Hemi-cellulose
			Heritability			
Conc.	-	0.61	0.69	0.87	0.60	0.63
IVD	0.86	0.69	0.65	0.09	0.63	0.69
			Clone x year interaction			
Conc.	-	**	*	ns	ns	ns
IVD	ns	**	**	ns	**	ns

*,**,ns Mean square significant at the 0.05 or 0.01 probability level or nonsignificant.

planting (Reich and Casler, 1985). It is apparent from this comparison that replication over years in the same planting may not provide an accurate picture of CxY interactions. Future evaluation of IVD and concentration of NDF and ADF, as well as IVD of cellulose, should be conducted over at least two years, preferably on different plantings.

Selection for divergent IVDMD was successful in this population (Ehlke and Casler, 1985). Increased IVDMD was associated with decreased NDF, ADF, lignin, and cellulose, but no change in hemicellulose (Table

Table 2. Mean concentration and in vitro digestibility of dry matter and cell wall constituents of high and low IVDMD clonal groups.

Clonal group	Dry matter	NDF	ADF	Lignin	Cell.	Hemi.
			%			
			Concentration			
Low	-	64.0**	36.6**	3.6**	33.0**	27.4
High	-	62.8	35.4	3.1	32.3	27.5
			In vitro digestibility			
Low	67.5	47.2	45.1	4.5	49.3	49.9
High	71.8**	51.2**	48.8**	3.3	53.0**	54.1**

** Difference between high and low groups significant at the 0.01 probability level.

2). Lignin was the constituent most closely associated with IVDMD ($r = -0.94$, $P < 0.01$). A 1-percentage unit increase in lignin was associated with a decrease of 7.0 percentage units of IVDMD. Lignin was the only cell wall constituent found to differ between the polycross progenies of the high and low IVDMD clonal groups (Ehlke and Casler, 1984, unpublished data). The results of both studies were similar for lignin expressed as a percentage of dry matter or the cell wall.

The divergence among clonal groups for IVDMD was associated with changes in IVD of each constituent except lignin (Table 2). Differences between clonal groups for IVD of these constituents were remarkably similar, ranging from 3.7 to 4.3%. With the exception of lignin, phenotypic correlations among IVD of cell wall constituents were all 0.87 or above. Although the IVD of each cell wall constituent except lignin correlated well with IVDMD ($r = 0.72$ to 0.79), none was highly correlated with concentration of the same constituent ($r = -0.52$ to 0.22). The only significant correlation between concentration and IVD of a constituent was for ADF ($r = -0.52$, $P < 0.05$). Thus, the concentration of each cell wall constituent seems to have little influence on its IVD. However, lignin concentration influences the IVD of NDF, ADF, cellulose, and hemicellulose ($r = -0.60$ to -0.73). Based on regressions over all clones, a 1-percentage unit increase in lignin would be expected to decrease the IVD of NDF by 4.7%, ADF by 5.0%, cellulose by 4.9%, and hemicellulose by 4.0%. Although the regression coefficients for cellulose and hemicellulose were not significantly different, the larger observed effect of lignin on IVCD than on IVHD is contrary to biochemical expectations, which hold that lignin and hemicellulose are closely-linked, while lignin and cellulose are not chemically bound with each other (Morrison, 1979). The apparent contradiction between these studies may be due to species effects and the crudeness of the detergent system in estimating cell wall constituents.

Genotypic variability for rate and lag of in vitro cell wall digestion could not be detected by analysis of variance at the 0.10 significance level. Because clone x year interactions for rate and lag were also nonsignificant and the range among clone means was considered large, the lack of significant variation was attributed to extreme laboratory and field variability. The range among clone means for rate of digestion was 5.2 to 8.2%/hr, a difference about 1.7 times greater than the 10% L.S.D. This led to a difference, after 24hr of incubation, of 19 vs. 34% of the potentially-digestible cell wall yet undigested. The clone with the faster rate reached 70% digestion after 18.7 hr, compared to the slow clone which required 26.7 hr. Based on these dramatic potential effects, breeding for a faster rate of digestion in smooth bromegrass appears

to be a desirable goal. Future emphasis should be placed on technical refinements to reduce error variability.

The range among clone means for lag time was also large, exceeding the 10% L.S.D. by about 1.7 times. However, two clones with extreme lag times and similar rates were digested to a similar extent after 24hr, and reached 70% digestion at nearly the same time. Thus, breeding for shorter lag times for initiation of cell wall digestion does not appear to be a viable method to improve smooth bromegrass nutritive value.

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INVESTIGATIONS OF QUALITY PARAMETERS IN LOLIUM PERENNE BY MEANS OF NIR

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ABSTRACT

Near infrared reflectance (NIR) spectroscopy has been used to investigate quality parameters in L. perenne. Calibration of a Technicon 4oo R was done for crude protein (CP), crude fibre (CF) and in vitro digestibility (IVDMD). Standard errors of calibration (SEC) were o.25, o.42 and o.79, whereas those for predicted samples increased to o.38, 2.34 and 3.o8, respectively. A set of 2o unselected PX-progenies were grown in a field experiment for 3 years. From every 1st cut samples were taken from each of the 3 reps. NIR-analysis was done in duplicate. Sampling error accounted only for a small portion of the total variance. Genotypic variance in the 3-year Anova were 35, 24 and 31 % of the phenotypic variance, thus indicating possible selection improvement.

INTRODUCTION

Among plant breeders it is widely accepted, that the most important breeding objectives are total production and seasonal spread, persistency and resistance to diseases and environmental stress. The nutritive value, though regarded important too, is mostly neglected. This is mainly because quality analysis, at least the standard methods, are very costly and laborious. Furthermore, plant breeders are not sure whether such expenditures will pay, especially, having in mind, that breeding progress in forage crops can be easily ruined by mismanagement of the farmer.

In recent years forage quality received increased attention. First, because of the introduction of new, fast and cheap

screening techniques, and second, because of a tremendous change in the economic conditions for milkproducing farms.

The role of plant breeding in improving the nutritive value of forages has been summarized by SHENK (1977). A description about calibration and the use of NIR in forage analysis was published by SHENK et al. (1981).

MATERIAL AND METHODS

A TECHNICON 4oo R with 19 filters, covering the wave-length from λ 1445 to 2348 nm and interfaced to a HP 9815 A minicomputer was establish in our lab in 1981. Calibration was done for crude protein (CP), crude fibre (CF) and in vitro digestibility (IVDMD). (In the meantime ADF could be established, too.) The calibration samples had been analysed using standard chemical analysis like Kjeldahl (CP) and Weende-method (CF). In vitro digestibility was estimated according to MENKE et al. (1979). A second set of L. perenne samples were used for prediction. While these two sets of samples were dried at 7o ^{o}C, samples from the experiment under investigation were dried at 1o5 ^{o}C, as for dry matter determination. According to the results of WINCH & MAJOR (1981), a very homogenous particle size is of great importance for the accuracy of NIR-measurements. Therefore, after coarse grinding with a Brabender mill, fine grinding was done using a Cyclotec mill with o,5 mm mash size.

A set of 2o entries (16 PX-progenies and 4 checks) were sown in a field trial in 198o with plot size of 5 sqm and 3 reps. Quality investigation was done from first cut samples in the three following years (1981-1983). Harvest time was at early heading stage. Samples were taken from each plot and NIR-measurements were carried out on 2 subsamples. Statistical analysis with sampling was carried out according to STEEL & TORRIE (198o).

RESULTS & DISCUSSION

1. The NIR-techniques

In Table 1 the results of calibration and prediction for the three quality parameters are given.

Table 1:

			Calibration			Prediction	
	λ	N	Range	SEC	R^2	SEP	R^2
Protein	7	94	4.4 - 16.5	o.25	o.98	o.38	o.94
Fibre	1o	134	17.7 - 33.7	o.82	o.93	2.34	o.83
IVDMD	8	69	62.9 - 79.o	o.79	o.96	3.o8	o.9o

For IVDMD the predicted values are presented as a scatter diagram (see Fig. 1). As was found, by WINCH & MAJOR (loc. cit.) too, CP can be predicted with higher accuracy than the other traits. Standard errors and R^2 of prediction are similar to the data reported by SHENK et al. (loc. cit.). Because of cheap and rapid determination of several quality parameters in one time, NIR-technique could serve as a valuable tool in screening breeding material.

2. The Experiment

Mean values of the three year average ranged from 14.6 to 18.3 % (CP), 2o.8 to 25.7 % (CF), and 64.2 to 7o.7 % (IVDMD), respectively. Least significant differences were 1.o5, 1.84 and 1.49. In the ANOVA (see Table 2) only for CF and IVDMD in 1983, mean squares were not significant and for the former, negative variance components occured and thus were set to zero. Variance components (VC) were transformed into % of the total variance. Sampling errors in the lab are of minor importance and may be neglected, whereas field plot errors are of greater magnitude, indicating that sampling in the field has to be done as carefull as possible. Genotypic variance, expressed

as VC % are highest in the first harvest year. Absolute values were o,97 (CP), 4,76 (CF) and 9,37 (IVDMD). The decrease in variability, especially for CF and IVDMD in the second year may be due to a somewhat delayed harvest time. In crude protein VC-values decline from 1981 to 1983 from o.97 to o.51 (56 to 27 VC %), though in 1981 and 1983 havest time was at the same maturity stage (d.m. %). Further investigations are necessary to proof whether this is due to ageing effects or only a single result.

In Table 3 the combined ANOVA is given for the three quality components. All mean squares, except the G x Y-interaction for CP, are significant. Similar findings were made by SOH et al. (1984) in Tall fescue and by SHENK & WESTERHAUS (1982) in Orchardgrass. Genotypic variance accounted for 35, 24 and 31 % of the phenotypic one. Estimates of broad sense heritabilities were o.79, o.57 and o.64 for CP, CF and IVDMD, respectively.
Besides information of the quality parameters per se, their interrelationship is of great interest to the plant breeder. Instead of calculating correlation coefficients, scatter diagrams are presented in Fig. 2, showing the relationship between CP (CF) and IVDMD. The entries with highest D-values are those, having low crude fibre and high protein content. Though IVDMD is probably the most important quality trait, NIR-technique provides additional information, without additional costs, which could facilitate quality selection.

VAN BOGAERT (1977), working with Timothy, has demonstrated, that selection improvement for IVDMD is possible. But, because of the well known negative correlation between yield and quality, the high quality selections had lower yields.

In Lolium perenne, however, having already a reasonable good quality, yield improvement, without loosing quality, still seems to be more appropriate.

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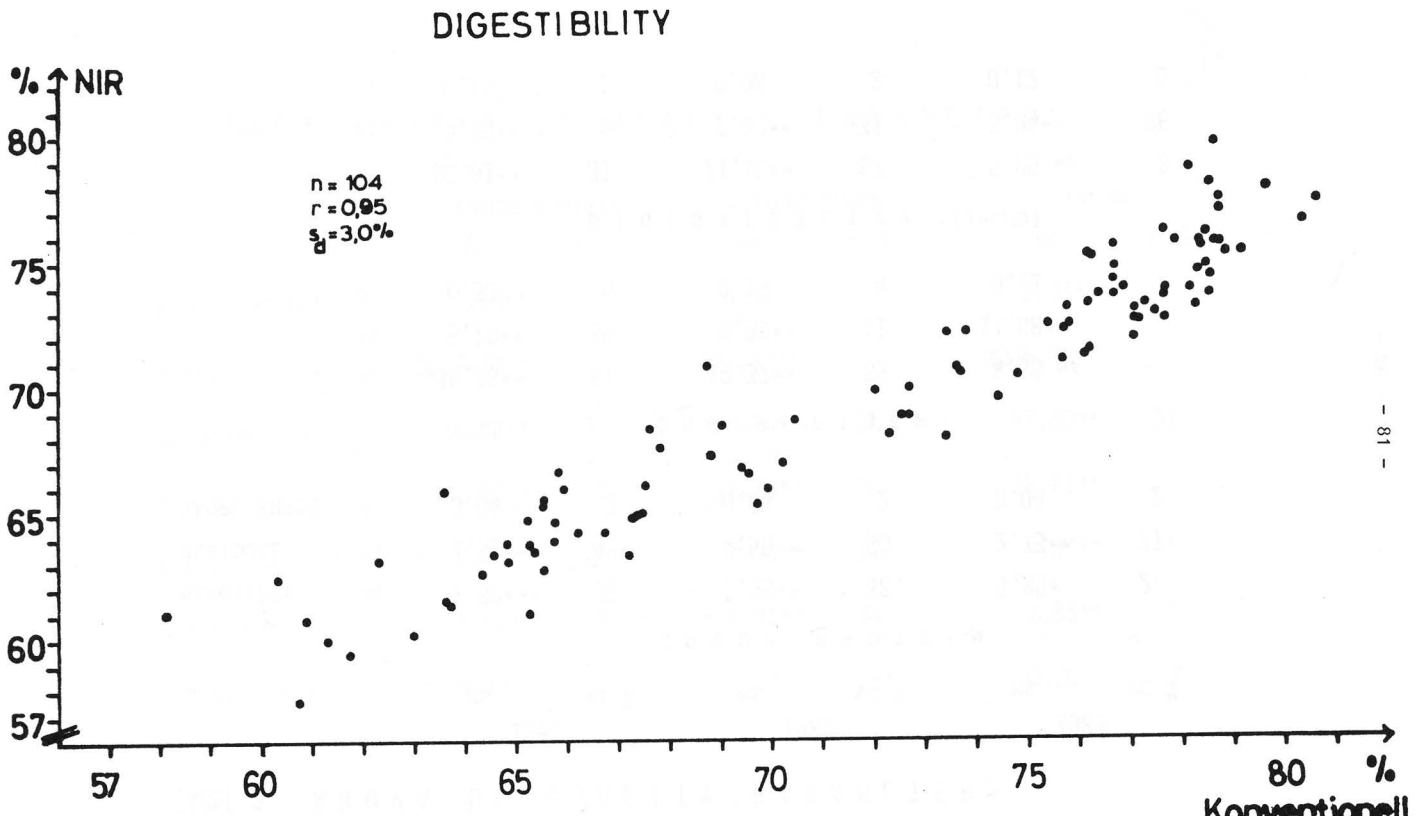

DIGESTIBILITY
% NIR
n = 104
r = 0,95
s_d = 3,0%
80
75
70
65
60
57
57
60
65
70
75
80
%
Konventionell

FIG. 1

TABLE 2: ANOVA OF QUALITY PARAMETERS

		1981		1982		1983	
		MS	VC %	MS	VC %	MS	VC %
				CRUDE PROTEIN			
GENOTYPES	A)	7.37***	56	7.36**	38	5.85*	27
RESIDUAL	B)	1.51**	42	2.56**	60	2.73**	71
SAMPL.ERROR	C)	0.04	2	0.04	2	0.04	2
				CRUDE FIBER			
	A)	34.36**	61	19.37**	85	8.40 NS	-
	B)	5.79**	35	0.93**	11	11.68**	-
	C)	0.30	4	0.13	4	0.11	
				DIGESTIBILITY (IVDMD)			
	A)	60.61**	81	21.36**	67	5.05 NS	8
	B)	4.36**	18	2.90**	31	3.94**	86
	C)	0.15	1	0.08	2	0.12	6

TABLE 3: ANOVA OF QUALITY PARAMETERS

	CRUDE PROTEIN		CRUDE FIBER		IVDMD	
	MS	VC %	MS	VC %	MS	VC %
REPS (R)	3.54**		5.96**		2.93**	
YEARS (Y)	1023.73**		181.72**		12236.94**	
GENOTYPES (G)	16.66**	35	36.74**	24	47.60**	31
G x Y	1.96 NS	0	12.70**	20	19.71**	37
G x R	2.40**	14	7.43**	17	4.87**	10
G x R x Y	1.97**	34	4.91**	34	2.83**	15
SAMPL. ERROR	0.39	17	0.37	5	0.56	7

3 - Y E A R - A V E R A G E

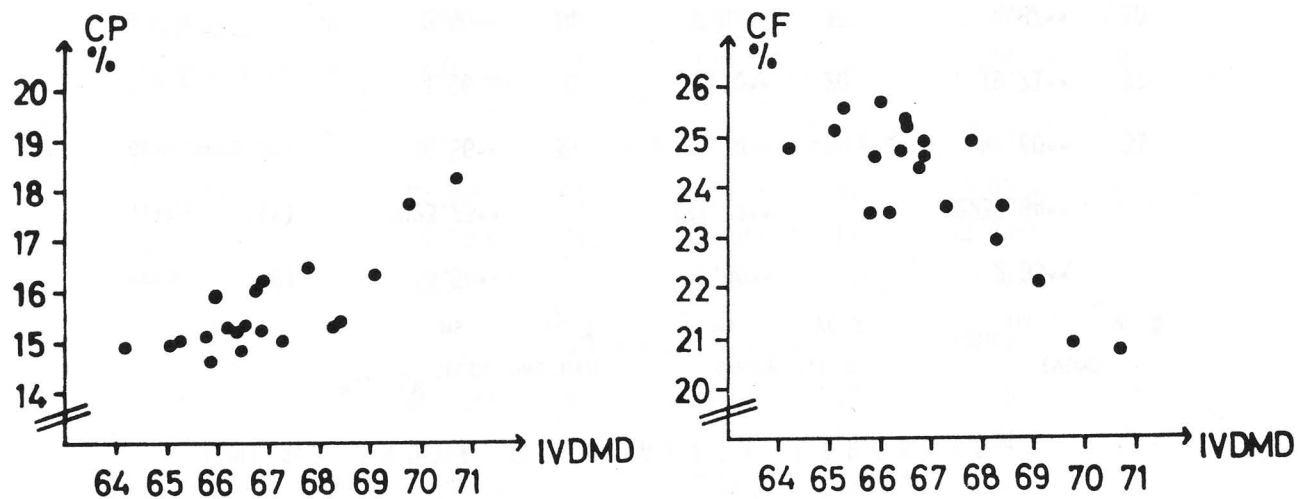


FIG. 2

Creating and Utilizing Variation in Grasses and Legumes via Intra- and Interspecific Hybridization

Norman L. Taylor, University of Kentucky, USA

The following is a summary of techniques and breeding methods used by University of Kentucky personnel in the breeding of forage grasses and legumes. Primary emphasis is given to the forage legumes, primarily Trifolium. Some of these results were given in greater detail by Taylor and Smith (1979), Taylor (1980, 1985). No attempt will be made to cover research on breeding for disease resistance or polyploidization (see R. R. Smith, this publication).

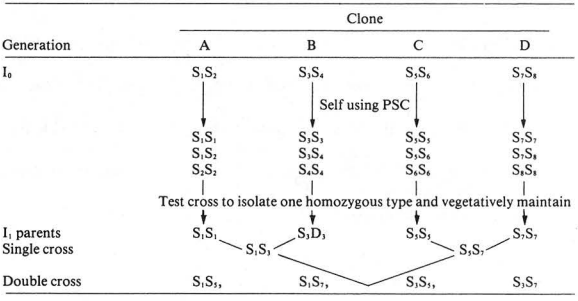
Inbreeding

Self-incompatible species may be inbred by application of heat to inflorescences. In red clover (Trifolium pratense L.) stems with unopened heads may be inserted in a heated chamber (38 to 40°C) where upon flowering, (in about 3 days) the heads are rubbed or flowers are selfed with a toothpick. Plants are then allowed to mature seeds at ambient greenhouse temperatures. In the clovers, selfing is usually prevented by the gametophytic S-allele system, but the application of heat overcomes the self incompatibility mechanism. One of the the primary reasons for selfing red, white or alsike clover is to allow the production of homogygous S-allele genotypes for single and doublecross hybrids.

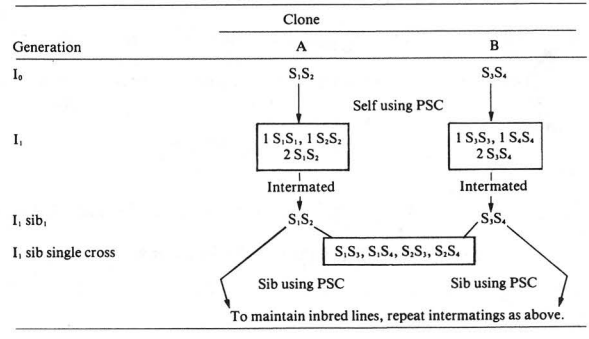
The investigation reported in this paper (No. 85-3-212) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

Production of Single and Double Cross Hybrids

After homozygous S-allele genotypes are identified by backcrossing to the non-inbred parental clone, the procedure for producing double cross hybrid red clover proceeds according to the following diagram (Townsend and Taylor, 1985):



An alternative method is to produce singlecrosses. In this case, selfs are sibbed, producing a heterozygous S-allele population (e.g. S_1S_2) which may be crossed with another population (e.g. S_3S_4). The resultant single cross is used to produce forage. Inbred lines are maintained by alternate selfing and sibbing. Contamination and changes in S-allele specificity may be a problem with seed maintenance of inbred lines (Taylor, 1982). See diagram (Townsend and Taylor, 1985).



In both methods, crosses may be made in field cages using bees (Apis melfifera L. or Bombus spp.). Three double cross hybrids of red clover have been tested but none were superior to the cultivar 'Kenstar' and were not released. The isolation of inbred lines with high combining ability requires extensive testing which may be beyond the scope of most clover breeding programs (Taylor and Anderson, 1980).

Interspecific hybridization

When variation is insufficient within a species, techniques for producing interspecific and intergeneric variation are available. In red clover, intraspecific variation for longevity (persistence) was inadequate and interspecific hybridization was begun at Kentucky in the early 1960's (Taylor et al., 1963). The first hybrid produced, T. pratense (2n=2x=14) x T. diffusum (2n=2x=16), was sterile as a diploid but fertile as an amphidiploid. The hybrid was less persistent than red clover. The second hybrid, T. pratense (2n=4x=28) x T. pallidum (2n=2x=16) was sterile and the amphiploid was never produced (Armstrong and Cleveland, 1970). These results and the difficulty of producing hybrids of red clover with perennial Trifolium species seem to indicate that red clover is more closely related to the annual than to the perennial species.

Techniques for overcoming interspecific barriers that have been successful in other species were investigated in Trifolium to aid in hybridization of red clover with the perennial species. Unsuccessful treatments included manipulation of mentor pollen treatments, ploidy levels, and compatibility and male sterility systems. These techniques were designed to affect prefertilization barriers, and the lack of effect may indicate that postfertilization barriers in Trifolium are of greater importance (Taylor, et al., 1980).

Hybridization among the perennial Trifolium species was also attempted

in the hope that bridge crosses might be successful. These hybrids included I. sarosiense (2n=6x=48) x I. alpestre (2n=4x=32), I. alpestre (2n=2x=16) x I. heldreichianum (2n=2x=16), I. alpestre (2n=2x=16) x I. rubens (2n=2x=16) and I medium (2n=9x=72) x I. sarosiense. All these hybrids were partially fertile but none could be crossed with I. pratense (Quesenberry and Taylor, 1976, 1977, 1978).

Interspecific and intergenetic hybridization has been successful in improving forage quality of tall fescue (Festuca arundinicea Schreb. (2n=6x=42)). Hybrids that have been produced include F. arundinacea x Lolium multiflorum Lam (2n=2x=14) and F. arundinacea x F. gigantea (2n=6x=42). Isozymes used for identifying parents and hybrids produced consistent patterns that were under nuclear control (Eizenga and Buckner, 1986a). This research has culminated in the release of several cultivars of which 'Johnstone' is the most recent. It is characterized by high quality coupled with absence of the endophyte Acremonium that has been implicated in fescue toxicity. The F. arundinecea X F. gigantea hybrid is still being evaluated (Eizenga and Buckner, 1986b).

Because conventional methods were unsuccessful, emphasis was placed on embryo rescue as an aid to hybridization in Trifolium. Early investigations showed that some plants of I. sarosiense when used in crosses with I. pratense would produce inviable shriveled seeds (Taylor, et al., 1981). Embryos were dissected from seeds about 14 days after pollination and placed on a nutrient medium. These embryos produced calli that regenerated sterile hybrid plants. The hybrid plants, intermediate between the parents in most characteristics, were doubled by the use of colchicine but were still sterile. The presence of rhizomes seemed to be dominant over absence and plants spread and lived under field conditions for two seasons (Collins, et al., 1981, Phillips., et al. 1982). I. pratense (2n=4x=28) has recently been

hybridized with I. medium (2n=10x=80) to produce a sterile hybrid (Merker, 1984).

In a different section of the genus Trifolium, the hybrid of I. ambiguum (2n=2x=32) x I. repens L. (2n=2x=32) was produced with the aid of embryo culture. In this hybrid, nurse endosperms facilitated the growth of hybrid embryos prior to dissection and transfer to nutrient media (Williams, 1978; Williams, et al. 1982; Williams and De Lautour, 1980). This hybrid has about 20% pollen fertility, and is intermediate between the parents. It has persisted for at least two years under field conditions in New Zealand. Backcrosses to I. repens and F_2 populations have been produced by Williams et al. (1982) and by Taylor during a sabbatical in New Zealand. These populations are being further investigated in Kentucky and at the Mississippi Agricultural Experiment Station in cooperation with USDA,ARS.

Protoplast Fusion

Another possibility of overcoming interspecific hybridization barriers is protoplast fusion. The specific objectives of this research at the University of Kentucky are: to develop the procedures for isolating, culturing, and regenerating entire plants from protoplasts of I. pratense and I. rubens L. (2n=2x=16) and to obtain a somatic hybrid between these two species. T. rubens was chosen because of its relatively low chromosome number, its perenniality, and its excellent regeneration capability. Sexual hybridization was not successful either by conventional means or by embryo rescue. Protoplasts were isolated from suspension-derived cells and mesophyll-derived cells. The latter possess chlorolasts which aid in identifying heterokaryons after fusion by polyethylene glycol. To date, whole plants have been regenerated from parental cultures and from homokaryons. Heterokaryons have been isolated and grown in microdrops to a 30 cell stage, (Grosser, 1984) or on feeder layers to a microcalli stage

(Myers, unpublished data). In vitro approaches to interspecific hybridization have been summarized by Collins et al. (1984).

Transformation of petioles is also being attempted to aid in screening for heterokaryons using cells transformed by the vector Agrobacterium tumefaciens. The disarmed A. tumefaciens vector is being used to introduce kanamycin resistance into I. pratense. Callus has now been produced from cut ends of I. pratense petioles growing in 300 g/ml kanamycin. As little as 50 g/ml kanamycin normally inhibits I. pratense callus growth.

Somaclonal variation

Whole plants of I. pratense have been regenerated from suspension cultures with low phosphorus levels. Many such plants are abnormal in appearance and sterile, and do not exhibit low phosphorus tolerance. Other plants are normal and these and other regenerates are being evaluated for degree and type of somaclonal variation. All whole plants have been regenerated from a single clone of I. pratense (B5C9) that exhibits greater regeneration capability than most genotypes.

Somaclonal variation also is being investigated in Festuca. Variation has been exhibited in inflorescence and leaf morphology (Eizenga, 1985).

Trisomics

Trisomics have now been produced in red clover from 4X-2X crosses that produce a relatively high frequency of triploids (Wiseman and Taylor, submitted for publication). Crosses of these triploids with diploids have produced to this date about 35 trisomics. Efforts are under way to isolate the 7 primary trisomics of the red clover genome, and to begin gene linkage investigations using genes recessive for the following characteristics: white flower, white seed, no leaf mark, dwarf, rudimentary corolla, and small pollen. More sources of single recessive gene markers are needed. Success in this program depends upon the ability to identify individual chromosomes

of the genome and to associate each additional chromosome of the trisomics with morphological, physiological or biochemical phenotypes.

Conclusions

Many techniques are now available to the forage breeder and those from the new biotechnology are being introduced from animal and other plant systems into forage plants. To date, very few if any of the new techniques have led to new cultivars. Because many characters of agronomic value in the forages depend upon quantitative gene action it is expected that progress will be slow. Conventional plant breeding techniques will continue to be required in conjunction with new techniques, and it is expected that conventional techniques will predominate in forage breeding in the near future.

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Breeding for Pest Resistance in Red Clover

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Losses caused by plant pathogens and insects have been and remain important constraints to increasing forage production and persistence. The most consistent and stable approach to overcoming these losses has been the development of resistant plant germplasm. Success in obtaining resistant germplasm is dependent upon available genetic variation and optimum genetic expression in the host, little or no genetic variation in the pest, and effective screening procedures.

Pest resistance in red clover (Trifolium pratense L.) is generally controlled by only a few factors (Taylor and Smith, 1979). In most existing germplasm sources of resistance occur at a low frequency. Consequently, one of the most effective breeding procedures used to develop pest resistant germplasm has been phenotypic recurrent selection (PRS). Resistant phenotypes are identified, usually as a result of selection under artificially-produced conditions, and intercrossed to produce a new generation of germplasm for further selection.

Phenotypic recurrent selection has been most effective in developing pest resistant red clover germplasm in the cooperative USDA-University of Wisconsin program. Inoculations and selections are conducted in the laboratory or glasshouse during the winter months and selected plants are intercrossed under isolation in the field to produce the subsequent generation. Those pests receiving primary attention in the program are the pathogens inciting northern

anthracnose (NA)(causal agent-Kabatiella caulivora (Kirch.) Karak.), target spot (TS)(causal agent-Stemphylium sarciniforme (Cav.) Wiltshire), powdery mildew (PM)(causal agent - Erysiphe polygoni DC ex St. Amans), and root rot (causal agent-Fusarium roseum Lk. emend. Synd. & Hans.) and the clover root borer insect (Hylastinus obscurus Marsh).

Northern anthracnose, target spot, and powdery mildew

The basic procedures and symptom expression scales (Disease Severity Index-DSI) used to develop germplasm resistant to the pathogens causing the foliar diseases of northern anthracnose, target spot and powdery mildew were documented respectively by Smith and Maxwell (1973), Murray, et al. (1976), and Hanson (1966). Artificial epiphytotics are created in the glasshouse and resistant phenotypes are selected for subsequent crossing. The response to annual selection for resistance to these diseases is given in Table 1. Progress per cycle of selection appears to be about the same for both NA and TS, however, selection for resistance to PM was most effective in the first cycle. As a result of continued selection, germplasm has been released with resistance to these diseases (Smith, et al., 1973; Smith and Maxwell, 1980).

Table 1. Response to annual selection in red clover for resistance to northern anthracnose (NA), target spot (TS), and powdery mildew (PM).

Cycle	NA	TS	PM
Syn 0	3.0*	4.6*	67**
Syn I	3.2	4.5	38
Syn II	1.8	4.1	36
Syn III	1.7	3.9	22
Syn IV	1.3	3.5	37
LSD (5%)	0.3	0.3	3
change/cycle	0.4	0.3	8

* Disease Severity Index: 1 = resistant, 5 = susceptible
** percent resistant

Root rot

The mature plant cut-taproot procedure of inoculation as described by Leath and Kendall (1978) was used to identify germplasm resistant to root rot caused by Fusarium roseum. Plants are grown in 10-cm clay pots. At four-to-six months of age the plants are lifted from the pots with the root ball intact. The lower portion of the ball is removed by cutting horizontally through the sod and taproot about 3-5 cm below the crown. The upper portion of the exposed taproot (approximately 1 cm in diameter) is then inoculated by placing a fungal inoculum strip (6x6 mm polyester cloth strips containing fungal mycelia) against the cut end of the taproot. The lower portion of the sod ball is placed against the strip and the complete ball is repotted. After a 21-day inoculation period in the glasshouse, the sod ball is again removed and separated. The taproot is cut longitudinally above the inoculation site to the crown. The degree of infection is determined by dividing the length (mm) of vascular browning by the total length of the taproot measured from the inoculum strip to the crown of the plant. Plants expressing less than 30% vascular browning are reinoculated by removing the callus which has developed on the inoculated end of the taproot, applying fresh inoculum and repotting. These plants are then re-examined 21 days later.

Twenty-five plants expressing the least amount of vascular browning (<20%) were selected and intercrossed (FUS 1). A second set of 25 plants (<30% vascular browning) were also selected and intercrossed (FUS 2). After only one cycle of selection these two populations were superior to the recommended cultivar, Arlington, for forage yield and percent stand at the end of the third year (Table 2). Subsequent selection was imposed on FUS 1 and FUS 2 and selected phenotypes are currently being intercrossed.

Table 2. Field performance of two populations of red clover after one cycle of selection for resistance to root rot.

Population	kg/ha+	% Arlington	% Third year stand
FUS 1 (syn2)	2797*	110	88
FUS 2 (syn2)	2953*	116	80
Arlington	2550	100	65

+ Two year total dry weight
* Significantly greater than Arlington at 5% level.

Clover root borer

Both the clover root borer and the root curculio (Sitona hispidula F.) are root feeding insects which cause considerable damage to red clover in the midwestern region of the U.S. A program was initiated in 1973 at the University of Wisconsin to develop germplasm resistant to the clover root borer. An individual preference test similar to that described by Leath and Byers (1973) was used to identify resistant (not preferred) red clover plants. All adult borers were collected in the spring or fall from field-grown Arlington red clover plants using the Berlese-funnel technique. Borers were held (not more than 6 weeks) on red clover root tissue at 4°C until used. A minimum of five borers was used per test but the actual number was dependent upon the number of plants evaluated per test.

A standard test would evaluate 42 plants and seven blanks in a 7 x 7 simple lattice design. Each block of the lattice consisted of one 15-cm-diameter petri dish fitted with seven 8 x 55 mm sterile filter paper strips (wicks) arranged to radiate out from near the center of the dish to the periphery. The strips were 5 mm apart at the center of the dish. Six test root pieces (6 mm in diameter by 4 mm thick) were placed on the outer edge of six of the

filter strips. Three to four ml of sterile, distilled water were placed on each root piece and the blank strip. The dish was then kept at 20°C for 1 hr to allow filtration through the root piece and down the strip to the center of the dish. After 1 hr five root borers were placed in the center of each dish and a smaller (5 cm) petri dish inverted over the borers and encompassing the interior 5 mm of each filter strip. The borers are then free to select respective strips at their preference. The dishes with borers are then placed in the dark at 20°C for three hours. At the end of the three-hour period the number of borers on each filter strip was determined and the borers moved back to the center of the dish. Another measure of borer response was to determine the degree of "chewing" observed on each strip based on a scale of 1 = no chewing to 10 = filter strip destroyed. After the first reading the test was repeated using the same root pieces and borers.

Initially, 250 plants from the cultivar Arlington were screened using the procedure just described. Six tests were conducted each with 42 plants. The three plants from each test exhibiting the lowest borer preference were selected and intercrossed. Also, the three plants from each test exhibiting the greatest borer preference were intercrossed. Subsequently, within each population, seed was composited from each selected plant and the composite polycross progeny evaluated and reselected on the same basis as the initial cycle. Then second cycle selected (nonpreferred and preferred) populations were then evaluated and reselected. Field evaluations are currently being conducted on the second cycle germplasm. The response to selection for number of borers feeding and chewing score after two cycles are presented in Table 3. The procedure has been effective in separating the two populations relative to preference by the root borer. The initial population, Arlington,

is intermediate to the two selected populations for both mean number of borers and chewing score. Preliminary field data would suggest that the nonpreference population will be higher yielding and more persistent than either the preference population or Arlington.

Table 3. Response of red clover germplasm after two cycles of selection for preference or nonpreference to the root borer.

Population†	No borers feeding††	chewing score§
Nonpreference	0.9*	2.0
Preference	2.5	4.7
Arlington	1.6	3.6

† Mean of 100 plants in selected populations and 20 plants in Arlington.
†† Mean number of borers out of 5 maximum.
§ 1 = no chewing, 10 = filter strip destroyed
* Significant at the 5% level.

Progeny test vs. phenotypic recurrent selection

While phenotypic recurrent selection has been effective in selecting for pest resistance, progress may be slow depending upon the number of genes controlling resistance and the effectiveness of the screening procedures. Selection is based on the plant phenotype thus allowing susceptible alleles to be retained in the population when resistance is controlled by a series of dominant factors. An alternative procedure would be to identify resistant parent genotypes using a form of progeny testing.

In order to test the effectiveness of these two procedures on selection for resistance to northern anthracnose in red clover, 60 resistant phenotypes from 1000 were selected. These 60 plants were intercrossed and equal amounts of seed from each were composited to represent the phenotypic recurrent selection procedure, and were also crossed to a susceptible tester clone. Test cross

and polycross progeny from each of the 60 clones were evaluated for their reaction to northern anthracnose. Two sets of 15 original resistant plants were selected based on the best polycross or test cross progeny performance. Each set of 15 selected plants were intercrossed to produce two synthetic populations. One population based on test cross progeny performance and one based on polycross progeny performance. These two populations together with the original population and the 60 clone PRS composite were evaluated for their reaction to northern anthracnose (Table 4).

Table 4. Response to selection for resistance to northern anthracnose of red clover synthetic populations derived from progeny testing and PRS.

Population	Mean Disease Severity Index*
Base population	2.81 c
Composite of 60 PX (PRS)	2.51 b
Intercross of Best 15 TC clones	2.29 a
Intercross of Best 15 PX clones	2.25 a

* 1 = Resistant, 5 = Susceptible; means with same letter not significant at 5%.

The level of resistance was significantly improved with the PRS procedure and further improvement was realized when the genotypes of the phenotypically selected plants were further elucidated as the result of test cross or polycross progeny performance. The latter procedure is more time consuming and costly, and therefore, perhaps not warranted when considering the progress achieved with the simpler and less costly PRS procedure. In addition, with the PRS procedure parent clones do not have to be maintained -- a difficult task in red clover.

Effect of annual selection procedures on perenniality in red clover

Phenotypic recurrent selection as applied on an annual basis has been effective in developing red clover germplasm resistant to several important diseases (Table 1). Our concern, however, was what effect, if any, did this annual selection pressure have on the agronomic performance and perenniality of the species. To address this question a field experiment was conducted using the germplasm described in Table 1. Plants were started in the glasshouse in January, 1977 and transplanted to the field in 50 cm rows with 25 cm between plants in May, 1977. Fifteen plants representing each cycle, the base cycle, and Arlington were included in each of eight replications of a randomized complete block design. Fifteen ramets of each of three clones were included in each replication to estimate the environmental variance, thus, allowing for the calculation of a genetic variance component for each cycle. Various characteristics were measured throughout the 1977, 1978, and 1979 growing seasons.

For brevity only second harvest yield in August, 1978, and spring vigor and persistence in 1979 are presented in Table 5. The annual selection procedure did not significantly alter the cycle means for yield, vigor, or persistence. It would appear that significant genetic variation existed within the cycles for yield and vigor when compared to Arlington. Even though the level of persistence was similar to Arlington after two growing seasons, it would seem appropriate to evaluate the selected population for agronomic performance and perenniality after several cycles of annual selection. This is the current procedure employed in our program in Wisconsin.

Table 5. Mean (x), standard deviation (s), and genetic variance (σ^2g) for yield (g/plant), spring vigor, and persistence for four cycles of PRS in red clover.

Cycle	Yield			Vigor(5-79)†			Persistence††
	$\bar{x}$	s	σ^2g	$\bar{x}$	s	σ^2g	(%) (5-79)
Cycle 0	123	83	4597	2.0	1.07	0.97	51
Cycle I	128	65	2197	2.0	0.94	1.24	54
Cycle II	152	75	3570	2.4	1.19	1.14	57
Cycle III	147	73	2875	2.1	1.06	0.72	61
Cycle IV	131	76	3764	2.2	1.42	1.68	58
Arlington	95	21	424	2.5	1.48	1.50	48
LSD (5%) or χ^2	ns	ns	*	ns	ns	*	ns

† 1 = most vigorous.
†† mean percent plants surviving.
ns = not significant, * = significant at 5% between cycles.

Response of sexually derived tetraploid germplasm to northern anthracnose

Tetraploid (4x) forms of red clover have been successfully produced sexually using 2n gametes in unilateral (2x-4x) crosses (Broda and Smith, 1980) or bilateral (2x-2x) crosses (Parrott, et al., 1985) in our program in Wisconsin. Evidence from numerous sources (Frandsen, 1945; Valle, 1959; Vested, 1960) have indicated that, without selection, the level of disease resistance in chemically produced tetraploids is improved over their counterpart diploids (2x). Germplasm developed in our program using 2n gametes would allow us to test this concept in response to the northern anthracnose disease.

The diploid cultivar Arlington is resistant to northern anthracnose and the diploid cultivar Kenstar is susceptible. These two cultivars were crossed and the subsequent progeny were intercrossed to produce a F_2 population

segregating for resistance at the diploid level. A tetraploid form of Kenstar, Kenstar 4x, (derived via nitrous oxide treatment) has been developed by N. L. Taylor, Lexington, Kentucky, and was used as the male parent in 2x-4x crosses with Arlington as the female parent providing 2n gametes. Tetraploid progeny from this latter cross were intercrossed to produce an F_2 tetraploid population segregating for resistance to northern anthracnose. Theoretically, the same gene compliments should be present in both the diploid and tetraploid F_2 populations.

These two F_2 populations along with Arlington and diploid and tetraploid Kenstar were evaluated for forage yield and their reaction to northern anthracnose (Table 6). Without selection, the tetraploid F_2 population was more resistant to northern anthracnose than the diploid F_2 (DSI) 2.98 vs 3.82). The tetraploid F_2 was similar to Arlington in disease reaction and forage yield.

Table 6. Forage yield and response to northern anthracnose of F_2 sexually derived diploid and tetraploid populations of red clover.

Population	Ploidy	Mean DSI†	Mean yield§
Kenstar	2x	4.40c††	152
(Arl. x Ken.)F_2	2x	3.82b	175
Kenstar	4x	3.68b	158
Arlington	2x	2.98a	181
(Arl. x Ken.)F_2	4x	2.65a	173

† DSI: 1 = Resistant, 5 = Susceptible.
†† Means with different letters are significant at 5% level.
§ Mean green weight (grams) per plant.

The nitrous oxide derived tetraploid Kenstar was more resistant than the diploid Kenstar but very similar in yield. Vestad (1960) reported a similar response favoring tetraploid red clover families when tested for their

reaction to clover rot (causal agent - Sclerotinia trifoliorum Erikss.). Such an improvement in resistance without selection in the tetraploids might be due to an increased frequency of dominant alleles, an increased frequency of higher order interactions between alleles, or both. Specific studies need to be designed using genotypes with know reaction to northern anthracnose in order to elucidate the cause(s) of this phenomenon.

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REPRODUCTIVE SYSTEMS AND BREEDING METHODS IN FORAGE GRASSES

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Amongst the various forage crop species utilized in temperate European agriculture outbreeding is the dominant mode of reproduction. Needless to say this has strongly influenced the choice of breeding method and system of variety construction. The synthetic, based upon a number of superior selected individuals, has predominated as the main system of variety construction being used in virtually all commercially successful varieties. For the grasses and legumes the breeding methods adopted in the selection processes have centred around mass phenotypic selection with or without subsequent progeny tests such as the polycross or inbreeding. These methods of selection and variety synthesis have been established on sound evidence that the major component of variation for the characters of interest is under additive gene action and hence is of a form which could be relatively easily exploited by these breeding methods (Breese and Hayward, 1972).

Recent studies at the Welsh Plant Breeding Station have shown however, that although considerable genetic variation exists within and between many populations, ecotypes and cultivars of such species as Lolium perenne, difficulties are often encountered in achieving the full potential expression of desired agronomic traits in a varietal form which can be commercially exploited. This failure occurs in two instances. Firstly in those cases where selection has been effective in raising the frequency of desirable genotypes in a population, under the conditions of relaxed

selection during seed multiplication, the mean performance of the population often regresses to that of the base from which it originated. We have shown this to occur for such characters as yield and quality components, in diverse selection lines where a marked improvement in performance had been achieved (Hayward and Abdullah, 1985). Secondly, where the breeder may be extending his range of variation by wide hybridization either of ecotypes within species, between species or even genera, heterosis is often exhibited in the initial cross which unfortunately our present breeding methods are unable to fully maintain during subsequent seed multiplication generations. Our recent experience of hybrids of Northern European with Swiss or North Italian ecotypes of perennial ryegrass have shown considerable heterosis to occur especially at certain growth stages (see for example, Humphreys, 1985). Similarly the hybrid vigour of Lolium multiflorum x perenne crosses is well known. What breeding methods are available which will allow us to ameliorate these difficulties of fixation or exploitation of heterosis? Before considering possible solutions to these problems it must be emphasized that in all cases where detailed genetic analysis has been conducted it has been found that the gene action responsible is nearly always of a form which could be theoretically utilized in a synthetic variety. In advocating alternative breeding practices these should be considered as systems of overcoming the protracted selection procedures which would otherwise be necessary.

The fixation of selected traits. As indicated earlier where selection has been effective, loss of performance may occur during seed multiplication due to the presence of residual variability and possible undesirable correlations with fitness characters such as

seed production. To overcome these difficulties the ability to rapidly recombine and fix the characters may be achieved by inbreeding. Unfortunately this is not easy with the majority of species we are concerned with! Two alternative approaches are possible to facilitate inbreeding. Firstly, by the production of doubled haploids through anther culture procedures. At the present time no really effective methods are currently applicable to the forage grasses. They are however being exploited in lucerne (Dunbier and Bingham, 1975). In the grasses an alternative approach is to introduce a self fertility gene into the species which will override the action of the incompatibility system. Within the Lolium genus as well as the agriculturally important outbreeders a number of closely related inbreeding species occur. It has been shown by Nitzsche (1983) that it is possible to transfer self fertility from L.temulentum to L.perenne. Our current programme is extending this work to L.multiflorum and also determining the genetic control of self fertility in L.temulentum. If it is due to a mutation to self fertility of either of the S or Z loci its manipulation in breeding programmes may be effectively carried out using the pollen/stigma fluorescence technique that we have used to determine the nature of the incompatibility system in Lolium (Cornish, Hayward and Lawrence, 1979). If a single gene mutation at the S locus is involved, the linkage of the latter to the PGI/2 isozyme locus (Cornish, Hayward and Lawrence, 1980) will greatly facilitate its transfer in a breeding programme. A similar approach is being adopted to the study of highly self fertile inbred lines of L.perenne. Preliminary results indicate that a major gene is involved (Thorogood, pers. comm.) although polygenic modifiers may also play a role. Once clearly identified manipulation of the controlling gene should be possible by orthodox

procedures for gene transfer. Whilst the prime objective of converting these species to an inbreeding habit is in order to make more efficient use of the gene action occurring it must also be mentioned that the subsequent uniformity which should be achieved would be advantageous in meeting statutory requirements for varieties to be uniform and stable.

F_1 hybrid production in the forage grasses. The production of F_1 hybrids in the forage grasses has for many years been a prime objective of breeders and various approaches have been adopted. These have included the use of male sterility, incompatibility or the production of 50% semihybrids (see review by Kobabe, 1983). These procedures have only had limited success the main one being the use of the incompatibility system in those species such as Phalaris (McWilliam, 1974) and Pennisetum (Burton, 1958) where vegetative propagation of the parents is possible. A procedure has been proposed by England (1974) to exploit the two locus incompatibility system which occurs in many grass species. The scheme involves the production of a population with a low level of 'within population compatibility' which when interpollinated with an appropriate pollinator should produce a high level of hybrid progeny; up to 83% under optimum conditions. There are however, certain anomalies within the scheme in that it is dependent on initially being able to inbreed an individual to produce the necessary homozygous S and Z genotypes followed by multiplication of the progeny without any form of self fertilization taking place. Such a situation is unlikely to occur in practice and indeed our experience indicates that the homozygous classes are rarely produced in the expected proportions.

Our recent determination of the operation of the incompatibility system in autotetraploid L.perenne shows that incompatibility occurs when a single SZ pair of alleles in the pollen are matched by the stigma (Fearon, Hayward and Lawrence, 1984). It has been shown by Lundqvist (1963) that such a mechanism can lead to a lower level of cross compatibility in a tetraploid population than in a diploid when an equivalent number of alleles are present. This attribute may be utilized in the scheme shown in Figure 1 for the production of a population of low cross compatibility. Within this population seed will only be produced by the plants which are monoallelic at the S locus which constitute at a maximum only 9.2% of the total.

FIGURE 1. Scheme for the production of a minimal cross compatible population of a tetraploid grass

1. Identify diploid incompatibility genotypes of the form:

$$S_{ij}\ Z_{kk} \text{ and } S_{ij}\ Z_{kl}$$

2. Treat with colchicine ——— tetraploid

3. Hybridize

$$\underset{\text{♀}}{S_{iijj}\ Z_{kkkk}} \quad x \quad \underset{\text{♂}}{S_{iijj}\ Z_{kkll}}$$

(Reciprocal cross is incompatible)

Progeny genotypes produced:

Genotype	Frequency % A	Frequency % B	Cross compatibility
$S_{iiii}\ Z_{kkll}$	2.8	4.6	+
$S_{iiij}\ Z_{kkll}$	22.2	24.5	-
$S_{iijj}\ Z_{kkll}$	50.0	41.8	-
$S_{ijjj}\ Z_{kkll}$	22.2	24.5	-
$S_{jjjj}\ Z_{kkll}$	2.8	4.6	+

A = Random chromosome assortment
B = Random chromatid assortment

Intermixing of this population with another of comparable genetic constitution but with differing incompatibility alleles should theoretically lead to the production of a hybrid variety containing less than 2% of each "within population seed". Even this level could be reduced by elimination of the monoallelic classes. This could most effectively be achieved in a large population by screening for isozyme phenotype at the PGI/2 locus, the original parents having been chosen with different alleles at this locus. This will allow identification of the monoallelic S classes except of course for those individuals which arise from gametes where recombination between S and PGI/2 has taken place.

CONCLUSIONS

The schemes proposed here could be applied to both within and between species hybrids in the L.perenne/multiflorum complex. Whether they would be applicable at the intergeneric level is dependent on functional homology occurring between the S and Z systems in what may be very distantly related species. These schemes are based on recent developments in our understanding of the genetics of the forage grasses. Their adoption however may well depend upon the timing and likely introduction of male gametocides to hybrid forage grass breeding.

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New Ways in Selecting Components for Synthetics.

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Breeding of synthetic varieties in fodder plants, especially in lucerne, certainly represents a perspective breeding procedure (ROD, VONDRAČEK 1982). It is an application of knowledge from the sphere of population genetics and represents an endeavour to guide their evolution in accordance with the biological and genetical character of fodder plants, like high heterozygosity of individuals and their predominant open-pollination. In the main components are looked for which create after intercrossing a population with a good yield and other effects. The success of realization depends on a number of genetic-biological and technical factors, which have been investigated by several authors. Nevertheless the deciding moment remains the choice of components, which have to be synthetized.

This task has been tackled and relevant procedures have been described (BUSBICE 1970; BUSBICE, GURGIS 1976). These are based on information gained in the generation of clones and in the generation of their generative progenies. Using the procedure published by the above authors, which enables a prediction of expected performance of a balanced synthetic population, we defined the given task as a selection of components and of their optimal number in such a way that the performance of synthetic population becomes maximal. Using the formula predicting yield of synthetic variety in equilibrium, we developed and proposed a mathematical and programme solution which has two steps: in the first, for all possible numbers of components those components are determined for which predicted performance of synthetic is maximal, in the second the optimal number of components is found, which corresponds to optimal performance of synthesis (ROD, VONDRAČEK 1981).

This solution requires information about the performance of the generation after inbreeding. Because of material and time difficulties this

generation is commonly not included in breeding programme. In addition the influence of this member on the performance of the synthesis according to the cited formula is minimal and can be omitted when the number of components is large. That means aiming at selection according to the performance of clones and their progenies after open pollination, best in a polycross. To heighten the effectivness of selection under these conditions we proposed a solution using a combination of both sources of information (ROD 1983; VONDRAČEK, ROD, CHLOUPEK 1984). It is based on performance tests which are a common part of breeding methodology. The only condition for applicability of the method is individual planting. The evaluation of experimental data by means of analysis of variance enables one to estimate the effects of clones and of their generative progenies. We have shown that the coefficient of intraclasscorrelation estimated by means of the given models can be interpreted when analysing the variability among and within clones as a measure of repeatibility, among and within progenies as a measure of their heritability.

Selection of components for a synthesis is based on this information and we showed that the selection value of a given component - clone is given by the performance weighted according to the source of information obtained. We derived the corresponding formulas enabling one to estimate selection values for clones and for their progenies after polycross. Using these characteristics of performance we can select clones in a synthetic population according to weighted means, which are sums of multiples of the performance of a given clone with the estimated weight of clones and the preformance of its progeny after polycross with the estimated weight of progenies.

All these procedures have a onedimensional character, which means that the components are selected according to individual traits independently. A multidimensional solution, that is the selection of components according to several traits simultaneously, can be solved in our opinion in three ways. Construction of selection index could formally be the most simple one. By means of selection index the problem could be transformed to a onedimensional one and the synthesis can be performed according to the procedures mentioned above. But the realization is difficult, as the suitable selection index as a function of given selection traits is to be found. This has to be determined on the basis of genetical elements

and according to the practical importance of single traits estimated by a team of experts.

The second possibility could be a modified method of tandem selection. It is necessary also here to estimate weights of single traits with respect to their genetical correlations and to coefficients of heritability. According to this information a higher number of components could be selected with respect to important genetically correlated traits and their number could then be reduced according to the guiding traits. It is of course necessary to judge the performance of a synthesis for each of chosen selection traits by means of the given procedures separately. The procedure can then be repeated on a computer with different entry components, that is with those which have been chosen according to the most important and positively correlated traits.

The most promising seems to be the method which we developed and verified on practical breeding material (ROD, VONDRÁČEK 1985). It is based on an analysis of a matrix of genetical and environmental covariances of traits, characterizing the performance of clones and of their progenies. We built genetically and environmentaly uncorrelated factors of heritability, which are linear combinations of the multidimensional trait observed. They are formally given as latent vectors of a bunch of quadratic forms of matrices of genetical and environmental covariances. It is possible to state in such a way a few independent genetical factors with the highest coefficients of heritability which exhaust the genetical information comprised in the multidimensional trait. The presumed contribution of individual clones-components for a synthetic population can be for these genetical factors (= artificial elements of heritability) estimated by means of the second above mentioned method for a one-dimensional case. This procedure is based on an analysis of genetical structure of the starting population. The genetical factors obtained have to be reasonably interpreted, for instance by means of their correlation with the quantities originally measured. In the final phase we select in a synthesis clones according to practically the most important factor, or according to two important factors. Thus the selection into the synthesis according to one genetical factor is not linked with the selection in the synthesis according to the other genetical factors. This makes it possible to choose different

ways of breeding strategy with respect to different breeding tasks.

It can be concluded that a onedimensional solution allows objective selection according to single traits individually and the components have to be empirically combined according to the breeding targets. A multidimensional solution allows on the contrary, selection of components according to traits with a high heritability, consequently with a real chance of success. Besides, components can be combined according to different breeding plans, in the main according to objective viewpoints.

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UNREDUCED GAMETES IN DIPLOIDS MEDICAGO AND THEIR IMPORTANCE IN ALFALFA BREEDING [1]

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SUMMARY

In Medicago genus several Authors have stressed the importance of 2n gametes both in evolution and breeding of cultivated alfalfa, which is a natural polysomic polyploid (2n=4x=32); however, only few data are available on the frequency of male and female 2n gametes in natural diploids. To obtain data on the frequency of 2n gametes, more than 12.000 2x-4x and 4x-2x crosses were made in 1982 at Madison (USA).

Crosses involved 8 populations of diploids Medicago, a tetraploid male sterile M. sativa clone and some vigorous tetraploid M. sativa plants. Each one of the 274 diploid plants was utilized both as male and as female. Of the 548 cross combinations, 266 produced extremely variable quantities of seeds which were sown in 1983 in a greenhouse at Perugia (Italy); the plants were space transplanted in the field in 1984. The identification of ploidy level of these genotypes was made on the basis of morphological characters, pollen stainability, plant fertility and chromosome counts.

The majority of the 515 analyzed plants behaved as normal tetraploids indicating that several mother plants produced either male or female unreduced gametes; the identification

[1] An extended version of this lecture will be submitted for publication to Theoretical and Applied Genetics.

of only 4 F_1 triploid plants confirms the presence of a very effective triploid block in alfalfa. As consequence, in alfalfa bilateral sexual polyploidization is a more likely alternative in respect to the triploid bridge hypothesis proposed by Harlan and DeWet for the origin of polyploids in angiosperm. Furthermore, the present study underlines the possibility of a quite simple identification, within natural populations of diploids Medicago, of genotypes able to produce high frequencies of unreduced gametes and usable in alfalfa breeding.

INTRODUCTION

Gametes with unreduced chromosome number (herein termed 2n gametes) have been reported to occur naturally at a low frequency in a number of plant species (Satina and Blakeslee, 1935; Rhodes and Dempsey, 1966; Bingham, 1969; Bringhurst and Gill, 1970; Quinn et al., 1974; Myers et al., 1984) and likely play a major role in the evolution of polyploid series (Harlan and DeWet, 1975).
In Medicago genus, several Authors have stressed the importance of 2n gametes both in the evolution (Stanford et al., 1972) and breeding (Bingham, 1968, 1979; Vorsa and Bingham, 1979; McCoy and Smith, 1983) of cultivated alfalfa, which is a natural polysomic polyploid (2n=4x=32); however, only few data are available on the frequency of male and female 2n gametes in natural diploids belonging to the Medicago sativa falcata (alfalfa) continuum. The purpose of the present study was to obtain more information on this matter and eventually to identify genotypes able to produce high frequencies of 2n gametes and usable in breeding programs.

MATERIALS AND METHODS

To obtain data on the frequency of 2n gametes, 12.437 2x-4x and 4x-2x crosses were made by hand in a greenhouse during the autumn 1982 at Madison, WI, USA. At diploid level, crosses involved the following 8 populations of Medicago (Table 1): 5 accessions of M. sativa subsp. coerulea

Table 1. Diploid populations utilized.

Population	Origin
M. coerulea	
315466	USSR
315462	USSR
325381	USSR
315465	USSR
243225	Iran
M. falcata	
262532	Israel
258754	USSR
M. sativa	
CADL	USA

(Lessing ex Ledebour) Schmalhansen, 2 accessions of M. sativa subsp. falcata (L.) Arcangeli and a population of cultivated alfalfa at the diploid level (herein termed CADL) developed from cultivated tetraploid over a 10-years period, using haploidy, with breeding and selection (Bingham and McCoy, 1979). At tetraploid level, a male-sterile clone (6-4 ms) and several vigorous M. sativa plants were utilized.
The 7 natural diploids accessions were kindly supplied by the Regional Plant Introduction Station, Iowa State University, Ames, IA, USA. For each diploid population a number of plants between 14 (M. coerulea 325381) and 79 (M. sativa CADL) was present.
Each one of the 274 diploid plants available was utilized both as male, on 6-4 ms, and as female, receiving pollen from tetraploids M. sativa plants. For each diploid plant at least 10 flowers were pollinated on 6-4 ms and 10 flowers were pollinated by pollen coming from 4x plants. The 1.454 seeds produced in 2x-4x and 4x-2x crosses were sown in a greenhouse at Perugia (Italy) in spring 1983 and the survived plants were space transplanted in the field in spring 1984.
During 1983-84 a preliminary identification of the progeny ploidy level was made on the basis of morphological characters, pollen stainability and plant fertility (evaluated by crosses with vigorous M. sativa tetraploid plants under isolation cages to prevent contamination). The plants which did not show a clear ploidy level on the basis of the preliminary identification were checked cytologically during 1985.

RESULTS

The percentage of diploid plants which produced seeds in interploidy crosses (Table 2) ranged between 0% (M. coerulea 325381) and 51% (M. coerulea 243225) when utilized as pollen sources, between 46% (M. falcata 258754) and 100% (M. coerulea 325381 and 243225) when utilized as eggs sources. Extremely variable quantities of seed were given by 266 out of 548 cross combinations.
Seeds set after interploid crosses was used as a rough

Table 2. Results of interploid crosses.

Diploid populations		Total no. of 2x plants utilized	% of 2x plants producing seeds	Average seeds set (%) (1)
M. coerulea				
315466	4x-2x	24	42	3
	2x-4x	24	83	19
315462	4x-2x	30	27	2
	2x-4x	30	70	7
325381	4x-2x	14	0	0
	2x-4x	14	100	13
315465	4x-2x	23	35	2
	2x-4x	23	74	9
243225	4x-2x	39	51	4
	2x-4x	39	100	25
M. falcata				
262532	4x-2x	32	34	3
	2x-4x	32	50	6
258754	4x-2x	33	9	0.5
	2x-4x	33	46	13
M. sativa				
CADL	4x-2x	79	37	30
	2x-4x	79	66	10

(1) Average seeds set = (no. of seeds produced)/(no. of flowers pollinated) x 100.

measure of 2n gametes production, as already done in Medicago by Bingham and McCoy (1979).
Average seeds set ranged between 2% (M. coerulea 315462, 2x-4x crosses and M. coerulea 315465, 4x-2x crosses) and 30% (M. sativa CADL, 4x-2x crosses) except for 4x-2x crosses involving M. coerulea 325381 which failed to produce seeds.
Unreduced gametes seemed therefore to be quite widespread in the analyzed populations; within each cross combination it was also possible to find at least one plant characterized by a seeds set higher than the average value of the population.
Even if data relative to the results of 2x-4x and 4x-2x crosses can be utilized to obtain preliminary information on the presence of 2n gametes in diploid populations, it is clear that this information must be utilized in a conservative manner. In particular, due to the use of 6-4 ms clone we can be reasonably sure that a large percentage of seeds produced in 4x-2x crosses were tetraploid originated by unreduced pollen from diploid parent, whereas it is possible that a certain amount of seeds produced in 2x-4x crosses were diploid originated by selfing. For this reason we decided to identify the progeny ploidy level (Table 3).
On the whole, ploidy level identification was possible for 515 plants which, coming from the 1.454 seeds produced in 2x-4x and 4x-2x crosses, survived during the period 1983-85. Assuming that all tetraploid seeds germinated and no tetraploid plant died during the experiment, which is a very conservative statement, the estimated percentage of 4x seeds ranged between 0% (M. coerulea 325381 and M. falcata 258754) and 33.33% (M. coerulea 315462) when the natural diploid populations were used as pollen sources and between 1.45% (M. coerulea 315466) and 29.95% (M. coerulea 315465) when they were used as female parents. These results confirmed the presence of 2n gametes in all the natural diploid populations; within the same materials was also possible to find some plants with 4x seeds set remarkably higher than the average both in 4x-2x and in 2x-4x crosses.
For what M. sativa CADL is concerned, this population showed the highest percentages of 4x seeds (Table 3); in particular, the high percentage of 4x seeds in 4x-2x crosses

Table 3. Number of tetraploid plants and percentage of tetraploid seeds produced in interploid crosses.

Diploid populations		No. of plants analyzed	No. of 4x plants detected	% of 4x seeds (1)
M. coerulea				
315466	4x-2x	3	3	23.08
	2x-4x	20	2	1.45
315462	4x-2x	4	4	33.33
	2x-4x	11	7	11.29
325381	4x-2x	0	0	0
	2x-4x	11	7	9.86
315465	4x-2x	4	4	36.36
	2x-4x	24	14	29.95
243225	4x-2x	10	9	31.03
	2x-4x	126	28	8.78
M. falcata				
262532	4x-2x	1	1	6.67
	2x-4x	14	11	28.95
258754	4x-2x	0	0	0
	2x-4x	69	10	7.09
M. sativa				
CADL	4x-2x	157	155	41.44
	2x-4x	61	52	31.14

(1) % of 4x seeds= (no. of 4x plants detected)/(no. of seeds produced) x 100.

was mainly due to the presence of two plants which produced 45 and 69 of the 155 tetraploid plants detected, respectively. These plants appeared therefore to be strong producers of 2n pollen. On the other hand, three different plants of the same population showed quite high levels of 2n eggs production being characterized by 4x seeds set of 31%, 41% and 70%, respectively.
In F_1 materials only four triploid plants were identified, one produced in 2x-4x crosses with M. coerulea 315466, one in 4x-2x crosses with M. coerulea 243225 and two in 4x-2x crosses with CADL.

DISCUSSION

Results of the crosses reported in this research would suggest that in Medicago sativa-falcata continuum unreduced gametes are quite diffused not only in artificial diploids such as CADL but also in natural diploid populations.
The presence of 2n pollen and eggs provides the natural diploid populations with the potentiality of undergoing a bilateral sexual polyploidization (BSP) process through 2x-2x matings with the production of tetraploid progeny. At the same time, the identification of only 4 F_1 triploid plants confirms the presence in alfalfa of a very effective triploid block which, as already pointed out in several species by Brink and Cooper (1947), Hanneman and Peloquin (1968), Von Wangenheim et al. (1960) and Johnston et al. (1980), operates in interploid crosses eliminating almost all triploid embryos due to endosperm imbalances. As consequence, in alfalfa BSP is a more likely alternative in respect to the triploid bridge hypothesis proposed by Harlan and DeWet (1975) for the origin of polyploids in angiosperm. Furthermore, the present study underlines the possibility of a quite simple identification within natural populations of diploids Medicago of genotypes which, distinct from the relatively rare occurrence of 2n gametes, are able to produce quite high frequencies of 2n eggs and pollen and could be usable in alfalfa breeding.

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In vitro propagation of Lolium multiflorum

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Many species of perennial forage grasses can be propagated vegetatively without any difficulty thereby giving the opportunity for easy maintenance of particular genotypes selected by the breeder. Clonal multiplication and persistence of clones offer valuable possibilities for plant breeding such as the propagation of infertile genotypes (male steriles) or inbreds with a high degree of selfincompatibility. If it is necessary to maintain special genotypes over years in order to produce identical populations (synthetic varieties) perennial species which can be cloned easily have great advantages.

Italian ryegrass (Lolium multiflorum) , however , is not a perennial species. Vegetative propagation by conventional means (separating big plants into small plantlets) is easily be done but this does not work sufficiently for a longer period of time. Therefore many attempts have been made to find better methods which can be useful for the plant breeder. Several authors have recommended the in vitro culture (CHEN et al. 1977 , DALE 1980, DALTON and DALE 1981, 1983 and others) .

Theoretically each kind of meristematic cells can be used for tissue culture. But since it is known that callus formation can induce mutations the breeder who is interested to get identical genotypes will prefer in vitro cultivation which avoids callus formation. Obviously immature inflorescences are the most suitable for direct regeneration of grass plantlets. The next step is to obtain roots and a sufficient number of rooted shoots. While the nutrient composition of the general culture medium is well established the yield of usable plants depends largely on the composition and on the concentration of special hormones to be added.

The first experiments have been conducted with different genera of forage grasses followed by investigations of different species of certain genera. The plant breeder, however, is more interested on informations concerning the performance of particular genotypes when they are cultivated and propagated in vitro. DALE (1980) already pointed out that there are genetic differences in tissue culture response.

The purpose of the present experiment was to examine the behaviour of different genotypes of Lolium multiflorum when cultivated in vitro. Four homozygous genotypes selected from inbred lines were involved in this experiment. Two of them were vigorous and had many tillers, the other two were weak with less tillering capacity. Unemerged inflorecences were taken and after surface sterilisation the explants were placed on an initial medium developed by LINSMAIER and SKOOG (1965). In order to stimulate cellgrowth 2,4 D (2,4 - dichloro - phenoxyacetic acid) , BAP (6 - benzylamonopurin) , and casein hydrolysate were added. After exposing these young inflorescences to a 12 ^{h} daylength with artificial light (2000 lux) the development of plantlets could soon be observed. About 60 Days later the shoots obtained on the initial medium were big enough to handle. They were separated and than transferred to another medium with different concentrations of BAP (0.0 - 0,5 - 1,0 - 1,5 ml/l resp.) . The purpose of the use of BAP was to estimate its influence on the rate of tillering, on the height of the plantlets and on root development. These characters were counted and judged respectively after another period of 60 days.

The data obtained from this experiment showed significant differences between genotypes and between BAP - concentrations as well. It is important to note that the interactions : genotype / tillering and genotype / height of the shoots were also significant. The response of the genotypes to the

increasing BAP concentration is remarkable different. While for genotype 2 the optimum concentration of BAP was 0.5 ml/l for genotype 1 1.5 ml/l was not sufficient to reach optimal tillering. Similar results were obtained for the other characters, although the differences in root development appears not to be meaningfull.

Sometimes green shoots were accompanied by albino shoots. But while DALE and DALTON (1983) observed ca. 10 % albinos in Lolium multiflorum their percentage in the present experiment was 2,5 % only. The reason for the appearance of albino shoots in tissue culture is not yet known. Similar observations can be made in inbred lines although after more than 10 selfing generations these lines should be nearly complete homozygous.

The regenerated plantlets were stored in a refrigerator (4-6° C) without light leaving them on a culture medium. These plants which were obtained last summer are still alive and now we try to induce new growth by cultivating them in the greenhouse.

This small experiment with its preliminary results indicates that it should be possible to develope methods of tissue culture which can be applied by the plant breeder. But the technique needs further improvement because it is not satisfactory when the rate of tillering under the condition of tissue culture is low with some genotypes and high with others. Perhaps there are connections between growing characters in the field and the suitability for in vitro culture. These are problems worth to be investigated in the future. If they could be solved the advantages of the use of in vitro culture for the multiflorum - breeding is obvious. Clonal propagation can be practised in winter and land reservation is not necessary. Many different genotypes can be stored in a small room and can be used as a source of genetic material around the whole year.

Since by utilizing the method described above no callus formation occured the regenerated plants should be expected to be free of mutations. If, however, enlargement of genetic variability by tissue culture via callus formation is required other methods (medium, hormones etc.) has to be applied.

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INTERSPECIFIC HYBRIDIZATION IN CLOVER BREEDING

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Introduction

Red clover is an important legume in Scandinavian forage production, where it is usually grown in mixture with timothy and meadow fesque. In the relatively hard Scandinavian climate the lack of good field persistance is a problem limiting the production of the species. The stand of red clover is often more sparse in the second and third years of the leys. Conventional breeding procedures with crosses within the species have been able to improve this disadvantage only to a limited extent.

Several wild relatives of red clover are perennial with good persistance and hardiness. In the Scandinavian flora two such species are represented viz. Trifolium medium and T. alpestre. T. medium, the zig-zag clover is a common species all over Scandinavia whereas T. alpestre is present only on a few sites in Denmark. In contrast to red clover, which has the basic chromosome number n=7 these species have the basic number n=8.

T. medium is decaploid with 80 chromosomes or octoploid with 64 chromosomes. All material of T. medium collected in Sweden is of the decaploid cytotype. T. alpestre is a diploid species with 16 chromosomes. A close relative of T. medium is T. sarosinense, a hexaploid species with 48 chromosomes. This species has a more southern and continental distribution and is not present in Scandinavia. All these species are perennial and have an effective system of vegetative propagation by means of rhizomes and are hence interesting for the improvement of red clover.

The first report on successful hybridization between red clover and a perennial clover species was published in 1981 by Collins et al. This group had developed embryo-culture techniques adapted to red clover crosses which made possible the production of hybrids between T. sarosiense and diploid T. pratense (Phillips et al. 1982). This successful hybridization stimulated Svalöf AB to start a program on interespecific hybridization in order to introduce new genetic variation into the breeding material of the clover species, especially into red clover.

Materials and methods

The crosses aiming at improving red clover were concentrated on the wild species T. medium, T. sarosiense and T. alpestre. T. sarosiense was obtained from the University of Kentucky, from the botanical garden in Vácrátót, Hungary and from the gene bank of Gatersleben, GDR. T. medium has been collected in Sweden and Norway, T. alpestre was collected in Denmark and was also obtained from the University of Kentucky.

As paternal parents the tetraploid Svalöf red clover varieties Sally, Fanny and Molly were used. The interspecific hybrids were backcrossed with the tetraploid varieties mentioned and also with the diploid Svalöf varieties Bombi and Björn. T. alpestre was pollinated with the diploid variety Bombi. Since all the clover material used in these crosses is self incompatible no emasculation was applied. This of course makes possible a higher number of pollinated florets. In all the crosses the embryo culture techniques described by Phillips et al. (1982) were used with some modifications.

Results and discussion

The first interspecific crosses were made in 1983 between T. medium and tetraploid red clover. From 2637 pollinated florets 363 embryos were dissected (Merker 1984). Large embryos of more or less normal size and development were shown to be the result of selfpollination and gave rise to normal T. medium plants with 80 chromosomes. Of the remaining approximately 300 embryos most were too small and weak to survive the embryo culture. From the more vigorous embryos three different hybrid genotypes were raised. These plants have the expected chromosome number 54.

In 1984 and 1985 further crosses were made. In addition to T. medium, T. sarosiense and T. alpestre were also crosses with T. pratense. The results of these crosses are presented in Table 1. From these crosses embryos of normal size were discarded as selfs and the rest were transplanted to embryo cultures. From the number of dissected embryos it is evident that fertilization in the two different cross combinations takes place in around 10 per cent of the pollinated florets.From the crosses of 1984 seven different vigorous hybrid genotypes from T. medium x T. pratense have been raised and are kept in the greenhouse. Four additional genotypes are weak and have not produced any floral structures. From the 681 embryos of the T. sarosiense x T. pratense crosses 11 different vigorous and five weak hybrid genotypes have been raised. The hybrids all have the expected chromosome numbers 54 and 38 respectively. The crosses between diploid T. alpestre and T. pratense gave no embryos at all. We have now produced tetraploids of T. alpestre and we will repeat this cross on the tetraploid level to see if this can be more successful.

The hybrids are generally resembling the maternal parents in their morphological characters. The T. sarosiense x T. pratense hybrids are flowering more polificly than the T. medium x T. pratense hybrids. Four of the latter have disturbed inflorescence development with reduced number of florets and many empty calyxes in the heads. One of them does not develop any normal florets, but only empty calyxes. One of the T. sarosiense x T. pratense also has reduced number of normal florets. Part of the T. sarosiense x T. pratense hybrids have disturbed clorophyll production being of a pale green colour, whereas

the T. medium hybrids have normal clorophyll. Some of the hybrids of both categories are forming rhizomes, but not to the same extent as the wild parents. This is of obvious interest for the further utilization of the hybrids in red clover breeding.

In 1985 the first backcrossing of the hybrids was made. The results are summarized in Table 2. From the figures it is obvious that the T. medium hybrids have a higher female fertility than the T. sarosiense hybrids, which are almost completely sterile. Another conclusion that can be drawn from the figures is that tetraploid T. pratense is much more effective in the backcrossing than diploid. The T. sarosiense hybrids did not produce a single embryo when backcrossed with the diploid variety, whereas the backcrosses with tetraploids gave four embryos, which were however small and weak. The large majority of the 194 embryos from the backcrosses of the T. medium hybrids came from two different hybrid genotypes both from the same T. medium individual. This indicates that there is a genotypic difference in the ability of the hybrids to produce backcross progeny. A few individuals from the T. medium x T. pratense backcross progeny has already been transplanted to pots in the greenhouse.

The results presented here are encouraging as far as the utilization of T. medium is concerned. Here there seems to be good possibilities to carry out a backcross program. To what extent this will entail a transfer of genetic material from T. medium to T. pratense remains to be seen. To get some indications about this we are planning to investigate meiotic pairing in the hybrids. The T. sarosiense x T. pratense hybrids on the other hand do not seem to be very useful for breeding purposes, since they produce only very few weak embryos in the backcrosses. They would demand a very extensive backcross program in order to produce a small number of backcross individuals. It is however possible that other hybrid genotypes of the same specific combination could be easier to backcross.

Acknowledgement - The present work has been supported by grans from the Swedish Plant Breeding Board.

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Table 1. Results of interspecific hybridization between T. medium and T. sarosiense (females) and T. pratense 4X (males).

		Pollinated florets	Dissected embryos	Per cent fertilization
T. medium x T. pratense	1984:	9466	805	8.5
	1985:	6944	756	10.8
T. sarosiense x T. pratense	1984:	5729	681	11.9
	1985:	5541	433	7.8

Table 2. Results of backcrossing of interspecific hybrids with T. pratense 2X and 4X.

		Pollinated florets	Dissected embryos	Per cent fertilization
T. medium hybrids	2X:	1171	13	1.1
	4X	1878	181	9.6
T. sarosiense hybrids	2X:	3969	0	0.0
	4X:	5789	4	0.07

PERFORMANCE OF SECOND AND THIRD GENERATION SYNTHETICS OF LUCERNE DERIVED FROM PARTLY INBRED PARENTS:FORAGE YIELD AND FERTILITY.(*)

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Introduction

The objective of the breeding program carried out at the Forage Crop Institute of Lodi is the constitution of cultivars for the northern and Central Italy irriguous plains, and therefore for intensive management systems. Methods and technical procedures are chosen in function of this objective;they are characterized by two principal aspects: the utilization of interplant competition during all the phases of breeding, and the utilization of two generations of selfing.

About the interplant competition, we said that it would increase the efficacy of selection, because it allows the appreciation of lucerne plants modelled by growing conditions as nearest as possible to the agronomic conditions (6,7,9,11).

About selfing, our working hypothesis was that a genetic homogeneity as greater as possible in an autotetraploid, for the physiological characters which are responsible for the resistance to the effect of cutting, should be the best way for mantaining the persistency of the meadow structure. In fact, a rich and well organized agriculture system can control the major part of natural environment factors. In these conditions, the effects of artificial factors, introduced by the farmers, become predominant. Among these, the high number of cuttings can be the most important.We know, of course, that a complete homogeneization is not realizable in an autotetraploid plant, and that interplant competition increases these difficulties, because it emphasizes the differences among the plants. Nevertheless, selfing and subsequent selection were proved indeed to enhance persistency and yielding capacity of the lucerne crop (5,8),in a first cycle of our breeding program.

A second cycle was initiated inorder to verify these results and at the same time to state the importance of the number of constituents of synthetic varieties in autotetraploid plants (1,2,3,4). Our first cycle of selfing and selection (5) showed the 4-clone Syn of the S_2 level as the best performing; so we have utilized two generations of selfing also in this second cycle,, the final steps of which are concernend in this paper.

(*) This work was supported by funds from Ministry of Agriculture (Piano Foraggero Zootecnico).

Materials and method

Thirty mother plants, selected after progeny test, were combined into 11 synthetic varieties according to the scheme of Table 1.

Table 1. Reference number of mother plants entered in Syn 1,Syn 2,Syn 3.

Number of parental clones	5	10	15
	Reference numbers		
Code of synthetics	A 1 to 5	G 1 to 10	L 1 to 15
	B 6 to 10		
	C 11 to 15	H 11 to 20	
	D 16 to 20		M 16 to 30
	E 21 to 25	I 21 to 30	
	F 26 to 30		

The mother plants were numbered from 1 to 30. The first five plants were intermated in a 5-clone Syn 1 (A), the plants from 6 to 10 were intermated in another 5-clone Syn 1 (B), and so on. After that, the first 10 plants were intermated all together in a 10-clone Syn 1 (G), the plants from 11 to 20 in a second 10-clone Syn 1 (H) and the plants from 21 to 30 in a third 10-clone Syn 1 (I). After having assured the seed setting of these 10-clone Syn 1, the plants from 1 to 15 were intermated all together to give a 15-clone Syn 1 (L), and the plants from 16 to 30 were intermated all together to give another 15-clone Syn 1 (M).

All the pollinations were made by hand, without emasculation, in greenhouse,

Each Syn 2 and Syn 3 was obtained by intermating 15 plants for each constituent of the respective previous generation.

75 plants for each 5-clone Syn 2 and Syn 3
150 plants for each 10-clone Syn 2 and Syn 3
225 plants for each 15-clone Syn 2 and Syn 3

Syn 1, Syn 2 and Syn 3 generation synthetics were tested in greenhouse, in concrete boxes/plots, 80 cm long, 25 cm wide and 60 cm high, at a density of about 300 plants per square metre. The results here presented concern dry matter yield and pod fertility.

Results and discussion

1.Pod fertility

An increase of pod fertility was observed from the first to the second generation, independently from the number of constituents of synthetics, in agreement with the theory concerning the autotetraploids.

From the second to the third generation we observed a variation in reponse of 5-clone synthetics, going from an increase of 31% (D) to a decrease of 23% (E). On average, 5-clone Syn showed a decrease of 15% on Syn 2. In 10-clone and 15-clone, the situation appeared to be stable (Figure 1). We underline that synthetic variety D shows the lowest pod fertility among the 5-clone synthetics.

In general, the average pod fertility increases with the increasing number of constituents, 5-clone synthetics showing a significantly lower fertility than 10-clone and 15-clone synthetics. In Syn 2 and Syn 3 also the difference between 10-clone and 15-clone synthetics becomes significant in favour of the 15-clone synthetics (Table 2).

Table 2. Average pod fertility in Syn 1, Syn 2 and Syn 3.

Number of parental clones		5	10	15
Generation of synthetics:	Syn 1	3.95	4.80	4.61
	Syn 2	6.66	7.22	7.77
	Syn 3	5.68	6.87	7.70

2.Forage yield

A general increase in yielding capacity was observed from Syn 1 to Syn 2 generation (Figure 2); by contrast, in Syn 3 several synthetics showed a decrease in yield when compared with their respective Syn 2.Consequently, no effect of the number of constituents was observed.

The trend from Syn 1 to Syn 2 is in agreement with the theory concerning the autotetraploids. The decrease in Syn 3 can be explained with the assumption that in Syn 2 the maximum level of heterozigosity is reached for some synthetics. In any case, the best synthetic variety in Syn 3 is based on 5 costintuents (A).

These Syn 3 results can be explained by different levels of allelic richness of the whole set of parental clones we utilized. When the parental clones are partially inbred, an increased number of constituent may be not sufficient to prevent inbreeding depression in Syn 3 generation. Such cases of inbreeding depression in Syn 3 could perhaps be avoided by using clones derived from parental populations very different in genetic origin and growth area.

Finally, the whole set of data at our disposal derived from Syn 1, Syn 2 and Syn 3, suggest that in autotetraploid plants the progeny test results have to be taken with caution because of a possible different level of inbreeding of the clones to be chosen as parents.

Conclusions

According to these results and to those obtained by other Authors , and by ourselves in other experiments, it is confirmed that in lucerne the utilization of parents partly inbred does not allow to reach the maximum level of heterozigosity at the first generation. This is true for pod fertility and for forage yield as well.

As it regards pod fertility, according to our results, the maximum level seems to be reached at the second generation. Concerning the optimal number of constituents, the best synthetic varieties are based on 10 (I) and 15 (L,M) parental clones.

Finally, we have to say that the seed production in the field does not reflect the panmittic pattern of pollination, but depends upon the selection operated by pollinators. So, it is advisable to use a number of constituent superior to that indicated by experiments made with material obtained by controlled pollination.

Regarding forage yield, our data suggest the following conclusions: with a material partially inbred, a synthetic variety narrow based (4-6 parents) will very probably show an inbreeding depression in Syn 3. The use of a greater number of parental clones should not modifiy the situation. A narrow based synthetic variety should be the best solution if the parental clones derive from populations very different as in genetic origin as in area of growth.

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Figure 1. Pod fertility (average number of seeds per pod)

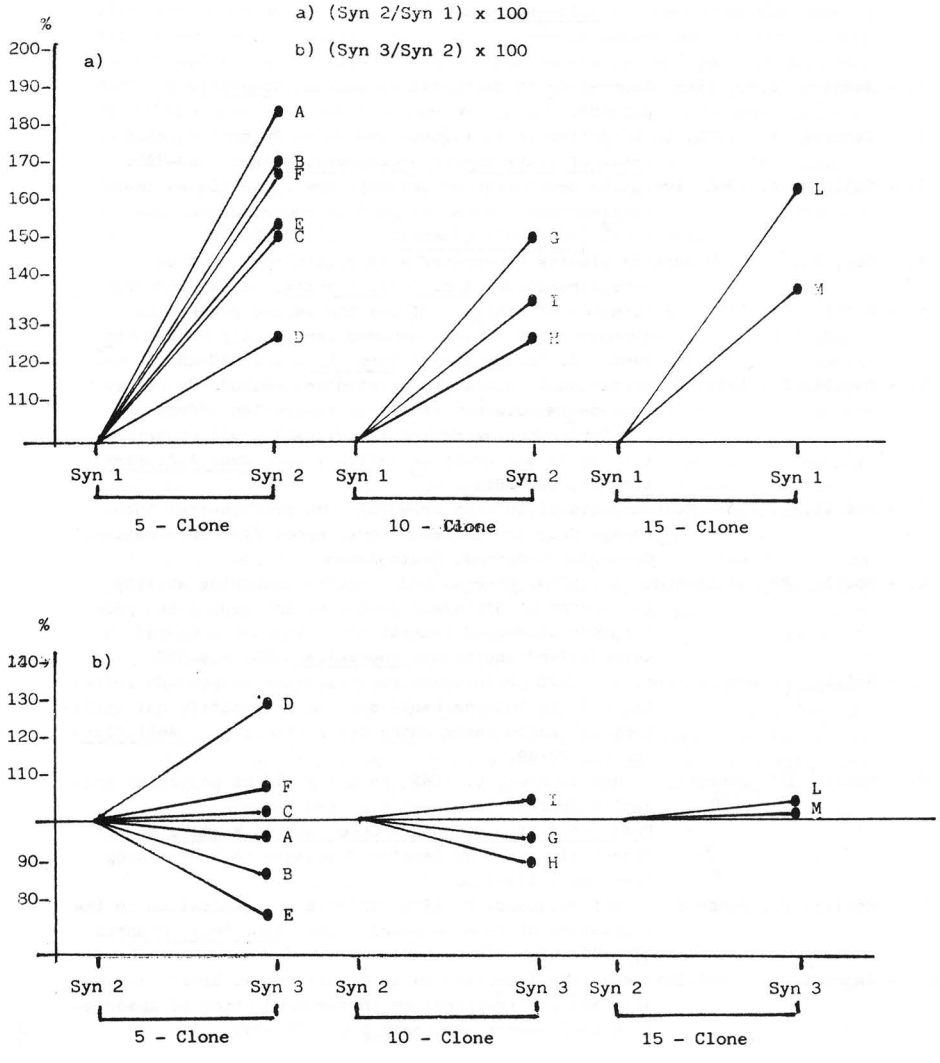
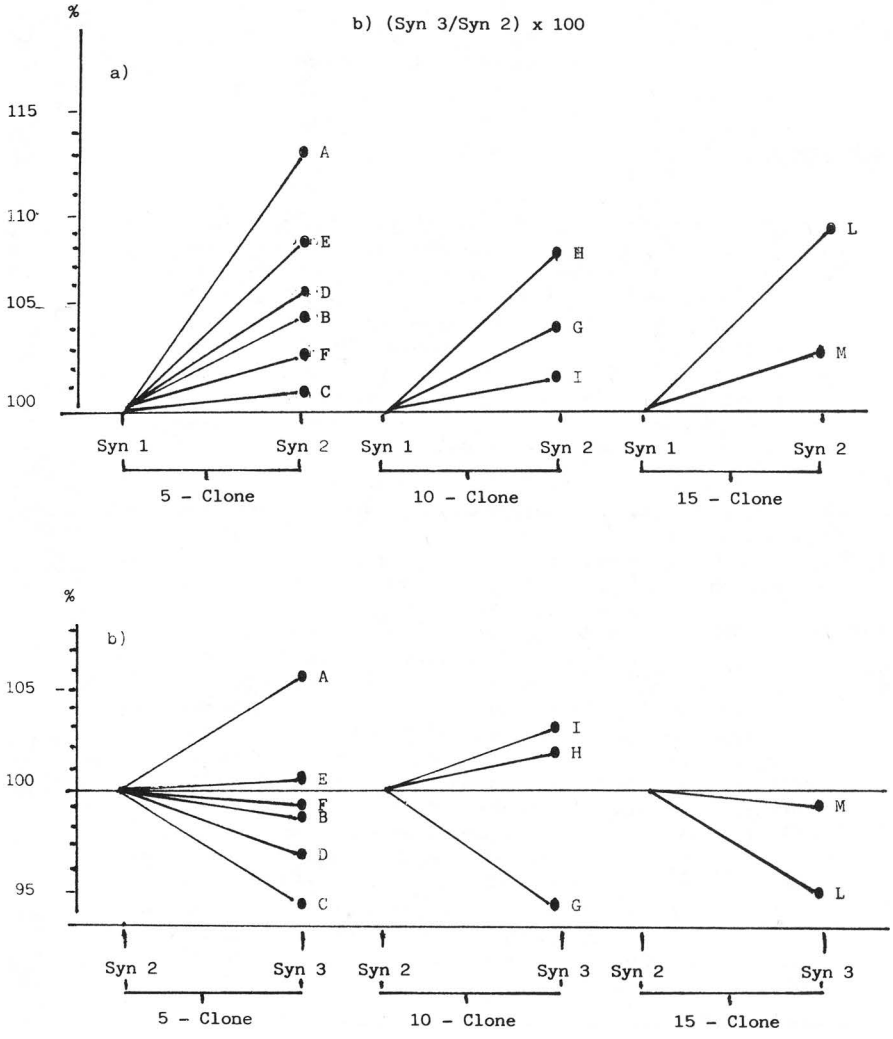


Figure 2. Dry matter yield (average of 6 cuttings).

a) (Syn 2/Syn 1) x 100

b) (Syn 3/Syn 2) x 100



LUCERNE MEADOW STRUCTURE. ANALYSIS OF AERIAL PART AND ROOTS.

I. DRY WEIGHT (*)

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Introduction

From the practical point of view, an individual plant of lucerne has no interest, because the exploitation concerns not an individual plant but the lucerne crop as a whole. In this case, the problem is to know the productiveness factors of the lucerne meadow. We made mny experiments in this direction, and we concluded that a lucerne meadow is not the sum of independent elements (that is the plants), but a society of plants, linked by a system of dynamic relationships which variate through the cuttings and years. The whole of these effects on physiology and morphology of aerial part and roots is defined as "meadow structure" (Rotili 1984 a).

The structure evolves during the cuttings as for vigour as for demography. The plants showing the highest vigour at the three first cuttings are, on average, the most peristent. This means that, at equal conditions of desease resistance, the persistency of individuals depends at a great extent, upon the position in the structure as whole (Rotili 1979),(Figure 1).

In addition, the topographical description of aerial part and roots shows the very strict correspondence between the upper and the lower structure of the lucerne meadow (Rotili 1984 b),(Figure 2).

Finally, mortality is positively correlated with the genetic heterogeneity of the utilized populations (Rotili-Zannone 1975).

Our hypothesis is that, at equal conditions of disease resistance, the death of a plant is the final result of the negative effects of competition cumulated through the cuttings.

Our previous results of a lucerne meadow structural analysis bring to a certain number of consequences on the lucerne breeding, concerning the choice of the meadow structure model; and consequently, the choice of the plant ideotype.Many Authors have studied the evolution of root reserves of lucerne through the cuttings (Demarly 1957,Smith 1962, Dobrenz and Massengale 1966, Cooper and Watson 1968, Talamucci 1970, Gosse et al. 1982).

(*) This work was supported by funds from Ministry of Agriculture (Piano Foraggero Zootecnico).

Concerning the model of the lucerne meadow, we think this model has to assure the stability either from the morphological or from the demographical point of view. We propose a model based on the genetic homogeneity for the characteristics indicated in Table 1 without considering the relationship with Rhizobium and the degree of desease resistance.

Table 1. Model of lucerne meadow

Genetic homogeneity for the following characteristics:

1. Moment of the root reserve recovery
2. Moment of regrowth
3. Velocity of growth and development
4. Number and lenght of stems
5. Moment of starting first blue bud stage

In connection with this model, the ideotype of the plant apt to give a vigorous and persistent lucerne meadow should have the traits indicated in Table 2.

Table 2. Ideotype of lucerne plant

1. Root reserve: total recovery at starting blue bud stage
2. Regrowth: early, conspicuous and stable through the cuttings
3. Number of stems: great and stable through the cuttings
4. Blue bud stage: early and homogeneous for all stems
5. Lenght of stems at the first blue bud stage: high, homogeneous and stable through the cuttings

The present experiments are aimed to verify the validity of such models and to improve them chiefly concerning the role of root reserve recovery for forage production and lucerne meadow persistency.

Material, methods and techniques

The program includes the following experiments:

1. Boxes: plots (diametre 40 cm, heigth 82 cm) with 30 plants at the distance of 2.5 cm (dense sward). These boxes are assembled to make a field (Figure 1). Cultivar utilized: Equipe.

2. Boxes: plots (diametre 40 cm, height 82 cm) with 4 plants per box. These boxes are isolated in the field. Cultivar utilized: Equipe.

3. Boxes: plots (diametre 40 cm, height 82 cm) with 36 plants. The boxes are isolated in the field. Cultivars utilized: Equipe (Italy), Victoria (Spain), Tula and Kometa (Poland), Sewa (Egypt), Yasdi (Siria).

4. The same cultivars of experiment n. 3 are studied in growth chamber with light and temperature under control, simulating the Central Europe and Mediterranean areas.

For lack of space, only experiments n. 1 and n. 2 are described, in which the aerial part and the root are studied at the follonwing biological stages:

1) flowering (about 50%)
2) 3 nodes in the stems (about 50%)
3) 5 nodes in the stems (about 50%)
4) 7 nodes in the stems (about 50%) (in the second year only)
5) green bud (about 50%)
6) blue bud (about 50%)

All these biological stages the following traits are studied on individual plant basis:
a) aerial part: height of all stems, dry matter (leaves and stems), protein and sugar content of leaves and stems.
b) roots: shape, lenght, presence of root nodules, dry matter, protein and sugar content.

In experiment n.1, 4 boxes/plots are studied at each stage in the first year, and 5 boxes/plots at each stage in the second year. In experiment n. 2 (spaced plants) at each stage at least 16 plants are studied.

Results and discussion

Figures 3 and 4 represent the trend of variation in dry matter of the aerial part (leaves + stems) and of roots during the different cycles.

The following observations can be made:

1. The curves concerning the aerial part and those concerning the roots are of the same type either in dense sward or in spaced plants.

2. At the first year in both experiments (normal stand and spaced plants) the aerial part decrease in weight during the cuttings, while the roots increase. In the second year by contrast either the aerial part or the roots decrease, in dense sward, from the first to the third cutting. At the 4 th cutting this trend is modified because mortality reaches 50%.

3. After each cutting, in both years, the dry weight of roots decreases during the first phases of regrowth. In the first year, the root reserves recovery is completed before blue bud stage; in the second year it is never completed after the first cutting till the 4 th one, when mortality affects the meadow structure.

4. During the winter time a part of root reserves is lost as it has been observed by many Authors. The decrease is about in spaced plants and about in dense sward. The regrowth after winter (first cycle) is not accompanied by an evident loss in root dry matter, as it occurs in the following stages of regrowth.
In Figure 5 it is represented the trend of dry matter weight of leaves and stems through the different biological stages during three cycles of the first year. At the green bud stage, in dense stand, the dry weight of leaves overcomes that of the stems. At the blue bud stage the opposite true. After this stage, any increase in yield is due to stems only.
5. The coefficients of correlation between the aerial part and the roots for dry weight are 0.83** at the first cutting (2.7% of flowering), 0.91** at the second cutting (47% of flowering) and 0.92** at the third cutting (50% of flowering).

Conclusions

At a first and summary reading of the presented data we can conclude that the analysis of the lucerne meadow structure gives great information either on the management criteria (exploitation) or on the objectives, the methods and the techniques of breeding.

The model of lucerne meadow (Table 1) and the plant ideotype (Table 2) should be improved when the dynamics of the root reserves recovery in the different seasons will be known. This is one aim of the program in progress. In any case, it is evident that the root reserves recovery at the first blue bud stage is important, for two reasons: first, because by this way the plant becomes resistant to the frequent cutting regime, and therefore a great demographical stability can be obtained; second, because by cutting at this stage we obtain a good quality of the forage and, at the same time, a great production of protein /Ha.

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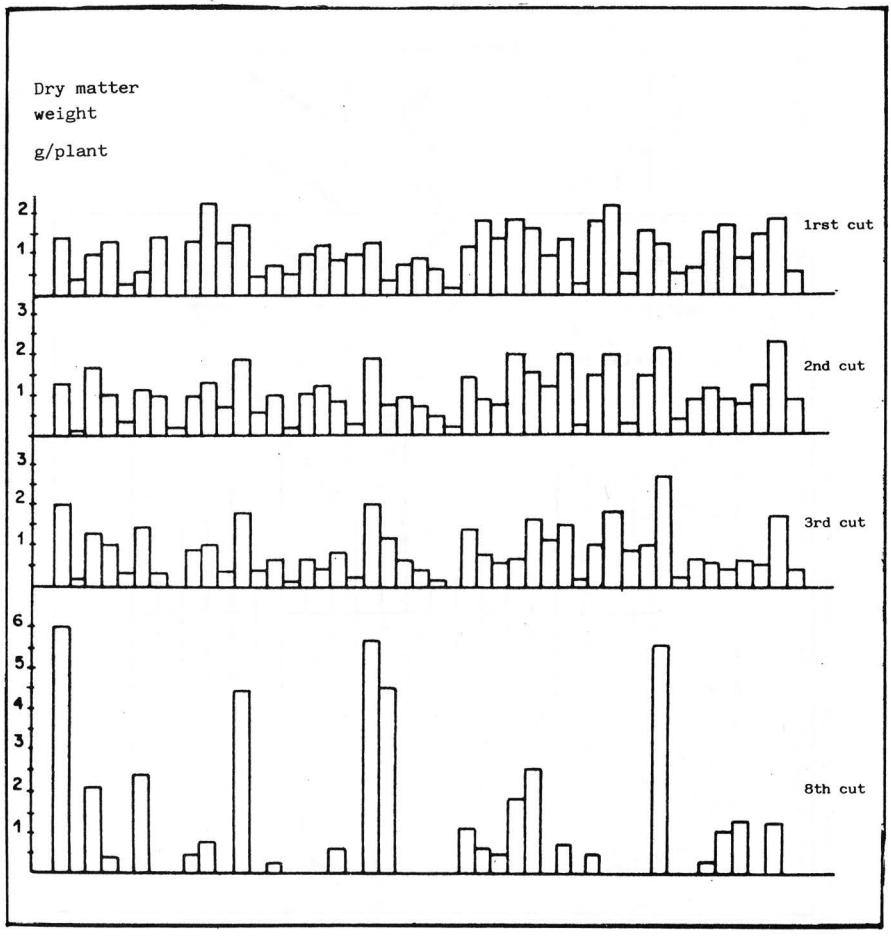

Figure 1. Variation in structure of a row of lucerne meadow, 64 cm long.
Abscisses: the position of individual plants, 1.4 cm apart.
Ordinates: the dry matter weight of individual plants, in grams.

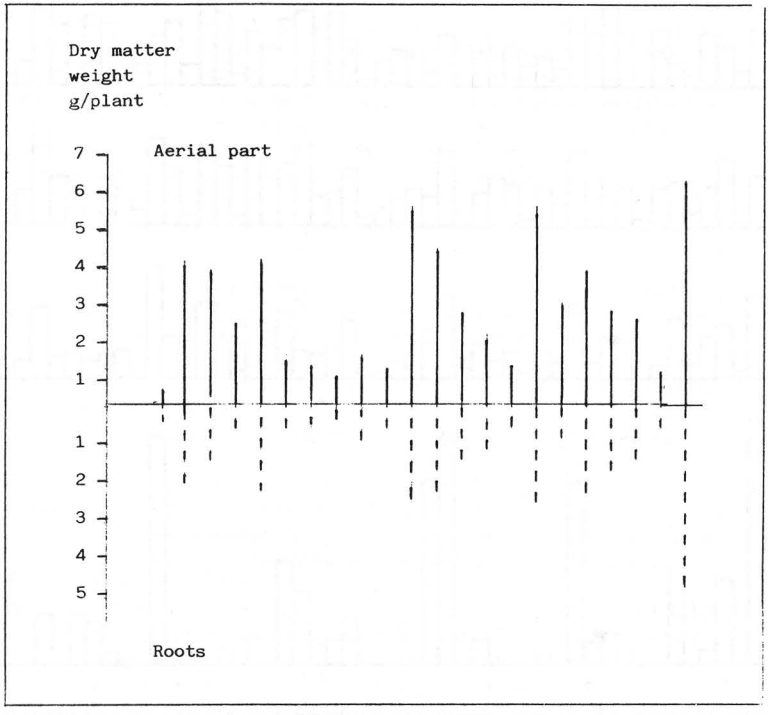

Figure 2. Dry matter yield of aerial part (leaves + stems) and roots of individual plants 2.5 cm apart on the row.
Abscisses: the position of individual plants on the row
Ordinates: dry matter weight in g/plant

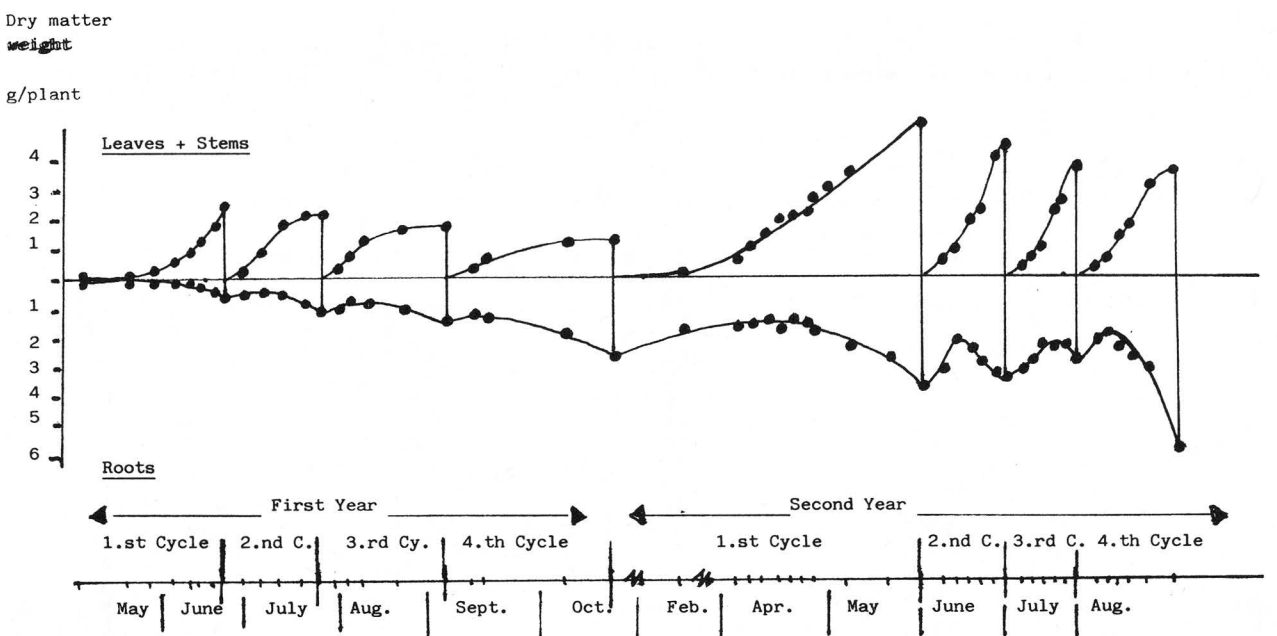

Figure 3. Lucerne dense sward. Trend of dry matter yield of aerial part (leaves + stems) and roots through the cuts. Abscisses: dates of cuttings. Ordinates: dry matter yield in grams.

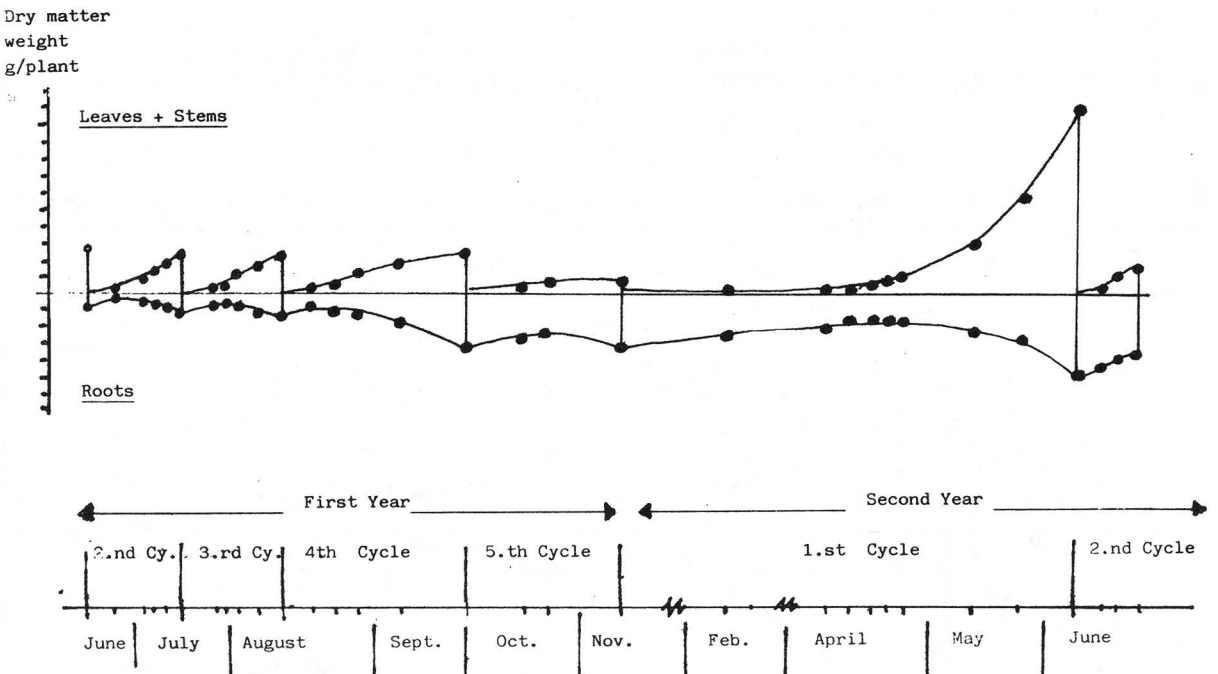

Figure 4. Lucerne spaced plants. Trend of dry matter yield of aerial part (leaves + stems) and roots through the cuts. Abscisses: dates of cuttings. Ordinates: dry matter yield in grams.

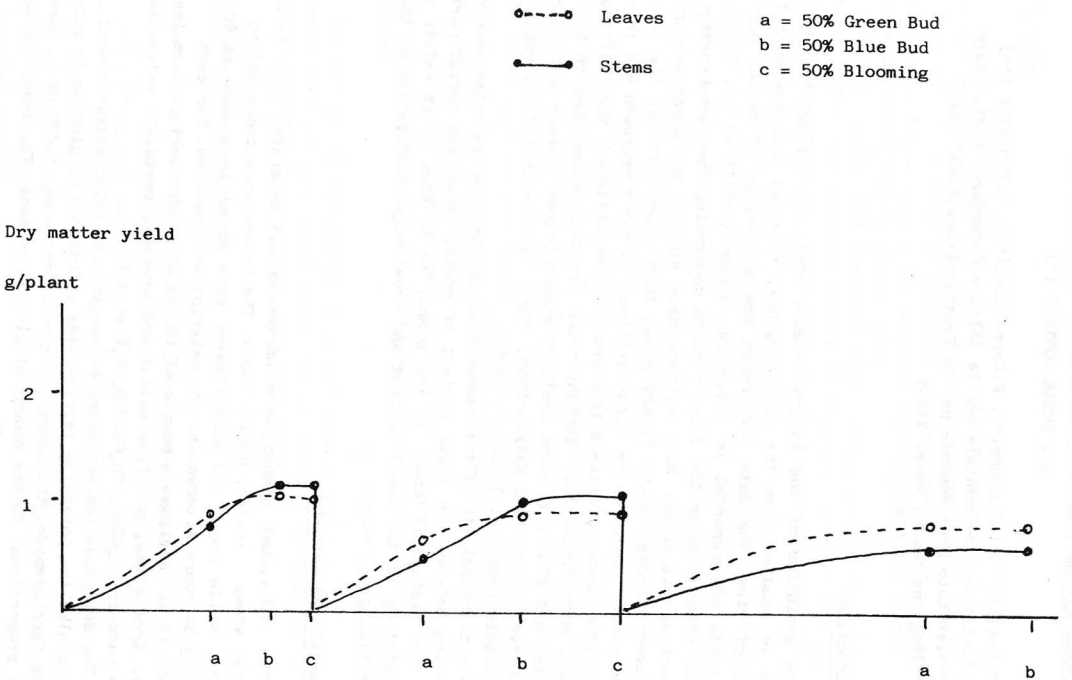

Figure 5. Lucerne dense sward. Trend of dry matter yield of leaves and stems through the cuttings. Abscisses: stages of cuttings. Ordinates: dry matter yield in grams.

LUCERNE MEADOW STRUCTURE. ANALYSIS OF THE AERIAL PART AND ROOTS. II. SUGAR CONTENT (°)

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Introduction

The evolution of the lucerne meadow structure through the cuttings is studied from the morphological, chemical and biochemical point of view. The data concerning the morphology of aerial part and roots are reported in a previous paper (Rotili et al. 1985). In the present paper the first results concerning the carbohydrates content are reported. Many Authors have studied the sugar content in lucerne roots. Smith (1962) shows that after cutting, the root carbohydrate reserves are first utilized in shoot regrowth and then replenished once sufficient leaf area is restablished. When alfalfa is cut more frequently, carbohydrates are not replenished to high levels, and plants becomes smaller, stands become thinner and yield drecreses (Cooper and Watson 1968, Ueno and Smith 1970, Rapoport and Trovis 1984, Rotili et al. 1985).

The principal aim of our research is :a) to know the relationship existing between the sugar content of roots, crown and aerial part and the biological stage of the plant; b) to know if it exists a variability in the level of root and crown sugar restoration at the same biological stage.

Material and methods

The individual plants were harvested and separated into three parts: stems + leaves, crown, roots. The crowns were conserved at -20°C, while roots and stems+leaves were dried in a owen at 60°C during 84 hours.Afterwards, the material was powdered. For each plant it was analysed a sample of 100 mg of powder derived from leaves, from stems, and from distal and proximal roots.The extraction was made by CH_3COON_2-CH_3COOH-H_2SO_4(Figure 1).

The determination was based on the inverse colorimetric method in which alkaline potassium ferricyanide is reduced by glucose to colorless ferricyanide. The decrease in color measured at 420 nm is directly proportional to the amount of glucose present. The apparatus was a technicon autoanalyser 2°.

(°) This work was supported by funds from Ministry of Agriculture (Piano Foraggero Zootecnico).

Results

In the present paper the first results concerning the spaced plants are reported. Figures 2,3,4 show the results of the first cycle and of the first biolgical stage after the second cutting (3 nodes). The materials are the same already described in the previous note (Rotili et al. 1985).

Figure 2 shows that the total sugar content is more than four times higher than reducing sugars. This is well evident both in aerial part and roots. These results are in agreement with those obtained by others Authors.

The analysis made separately for the distal and the proximal part of roots (Figure 3) shows the following characteristics:

a) The total sugar content is about four times higher than the reducing sugars.

b) The total sugar content of distal roots is almost always over that of proximal roots.

c) The trend through the biological stages is quite different in total sugars compared with reducing sugars: in total sugars it is evident a significant depression at the first stage after the cut, but already at the second biological stage after the cut the total sugar content reaches again the initial values (first cut, full blooming stage). By contrast, the reducing sugar content shows a great stability.

Finally, in the aerial part of the plant (Figure 4) both total and reducing sugars are highly stable through the biological stages.

Concerning the variability, Figure 5 shows a great difference between proximal and distal roots: the dynamics is the same but a greater variability is evident in distal roots.

Discussion and Conclusions

The most interesting aspects of these first results are:

a) A higher variability in the decrease of sugar content just after the cutting and during the following phase of restoration.

b) No correlation exists between the dry matter yield of the plant and the sugar content.

c) No correlation exists between the biological stage of the plant and the sugar content.

A better knowledge will be possible when the data concerning different seasonal cycles as well data concerning dense sward will be at our disposal.

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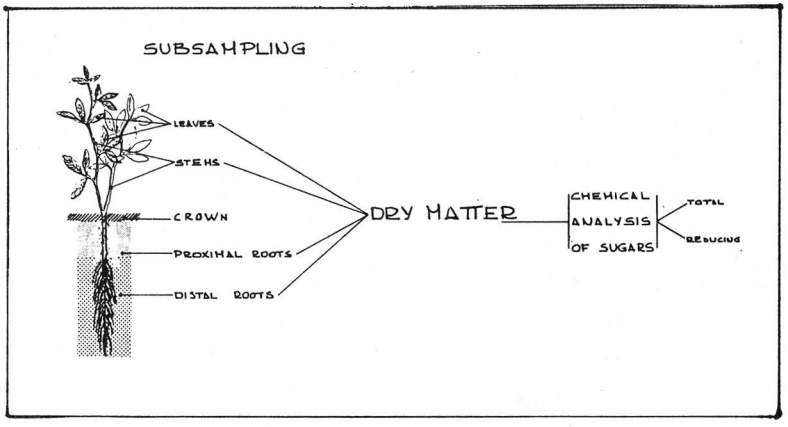

Figure 1. Description of the aerial part and root subsampling for sugar content analysis.

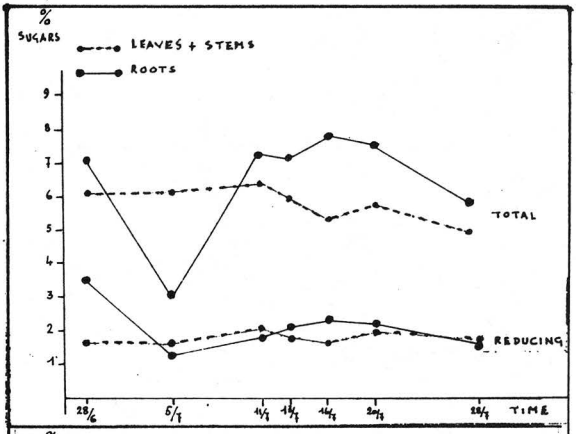

Figure 2. Trend of sugar conten through the different stages.

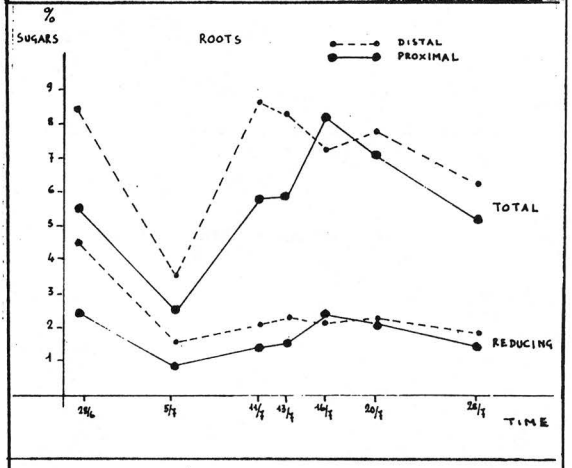

Figure 3. Trend of sugar conte in proximal and dist roots.

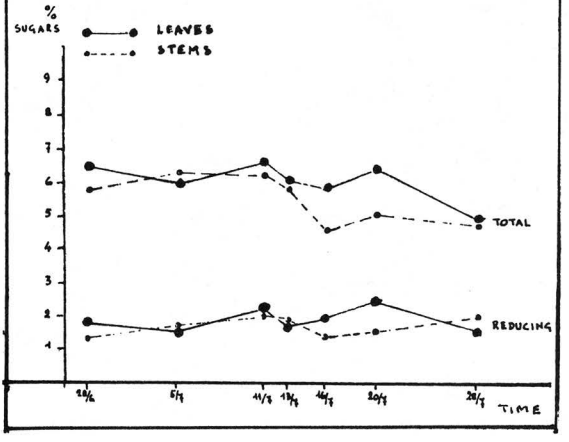

Figure 4. Trend of sugar conte in leaves and stems.

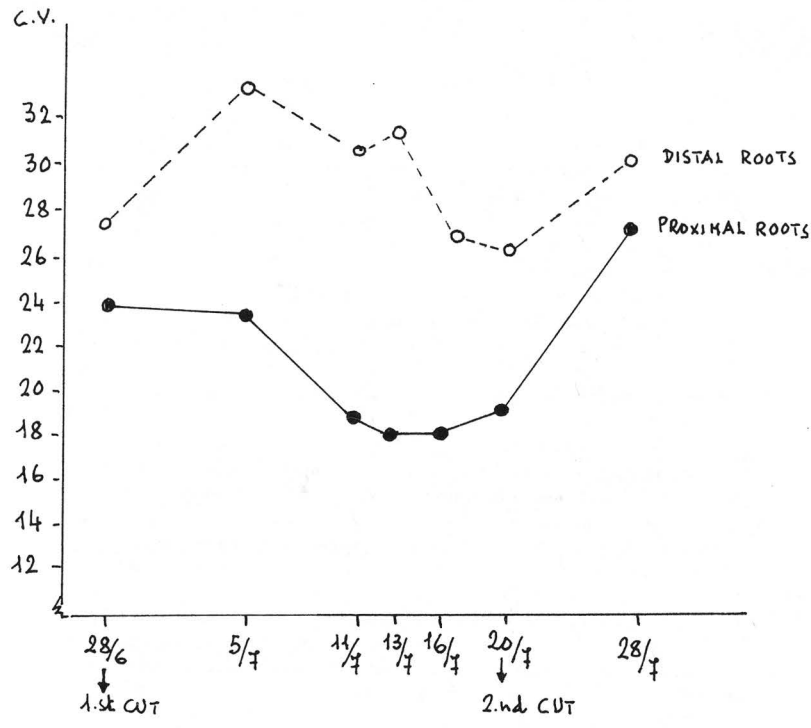

Figure 5. Trend of the coefficient of variability of the sugar content of proximal and distal roots through the different stages.

EVALUATION OF THE GRASS-AZOSPIRILLUM ASSOCIATIONS.(°)

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Introduction

The study of the associations between Azospirillum spp. and forage grasses began at the Istituto Sperimentale per le Colture Foraggere in Lodi since 1983.

Our aim was to verify the effectiveness of the inoculation with Azospirillum in the intensive farming system of the temperate regions.

In fact an active diazotrophic association between Azospirillum spp. and the root system of various Gramineae was proved (Döbereiner and Day, 1976).

Besides, we wanted to test the existence of specificity in plant-bacteria association both at the species or varieties level.

Previous experiments carried out at Lodi (Marocco et al. 1983) with the forage grasses Festuca arundinacea and Dactylis glomerata and two Azospirillum brasilense strains, showed a significant positive effect of the association with both the strains in Festuca. Dactylis glomerata responded positively only to one strain inoculation: this result could indicate that a plant-bacteria specificity exists.

The highest increases were found at intermediate nitrogen fertilization (60 U/ha) and at the first cut; no correlation seemed exists between the effect of the inoculation and the survival of bacteria in the rizhosphere.

The present paper reviews the whole set of experiments carried out at Lodi in 1984-85 to test the previous results.

Materials and methods

The materials and methods utilized in these experiments are summarized in Table 1.All the azospirillum strains were testes in the laboratory for the nitrogenase activity.

(°) This work was supported by funds from Ministry of Agriculture (Programma Speciale Tecnologie Avanzate).

Table 1. Description of the experiments.

	Experiment 1	Experiment 2	Experiment 3
Year	1983-84: greenhouse	1984 : outside	1985 : outside
		concrete boxes : 150 lenght, 25 cm large, 40 cm depth	
Plant species	Festuca arundinacea cv. Manade Dactylis glomerata cv. Dora	Festuca ar.:"european" cv.:Magno, Manade, Kenwell, Clarine :"mediterranean"cv.:Gloria,M.Kasba	Festuca ar.: Magno, Manade, Kenwell
Azospirillum	Azsp.brasilense SpF267 gentamicine resistant mutant	Az.br.:CD(pink coloured mutant),SpF 267, 242, Sp 7, Sp 13T, 107 Az. Lipoferum: Al 59, A3a, USA 5a	Az.br.: CD,SpF 267, Sp 7, MEA 4/8 } metilammo MEA 4/11 } nium res.
Treatments	N_o-Sp_o (control); N_o-Sp_1 N_1-Sp_o ; N_1-Sp_1	54 associations; 6 controls	15 associations; 3 controls 5 inoculated soil without plants
Experimental plot size	27 plants in single row	18 plants in single row (2.8 cm between plants)	36 plants in two rows of 18 (2.8 cm between plants ;15 cm between rows)
		In each box, inoculated with the same Azsp. strain,all the cv.are always present	
Experimental design	Randomised block (12 replications)	Randomised block (5 replications)	Randomised block (9 replications)
Sowing date	15/12/1982	25-27/10/1983	3-5/4/1985
Fertilization	P_2O_5 150 u/ha ; K_2O 250 u/ha N: 30 u/ha at spring regrowth and after each cut		P_2O_5: 200 u/ha; K_2O 400 u/ha N: 30 u/ha 45 days after sowing and " each cut
Inoculation	10^8 cells diluted in 2ℓ/box at the same time of N fertilization	10^8 cells diluted in 3ℓ/box at spring regrowth and after the 1st, 3rd, 4th cut	10^6-10^7 cells in 3ℓ/box at the same time of N fertilization
N of cut and days between cuts	6 (26-37)	6 (29-41)	4 (28-29)
Characters examined	D.M. yield (g/plot); ear number at 1st (and 2nd) cut		nitrogen content bacterial counts on roots before and after sterilization

Results and discussion

Experiment 1

No significant effect of the inoculation with Azospirillum was found irrespective of the nitrogen fertilizer level (0, 30 U/Ha) both in Festuca and in Dactylis. On the contrary, the nitrogen fertilization increased significantly the yield both in inoculated and not inoculated treatments (Figure 1).

These results differ from the previous ones (Marocco et al. 1983), but it can be noticed that, despite the quantity for application was the same (30 U/Ha), the total quantity and the frequency of nitrogen fertilization was higher in 1984 than in 1983 (180 U/Ha in 6 times vs 60 U/Ha in 2 times). Nevertheless, the inoculation with Azospirillum had, after the first cut, a stable and positive effect that ranged from 3 to 11%, in Festuca without nitrogen. It can be noticed that there was no difference in the ear number between treatments in Festuca at the first cut, while Dactylis glomerata not added with nitrogen remained at a vegetative phase compared to fertilized treatments.

Experiment 2

The effect of inoculation with Azospirillum strains was not significant on average (Table 2), but the interaction variety x Azospirillum stra ins was significant (Figure 2).

Each strain had a specific and different effect on the various cv. of Festuca; 26 plant-bacteria associations, compared with the whole 54, performed better than the control not inoculated; the effect of inoculation on yield ranged from +19% to -34%. The differences between treatments were evident since the 1.st-2.nd cut and remain substantially stable during the cuts (Figure 3): the ear number was nor responsible for these differences, at the first and second cut.

These results seem to indicate that the Festuca-Azospirillum association doesn't imply the nitrogen fixation only, but more complex relationship that can lead also to a decreasing yield (Patriquin et al. 1982).

Experiment 3

To test the results of experiment 2, three cultivars, with a specific and different behaviour in association with Azospirillum strains, were chosen among the previous six.

The effect of inoculation is significant, but negative (from -2,5% to -9%) compared with control not inoculated : no association yields more than the control (Table 3, Figure 4).

The variety x Azospirillum strain interaction is not significant and the rank of associations doesn't agree with the experiment 2. It can be noticed that the association with Azospirillum resistant to metilammonium are not different from the control.

The survival of bacteria in the rizosphere is followed collecting two plants for each treatment and computing the number of bacteria before and after the sterilization of the roots. The isolation of inoculated bacteria in non-sterilized soil is difficult except for mutant strains as CD and SpF 267: the results (Table 4) show a general decrease of bacteria population from the inoculation to the cut (28-29 days), till to complete loss.

Conclusions

- The association with Azospirillum strains has an effect on the forage grasses Festuca arundinacea and Dactylis glomerata; this effect can be positive or negative and not always significant, but detectable.
- From our experimental data, it doesn't seem possible to speak about a plant-bacyeria specificity because the results of the two experiments carried out in 1984 and 1985 are contradictory.
- The study of plant-bacteria associations in field conditions demands, to be successfull, more suitable techniques to study the different factors concerning the association "in situ" and above all the dynamic evolution of the demography of bacteria populations.

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Patriquin, D.G., 1982. Review in Adv. in Agricultural Microbiology, SUBBA RAO Ed. 139-175.

Table 2. Experiment 2. Significance of ANOVA at each cut and on the average of 6 cuts 1984.

Progressive number of cut	1	2	3	4	5	6	Average		Average of associations of experiment 3 only
Treatments (azosp.strains + control)	1.04 ns	0.22 ns	0.19 ns	0.18 ns	0.13 ns	0.22 ns	0.09 ns		0.45 ns
Varieties	136.33 **	45.01 **	133.17 **	197.66 **	177.19 **	77.85 **	140.56 **		2.73 ns
Treatment x Variety	1.04 ns	1.35 ns	1.57 *	1.31 ns	1.65 **	1.32 ns	1.47 *		2.01 *

Table 3. Experiment 3. Significance of ANOVA at each cut and on the average of 4 cuts 1985.

Progressive number of cut	1	2	3	4			Average		
Treatments (Azosp.strains + control)	8.25 **	2.60 *	3.22 **	2.84 *			7.94 **		
Varieties	19.76 **	51.50 **	104.71 **	112.99 **			120.65 **		
Treatment x Variety	0.31 ns	0.45 ns	0.85 ns	0.48 ns			0.82 ns		

* = P 0.05

** = P 0.01

		1st cut			2nd cut			3rd cut			4th cut		
		Inocul.	NS root	S root	Inocul.	NS root	S root	Inocul.	NS root	S root	Inocul.	NSroot	S root
	MAGNO	$1.2x10^6$	-	-	$3.9x10^6$	$1x10^3$	-	$1x10^6$	$1.5x10^3$	-	$1x10^6$	-	
	MAN		$1x10^4$	-		-	-		$1.5x10^3$	-		-	
	KENW		$2x10^4$	$2x10^3$		$5x10^3$	-		-	-		-	
	AZSP. without PLANTS		-			-			-			-	
	MAGNO	$0.7x10^6$	-	-	$0.4x10^6$	-	-	$0.4x10^6$	-	-	$0.4x10^6$	-	
	MAN		-	-		-	-		$5x10^3$	-		-	
	KENW		-	-		$1x10^4$	-		$5x10^2$	-		-	
	AZSP without PLANTS		-			$2x10^3$			-			-	
	DATE	14/5	17/6		18/6	15/7		18/7	5/8		12/8	4/9	

NS root = non sterilised root

S root = sterilised root

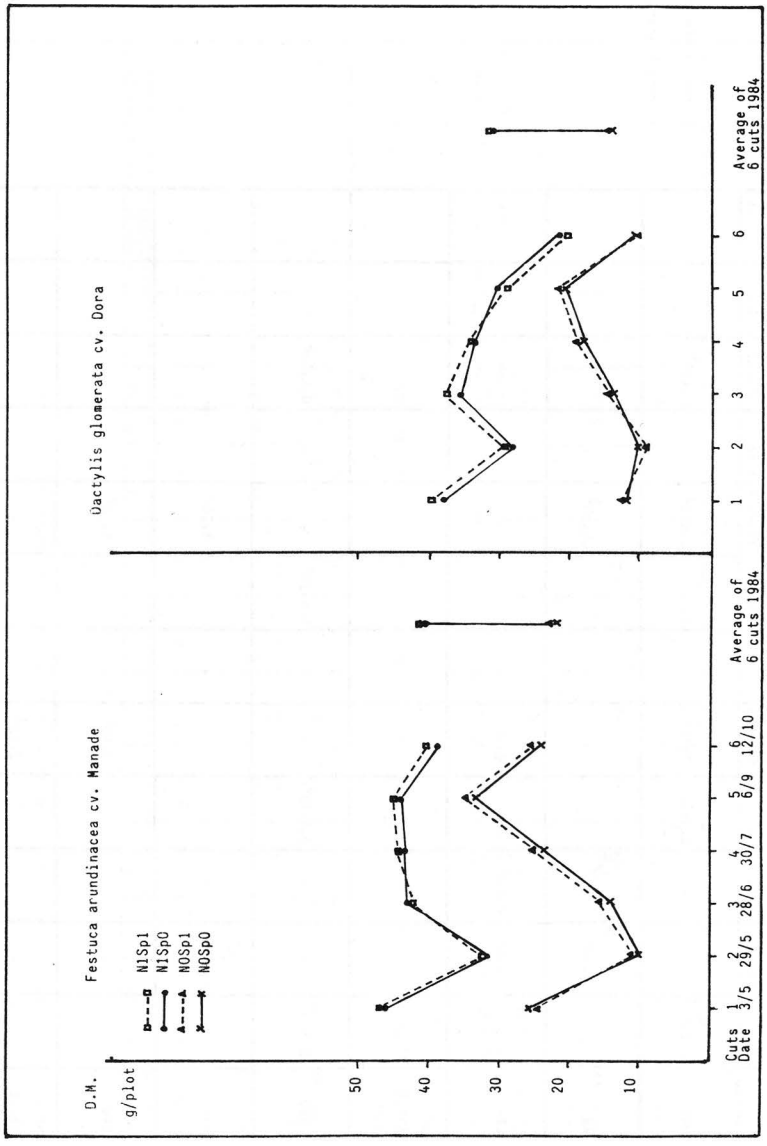


Figure 1. Experiment 1. Dry matter yield at the different cuts and on the average of 6 cuts 1984.

Figure 2. Experiment 2. Dry matter yield of Festuca ar.-Azospirillum associations (averages of 6 cuts 1984).

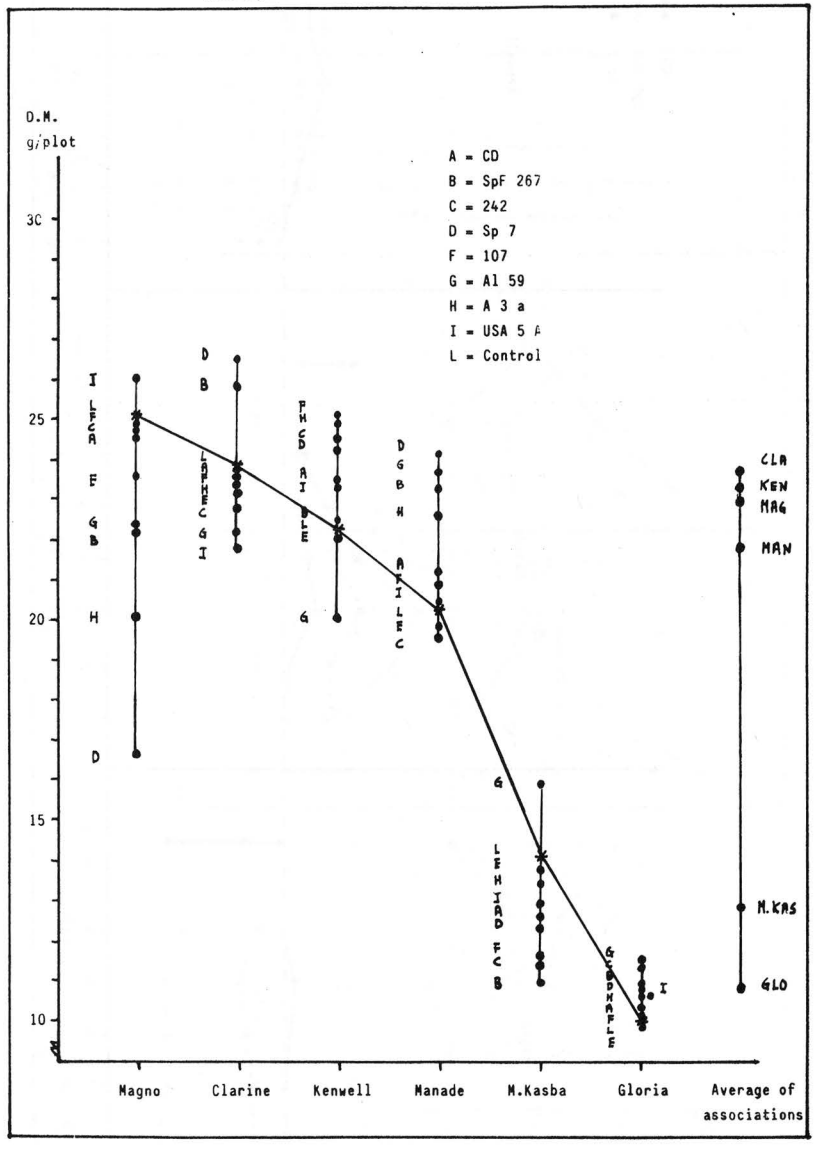
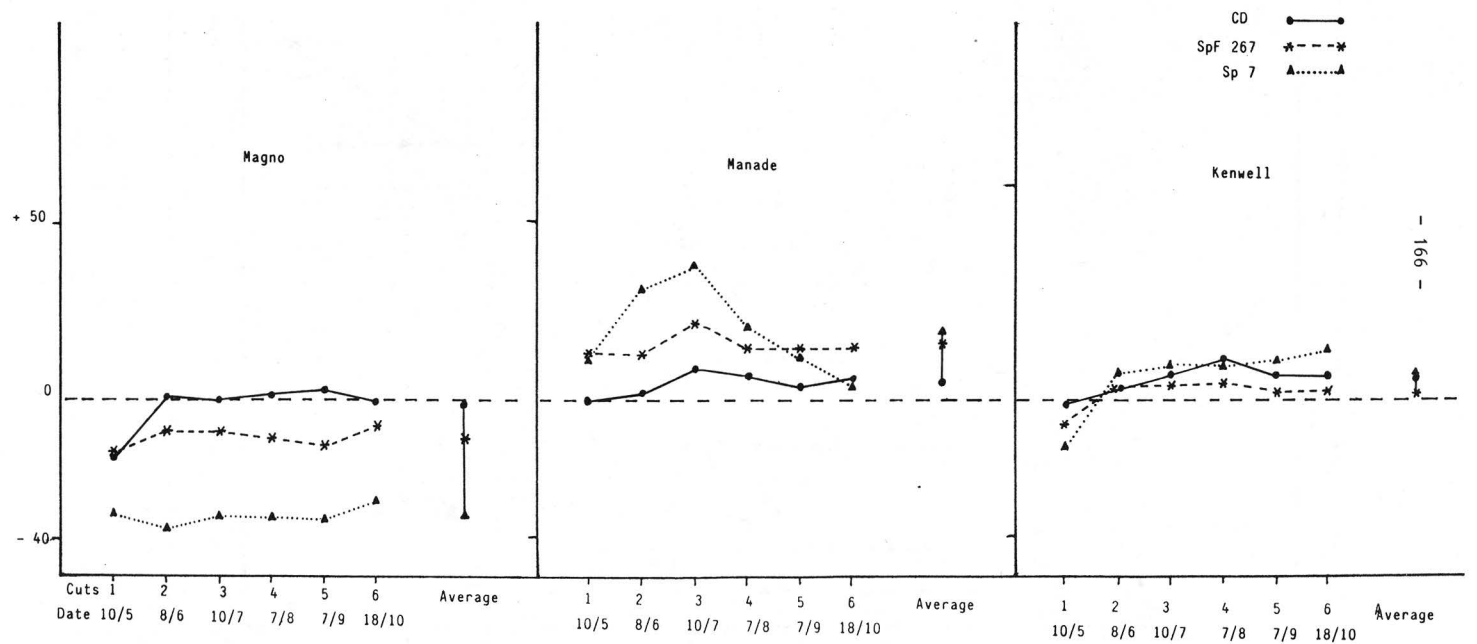


Figure 3. Experiment 2. Dry matter yield of the plant-bacteria associations expressed in % of control not innoculated.



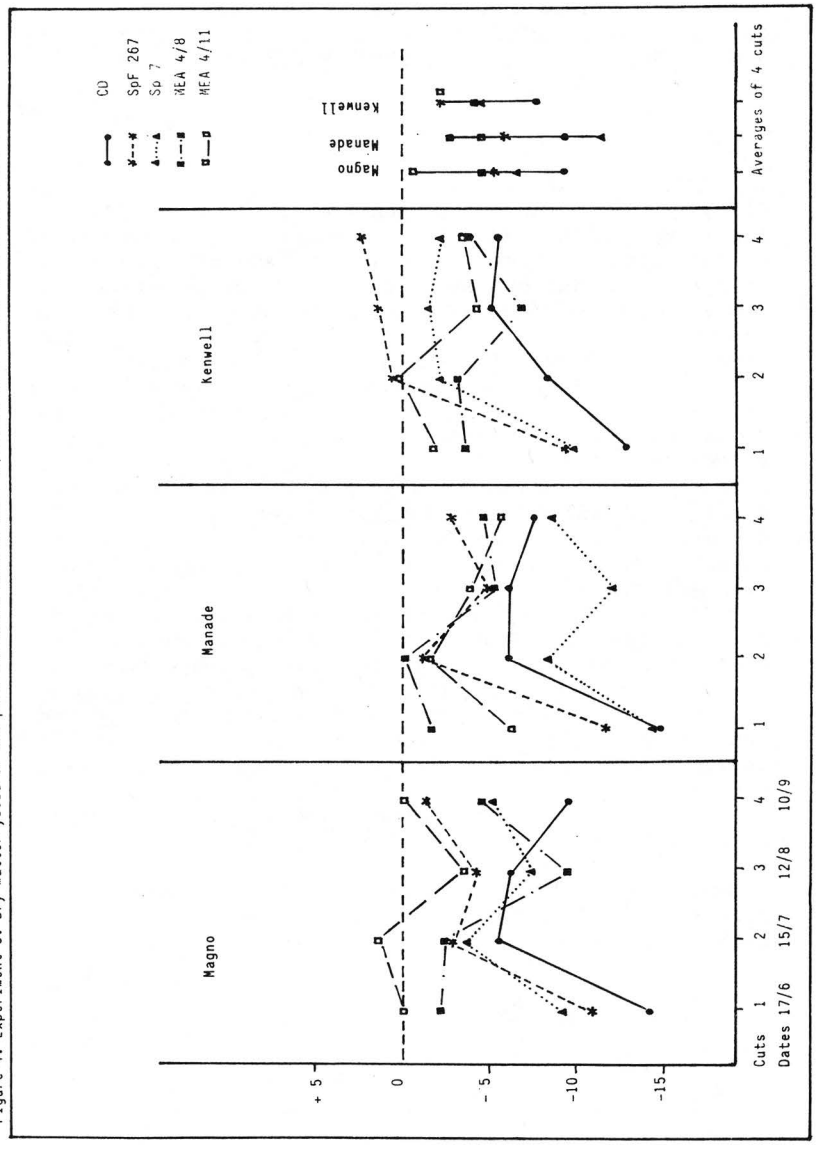


Figure 4. Experiment 3. Dry matter yield of the plant-bacteria associations expressed in % of the control not inoculated.

Enzymatic and Ribosomal DNA Spacer Length Polymorphism in Natural Populations of Ryegrass

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Introduction

Electrophoretic analysis of genetic variation at allozyme loci let, beside a study of biosystematic, the knowledge of geographic pattern of variability and of the genetic structure of natural populations. Since adaptive and evolutionary processes of the species are related to the ecological environment it is of interest to study the genetic structure of populations and investigate the possible relationships with environment. Previous papers showed, in some species, associations between allozyme variation and environmental factors (1,2) suggesting a better way for sampling and using germplasm. More recently methods based on DNA analysis, that allow a new class of genetic polymorphism, have been developed; the detection of genetic variation by this way is expected to reveal a higher level of resolution. This paper deals with the isozymatic characterization for 2 loci (PGI-2 and GOT-3) of 32 populations of ryegrass (Lolium perenne L.) sampled in Italy from many geographical and ecological areas and with preliminary experiment to characterize DNA polymorphism for ribosomal DNA (rDNA) spacer length. Aim of this work is: 1) to know the genetic variability of natural populations at the two loci; 2) to assess possible relationships between particular isozyme phenotypes and environmental variables; 3) to detect the presence of rDNA spacer length polymorphism.

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* work partially carried out in the lab directed by prof.R.W.Allard, Department of Genetics- University of California, Davis.

Materials and Methods

Seed samples of 32 populations of ryegrass were collected in as many sites scattered on the Italian peninsula which environmental data are reported in table 1. Enzyme extraction, electrophoresis and staining have been carried out as described by Hayward and McAdam (3). For each locus Shannon-Weawer diversity index (H), cluster (UPGMA) and principal component analysis have been carried out. Means of environmental data and of H of populations clustered togheter have been compared with the "t" test. Ribosomal DNA spacer length variation was assaied on only two populations (21 and 23), DNA preparation, digestion, electrophoresis, blotting and autoradiography was carried out as described by Saghai-Maroof et al (4).

Results

1. Both for PGI-2 and GOT-3 5 alleles were found, the three (A,B,C) observed by Hayward and McAdam (3) plus a faster (X) and a slower (D) allele. B allele is in general the most frequent. The rarest ones (X and D) are particularly frequent in the Southern populations.
2. The allelic diversity index ranges from 1.01 to 2.04 for PGI-2. The highest values of H are those of Southern populations. GOT-3 H values, ranging from 0.00 to 1.21, vary regardless the population origin (tab.1).
3. Cluster analysis, based on allelic frequencies, allows to group the populations in four clusters (1,2,3,4), each of them characterized by a predominant presence of one allele . The cluster 1 (characterized by B allele predominancy) includes the large majority of populations; for PGI-2 locus, differently respect to GOT-3, in the clusters 2,3,4 are present South-Italian populations only (fig.1,2).
4. Mean values of almost all environmental parameters characterizing the populations belonging to PGI-2 cluster 1 are significantly different respect to the other three clusters, which substantially do not differentiate each other. This phenomenon is less evident for GOT-3. These results suggest a relationship between allelic frequencies and environment (tab.2). H values for PGI-2 averaged on populations pooled on the base of their Thornwaite's humidity index (Im) decrease with the

increase of humidity, as matter of fact humid group ($Im \geq 100$) and semi-arid group ($-20 \leq Im \leq 0$) have significantly different values of H (1.34 vs 1.60).
5. Analysis of rDNA spacer length displaies six differently sized variants ranging from 7.7 to 6.2 Kbp. Intergenotipic polymorphism is observed. The two populations, although coming from close, both geographically and environmentally, sites can be differentiate on the base of the frequencies of the variants(tab.3).

Discussion

PGI-2 locus shows, differently from GOT-3, a variability for allelic distribution moving from the northern, cool and humid, areas to the semiarids of the South. It suggests a possible role of PGI-2 or of a linked locus on population evolution, the most interesting result is, however, the increasing of diversity index in the drier areas that let assume a different kind of environmental selective pressure and/or a different kind of population reproductive biology. DNA analysis, that for the gene examined results suitable for genetic of population studies in ryegrass,could supply more adequate tools to detect these phenomena.

Aknowledgements: thanks are due to prof. R.W.Allard for his kind hospitality and to dott. P.D.Cluster for his valuable advices.

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Table 1. Environmental data for 32 sites in which collection was made: 1. latitude(°'N), 2. elevation(m a.s.l.), 3.average year temperature(°C),4. average coldest month temperature(°C), 5. average hottest month(°C), 6. annual rainfall(mm), 7. annual evaporation(mm), 8. Thornwaite's humidity index; 9 and 10. Shannon-Weawer diversity for PGI-2 and GOT-3.

sites	1	2	3	4	5	6	7	8	9	10
1	2631	410	14.7	6.0	23.9	1209	821	56	1.46	0.67
2	2646	200	15.3	6.9	23.8	1326	813	69	1.54	0.87
3	2672	280	13.0	4.5	21.6	1675	754	125	1.59	0.43
4	2704	250	12.6	0.7	24.0	844	785	14	1.59	0.94
5	2758	640	3.8	-4.7	12.0	833	661	26	1.02	0.68
6	2709	60	13.3	1.1	24.3	635	418	-8	1.41	0.99
7	2724	30	13.2	1.8	23.5	872	794	18	1.47	1.01
8	2574	800	12.6	4.4	21.6	1050	731	52	1.70	0.99
9	2574	900	10.6	0.5	19.8	1070	700	49	1.48	1.20
10	2541	0	15.5	7.2	24.6	611	836	-10	1.38	0.78
11	2545	100	14.9	6.4	23.8	907	807	24	1.34	0.94
12	2596	310	14.9	5.8	24.6	827	834	-4	1.68	1.02
13	2619	1050	11.9	2.6	21.2	1220	718	76	1.31	0.69
14	2630	0	12.9	3.8	22.0	768	750	13	1.54	0.86
15	2558	660	11.8	2.0	16.0	839	713	26	1.58	0.70
16	2558	1450	11.0	0.2	15.0	890	690	49	1.58	0.44
17	2574	200	14.9	5.8	24.3	814	831	9	1.62	0.59
18	2569	120	15.4	6.4	25.0	774	851	4	1.26	0.51
19	2520	0	16.1	7.5	25.3	720	862	0	1.69	0.67
20	2501	0	15.1	6.6	25.3	542	854	-17	1.43	0.49
21	2473	540	15.3	7.2	24.4	595	833	-12	1.49	0.71
22	2460	0	16.4	9.0	24.8	592	869	-13	1.93	0.70
23	2445	500	14.5	6.5	23.5	651	801	- 1	1.90	1.21
24	2422	0	16.5	9.0	25.9	567	880	-19	1.49	0.89
25	2345	60	17.8	10.1	26.6	673	916	- 5	1.94	0.57
26	2352	1300	8.8	0.6	17.5	1411	592	148	1.41	1.08
27	2338	300	16.6	9.2	24.8	1168	854	50	1.08	0.20
28	2388	350	15.3	6.5	25.0	969	803	36	0.92	0.00
29	2438	840	12.5	3.9	21.8	741	729	15	1.71	0.66
30	2508	1050	12.0	3.3	21.6	992	715	50	2.04	0.73
31	2733	450	13.2	2.3	23.4	1110	783	32	1.45	0.81
32	2735	300	12.7	2.6	22.9	1702	766	120	1.01	----

Table 2. Significance test among the average values of environmental parameters of the populations grouped in 4 clusters.

	PGI-2 cluster				GOT-3 cluster			
	1	2	3	4	1	2	3	4
Average annual temp.	a	b	b	b	a	a	b	b
Average coldest month	a	b	bc	c	a	a	b	c
Average hottest month	a	bc	ab	c	a	a	b	b
Annual rainfall	a	b	b	ab	a	ab	bc	c
Annual evaporation	a	b	ab	b	a	b	c	c
Thornwaite's index	a	b	ab	b	a	a	b	c

Clusters with same letter do not differ significantly per $P \leq 0.05$

Table 3. Frequencies % of the rDNA spacer variants in the populations 21 and 23.

	Length Kbp					
rDNA spacer variants	7.7	7.4	7.2	7.0	6.5	6.2
Pop. 21	-	-	100	50	75	100
Pop. 23	39	6	78	22	94	78

Fig.1 Global structure of PGI-2

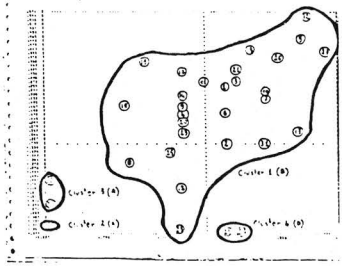
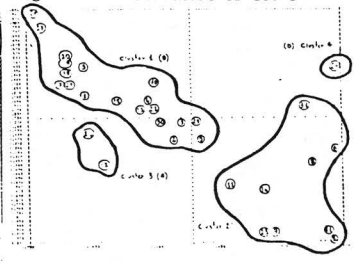


Fig.2 Global structure of GOT-3



Transformation of grasses

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Introduction

In the last few years several methods for plant transformation have been presented.
The best known method is the infection of wounded plants with agrobacterium tumefaciens which induces a tumor at the wounded site by introducing a T-fragment of this Ti-plasmid into the plant chromosome. This method seems very efficient and stable, but infection of monocots is practically impossible (1).

In order to overcome these host problems, protoplasts are transformed by cocultivation with A. Tumefaciens or plants are transformed directly by introducting naked DNA into protoplasts. Until now however, the production of protoplasts and further regeneration of gramineous plants is, with a few exceptions, not possible (2).

Therefore we developed a system of transformation of seed with naked DNA in order to overcome regeneration problems (3).

Transformation of grasses by the seed incubation method.

In an attempt to transform monocotylous plants, we used the method of incubation with germinating seeds. Therefore peeled axenic seeds are incubated in a 0.01 M NaCl solution with 200 µg vector (plasmid) DNA/ml. After two or three days the seeds are sown on perlite or agar with a mineral medium (brown). As vectors, we used different plasmids of the pBR325 family, including new constructions. Control plants were treated with 0.01 M NaCl. As receptor monocotyledon we used the grass timothy (Phleum pratense). This species seems to have a low nuclease content (4). After transformation we looked for β-lactamase activities (coded by the

Amp gene of the different plasmids) and we tried to select for transformed plants on chloramphenicol or Kanamycin. In order to look for single transformed cells, callus culture methods were developed. The investigation of the DNA of transformed plants or calli is in progress.

β-Lactamase activity

The following method was used for measuring β-lactamase activities. Plants of 3 weeks old are homogenised in an ultra turrax, then in a potter with a 0.025 M phosphate 0.25 % triton x-100 buffer. In the case of calli, no ultra turrax was used. The fraction of this homogenate that precipitates between 45 and 90 % of ammonium sulfate was taken. To this fraction, nitrocefin was added. Nitrocefin absorbs normally at 390 nm. The β-lactam ring of nitrocefin is cleaved by the action of β-lactamase. The in that way modified nitrocefin absorbs at 486 nm. The increase of absorption at this wavelength was measured in function of the time. The mean activities, found with different plasmids after the first, second and third cut are given in table 1. By this method 1 gr of fresh plants results approximately in 496 $\pm$ 80 µgr of protein.

The specificity of the enzymatic reaction was detected by adding a specific antiserum and proved by an inhbition of the reaction.

The molecular weight of the β-lactamase was estimated by column chromatography (Sephadex G75) to be approximately 25.000 (see fig 2). This corresponds with the normal molecular weight of the active enzyme of the R-tem type (22.000).

We also observed a slight inhbition of the β-lactamase enzyme by the plant homogenate which caused difficulties in measuring the real β-lactamase activity.

Afterwards, the plants were transferred to the field and seeds were harvested to grow F_1 progenies. In some cases, a β-lactamase activity was found in these F_1 progenies (see table 2). These progenies will be further examined. The seeds will also be sown for the F_2 progenies.

Selection for tranformed plants

Until now β-lactamase activity measurement was done on lots of 20 or

more plants, no clones of single transformed plants were examined. Therefore the sensitivity of different antibiotics was measured in order to obtain selection systems for transformed plants. Growth sensitivity was tested on different concentrations of chloramphenicol, Kanamycin, Gentamicin, Trimethoprim and G418 (5).

The select for single transformed cells, callus growth was assayed on B5. Medium (6) with a supply of different concentrations of 2,4 D and kinetine. The best callus growth could be obtained with 0.00 or 0.01 mg/1 kinetine and 1.0 mg/1 2,4 D. Callus growth was also tested on different antibiotic concentrations as mentioned above (5).

Selection of chloramphenicol

The first assays for selection were done on chloramphenicol because an easy method for determining the chloramphenicol acetyl tranferase activity was available (7). The selection was done on B5 plates with 5.10^{-4} M chloramphenicol. After several weeks we looked for callus formation, germination and shoot formation. The results are given in table 3. Only a few calli were formed and those were very small in comparison with calli grown on B5 control plates (without chloramphenicol). When we investigated these calli for chloramphenicol acetyl transferase activities no real positive answer was found. Further there was an inhibition of the enzymatic activity by the callus or plant homogenate and plants appeared to contain aspecific acetyl transferases.

Selection on Kanamycin

Plasmid treated seeds are germinated on brown (for plants) or B5 (for calluses) medium with 60 or 80 µg Kanamycin/ml.
On these concentrations no resistant plants could be found. On the other hand small calli were formed on B5 + 80 µg Kana/ml medium. However, these calli grew very slowly and no real selection could be done on this medium.

Nevertheless, treated and control plants grown on brown medium with 20 µg Kanamycin/ml grew first and died after two months. At this time 4 plants, treated with pLGVR7, were still green and kept on growing. The plasmid pLGVR7 contains a ribosomal plant DNA and a Kanamycin phosphotransferase gene under the control of the nopaline synthase

promotor. These resistant plants will be studied further (presence of kanamycin, phosphotransferase activities). On the other hand, it was not possible to use such a low kanamycin concentration for selection in tissue cultures.

Discussion

The monocotyledon timothy was transformed, by incubating germinating seeds with different plasmids of the pBR325 family.
With some plasmids a specific β-lactamase activity (coded by the Amp-gene) was measured. The molecular weight of this β-lactamase was about 25.000. Until now, no clear chloramphenicol acetyl transferase activity (coded by the Cm gene) could be found. The promotor of the Cm gene is probably not efficient in these plants.
By using the gene for kanamycin phosphotransferase under the control of a plant promotor (from nopaline synthase) some plants could be selected on low kanamycin concentrations.
In the future, the selection system will be ameliorated by using low concentrations of kanamycin or by using the kanamycin derivative G418. The plants selected in that way will be used for seed harvesting and DNA study.

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Table 1. β-lactamase activities.

Plasmid	First cut				Second cut		Third cut	
	-		Cm (2)		-		-	
NaCl	0,003 ± 0,002 (1)		0,001 ± 0,000		0,008 ± 0,002		0,010 ± 0,004	
pBr325	0,060 ± 0,020	4/7(3)	0,090 ± 0,010	1/1	0,015 ± 0,002	2/3	0,040 ± 0,010	2/2
pSV_2Cat^R	0,080 ± 0,010	3/4	0,040 ± 0,010	1/1	0,080 ± 0,002	1/1	0,050 ± 0,010	1/1
pBR 322	0,000	1/1	-		-		-	
pHV33	0,080 ± 0,010	1/1	-		-		-	
pMOL104	0,016 ± 0,002	1/2	-		0,000	1/1	-	
pMOL106	0,000	0/1	-		0,000	1/1	-	
pMOL105	0,000	0/1	-		-		-	
pMOL102	0,000	0/1	-		-		-	
pMOL101	0,240 ± 0,020	1/2	-		0,037 ± 0,004	1/1	0,020 ± 0,002	1/1
pCW2	0,014 ± 0,002	1/1	-		0,000	1/1	0,000	1/1
pCW 6	0,015 ± 0,002	1/1	-		-		-	
pMOL112	0,074 ± 0,004	1/1	-		0,010 ± 0,002	1/1	0,030 ± 0,002	1/1
pMOL113	0,000	0/2	-		-		-	
A2.5.	0,000	0/2	-		-		-	
pMOL114	0,000	0/1	-		-		-	
pMOL115	0,000	0/1	-		-		-	

(1) β-lactamase activity is expressed in OD/hr/gr plants

(2) grown on chloramphenicol

(3) a/x: a = number of positive experiments
x = total number of experiments.

Table 2: β-lactamase activities of F_1 progenies.

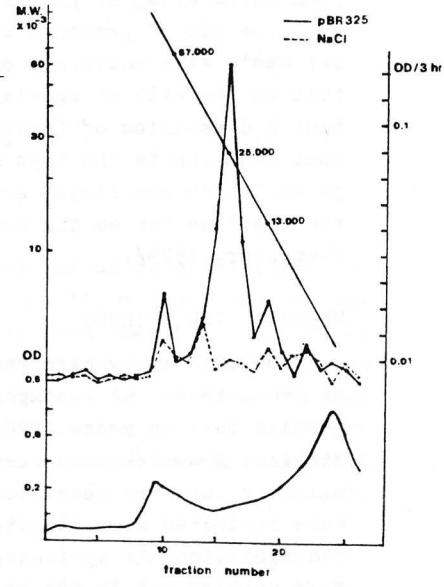
Plasmid		β-lactamase activity (1)		
NaCl	1	0,000	0/2	
"	2	0,009 ± 0,008	1/3	
"	3	0,007 ± 0,006	1/2	
"	4	0,000	0/1	
pBR325	1	0,050 ± 0,010	4/6 (2)	x (3)
"	2	0,000	0/3	
"	3	0,010 ± 0,005	1/3	
"	4	0,010 ± 0,005	1/3	
pBR322	1	0,009 ± 0,002	1/1	
"	2	0,000	0/1	
"	3	0,018 ± 0,001	1/1	x
"	4	0,021 ± 0,002	1/1	x
pBR328	1	0,017 ± 0,002	1/1	x
"	2	0,000	0/1	
"	3	0,021 ± 0,001	1/1	x
"	4	0,000	0/1	
pSV_2Cut^R	1	0,000	0/1	
"	2	0,015 ± 0,004	1/1	x
"	3	0,001	1/1	
"	4	0,002	1/1	
CM282	1	0,00	0/1	
"	2	0,000	0/1	
"	3	0,000	0/1	
"	4	0,010 ± 0,005	1/1	

(1) β-lactamase activity is expressed in OD/hr/gr plants.

(2) a/x: a = number of positive experiments
x = total number of experiments.

(3) activities indicated with x can be considered as really positive in comparison with the control treatments.

Fig 1. Molecular weight determination of β-latamase.



Note: β-lactamase activity was expressed as the increase of the optical density of nitrocefin at 482 nr in 3 hours.

YIELD AND QUALITY OF BRASSICA FODDER PLANTS USED IN POLAND

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In Poland there is a great need of winter catch crops cultivation. Among plants used in this sequence of crop rotation cruciferous plants, especially from the genus Brassica of such species as napus, campestris and oleracea, which are characterized by good winterhardiness and economical use of soil fecundity, and rather short vegetation period enabling their early harvest in spring and proper sowing time of main crop, seems to be the most promissing group of plants.

The aim of presentation of the results of field experiments with cultivars of Brassica napus and campestris is the will of sharing some doubts and bringing about a disscusion of feeding value of these plants. The most annoying is the high nitrate content in their green parts, which oscillates around 0,25% of DM and which is the limit as far as the health of animal is concerned /Lampeter, 1979/.

MATERIAL AND METHODS

Cultivars of winter rape and winter turnip rape used as green fodder were compared in the field experiments carried out in years 1976-1981 at the 19 Experimental Stations of the Research Centre for Cultivar Testing spread all over country /Lewandowski 1979, 1982/. Experiments were conducted according to the cultivation and manuring recomendations for agricultural practice. All experiments were carried out in the Split-block method.

For the presentation of the value of the cultivars were chosen: dry matter yield, crude protein and nitrate content in dry matter. Results given in this paper are arranged according to the best cultivar from the point of view of character considered in the first harvest /when the plants of the earliest cultivar formed flower buds /According to the COBORU scale: stage No.53, Heimann and Drobnik, 1985/. All cultivars were collected at the same time. The second time the cultivars were harvested separately at the stage of beginning of their flowering /Stage No. 61, Heimann and Drobnik, 1985/. The following cultivars were taken into account:

No. on Figs.	Name	Origin
Winter rape:		
1	Górczański	PL
2	Skrzeszowicki	PL
3	Akela	D
4	Girita	D
5	Lifura	D
6	Livera	D
7	Nevin	GB
8	Fora	S
9	Samo	S
10	Sv 7419	S
Winter turnip rape:		
1	Ludowy	PL
2	Szczeciński	PL
3	Solo	S
4	Rabra 1	Pl
5	Brachina	Pl
6	Perko	D
7	Buko	D
8	R 80/71 Tetra	Pl
9	Tyfon	Nl

The investigations on the influence of nitrogen fertilization on the yield and quality of Brachina were carried out at the 9 Experimental Stations/Heimann, 1985 /. Split-block and Split-plot methods were used. Nitrogen was given in autumn and in spring in the amounts given in the Tab. 1. Amount of nitrate and crude protein per cent of DM and yield of green and dry matter and yield of crude protein were estimated. The experiment was collected in the first harvest time.

RESULTS

Fig. 1 contains results of experiments with winter rape. Mean DM yield in the first harvest was 1.33 dt/ha and 3.60 dt/ha in the second. The best in both harvests was Swedish variety Sv 7419. Next to it were Polish traditional cultivar Skrzeszowicki and Górczański. DM yield of fodder rape varieties such as Akela, Lifura, Livera and Nevin was rather low in both harvests. They were not winterhard enough in Polish climatic conditions, and in spring they were growing rather slowly, as compared to the seed forms of rape. There were some cultivar differences in DM yield.

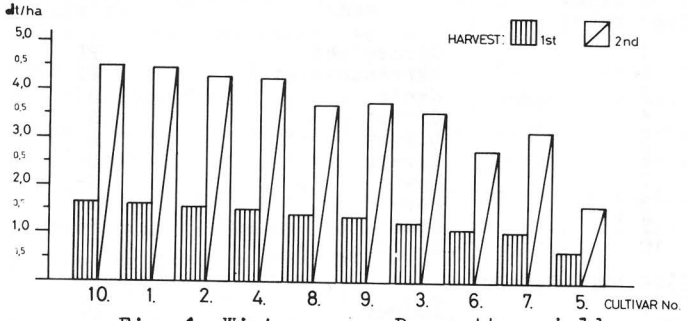

Fig. 1. Winter rape. Dry matter yield

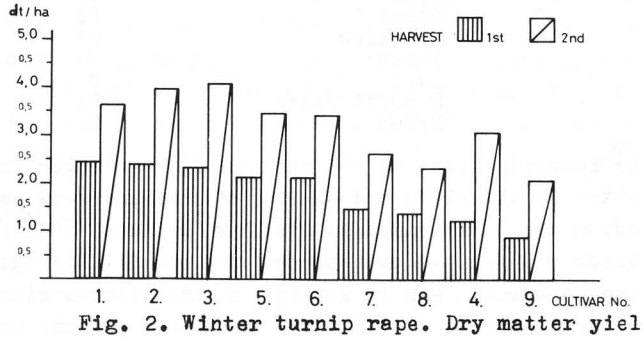

Fig. 2. Winter turnip rape. Dry matter yield

Fig.2 contains the results of experiments with winter turnip rape cultivars.DM yield was in the first harvest in general higher than of winter rape. Mean yield was 1.8 dt/ha and in the second harvest was 3.17 dt/ha.

In the first harvest the best were Polish cvs.Ludowy and Szczeciński and Swedish cv.Solo. The latter was also the best of all cvs. investigated in the second harvest. Brachina /Polish Perko on the diploid level/, Tyfon and Rabra-1 were rather lower yielding cvs., Tyfon, not winterhardy enough, and Rabra and R-80/71 Tetra rather slow in growth. Differences among winter turnip rape cvs were in both harvests greater among winter rape.

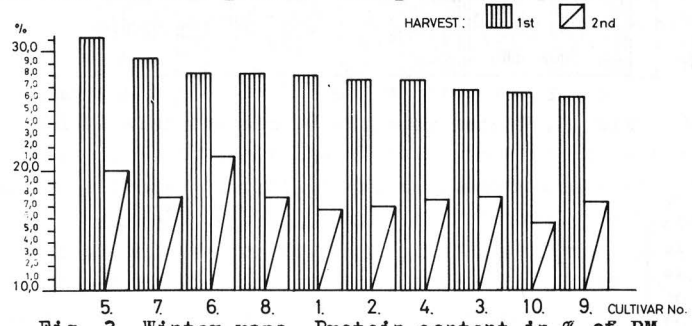

Fig. 3. Winter rape. Protein content in % of DM

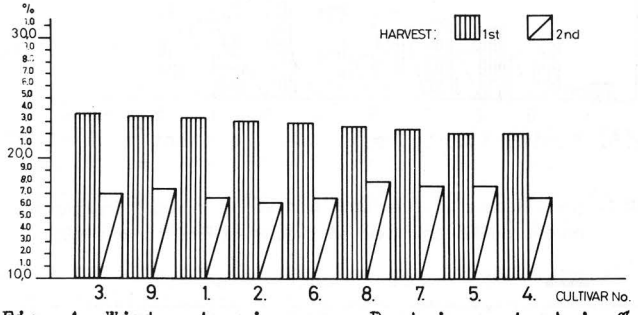

Fig. 4. Winter turnip rape. Protein content in % of DM

Figs. 3 and 4 concern crude protein content in DM. For the first harvest mean content was 27.6% for rape and much lower for turnip rape /22.9%/. In the second harvest - 17.6% for winter rape and 17.0% for winter turnip rape. Among rape cvs. the best was Lifura and among turnip rape - Solo. The differences among rape cultivars were greater than among winter turnip rape.

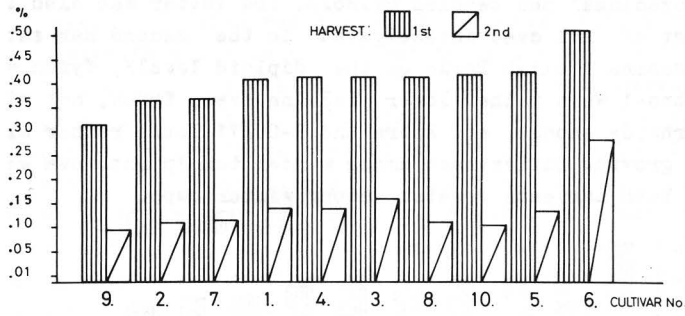

Fig. 5. Winter rape. $N\text{-}NO_3$ content in % of DM

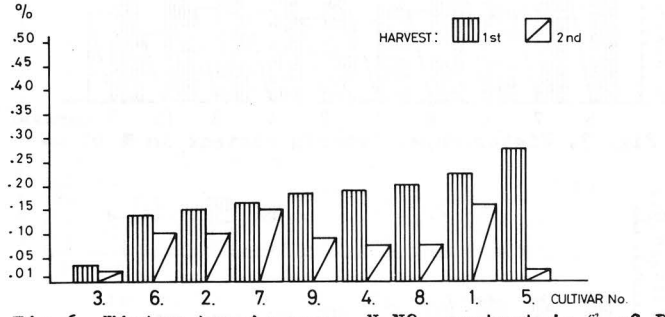

Fig.6. Winter turnip rape. $N\text{-}NO_3$ content in % of DM

Tab.1. The influence of an autumn and spring nitrogen ferlilization on the quality and yield of Brachina.(First harvest. 1985)

the best results — the worst results

QUALITY AND YIELD ASPECTS	NITROGEN IN kg / ha			
	40•+50••	90•+0••	40•+100••	90•+50••
$N\text{-}NO_3$ % OF D.M.	0,096	0,048	0,256	0,101
PROTEIN % OF D.M.	19,2	19,0	21,9	20,1
GREEN MATTER dt/ha	219,5	172,2	281,2	262,2
DRY MATTER dt/ha	25,1	18,3	27,8	26,1
CRUDE PROTEIN dt/ha	4,82	3,48	6,1	5,3

• in autumn
•• in spring

Figs 5 and 6 concern nitrate content in DM. In the two harvests mean content of nitrate was much higher in winter rape than in turnip rape. Its mean amounts were 0.403 in the first harvest and 0.135% in the second, while as far as winter turnip was concerned they were 0.191 for the first harvest and 0.099% for the second. There was great cultivar differentiation as far as nitrate content in DM is concerned, especially among winter turnip rape cvs. Remarkably low amount of nitrate was found in cv. Solo /Swedish variety/ and the highest amount in Brachina, especially in the first harvest.

As it is seen at the Table, the lowest amount of nitrate content was found in the case when there was no spring nitrogen fertilization.The results concerning the remaining yield aspects were the lowest. The highest amount of nitrate was found in the case of the highest nitrogen fertilization in spring. From the limit of nitrate content - this result was the worst. As far as the rest of the yield aspects - results were remarkably good.

CONCLUSSIONS

1. There were differences among cultivars of winter rape and winter turnip rape in all investigated yield and quality aspects /especially in $N\text{-}NO_3$ content in DM/ which suggest possibility of their change in the way of plant breeding /see also: Młyniec 1984/.
2. The results prove that the amount and time of nitrogen fertilization influences essentially the content of nitrate in DM and also the investigated yield and quality aspects /crude protein content in DM, and green matter, DM and protein yield/.
3. In the case of testing initial material for breeding, or material derived in the result of breeding work the level of nitrogen accessible for plants contents in soil and nitrogen fertilization should be precisely fixed.

4. Results obtained suggest the need of verifying the recommendations of nitrogen fertilization of plants of Brassica species.

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