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of the

EUCARPIA

FODDER CROPS SECTION MEETING

NATURAL VARIATION AND BREEDING FOR ADAPTATION

September 22nd - 24th, 1987
INRA - Station d'Amélioration des Plantes Fourragères
LUSIGNAN (France)

EUCARPIA

EUROPEAN ASSOCIATION FOR RESEARCH ON PLANT BREEDING

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PREFACE

In 1970 a most successful meeting of the Eucarpia Fodder Crops Section was held at Lusignan. With memories of that meeting still fresh in members minds it was the unanimous wish of members that we accept the kind invitation of Dr C Poisson of Station d'Amelioration des Plantes Fourrageres to hold our 1987 meeting at Lusignan.

Such is the magnetic attraction of Lusignan to fodder crop breeders that a record number of 120 members and guests from 23 different countries gathered together for the meeting held from the 21st to 24th September, 1987.

The main theme of the meeting was "Natural Variation and Breeding for Adaptation". This report contains the texts of the plenary and short papers presented during the various sessions together with a summary of the posters.

In contrast to earlier meetings of the Section, Workshop sessions were introduced to provide a greater opportunity for discussion on a range of topics allied to the main theme of the meeting. A synopsis of these workshops is also recorded here.

During the course of the meeting two visits were arranged, the first visit was to the INRA Station at Lusignan to see some aspects of the current research and breeding work. Although showery rain unfortunately interrupted the visit to the experimental fields it did nothing to dampen the enthusiasm of the participants for the research on view.

The second visit to the Marais Poitevins was to an environmentally sensitive region of low lying marshland. The problem of management of such areas was clearly presented and their importance to the pattern of farming well exemplified.

The meeting was most efficiently organised by Dr C Poisson and colleagues to whom we extend our thanks. It is a pleasure also to acknowledge the support of the Council of Lusignan for the use of their excellent civic facilities and to the following organisations for financial support.

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The Xavier BERNARD Foundation Council
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M D Hayward
President of the Eucarpia Fodder Crops Section
Aberystwyth
Wales

October 1987.

DESCRIPTION OF NATURAL VARIABILITY AND MANAGEMENT OF GENETIC RESOURCES

PAPERS

Description and distribution of natural variation in forage grasses

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SUMMARY

Forages are unique in that wild ecotypes have similar morpho-physiological characters to the more advanced crop cultivars and thus provide a wealth of genetic variation which can relatively easily be utilised for breeding. This variation in the form of differences in growth rhythm and tolerance to stress is found in the contrasting macro-climates of Europe and as discontinuous variation in microhabitats.

Tentative reasons can often be given to explain particular distribution patterns and thus provide information to help predict the location of specific variation. Even so, many unexplained anomolies remain, some of which can be resolved by more intensive characterisation of the collection environment and the ecotype itself.

Detailed characterisation of naturally occurring diploid and tetraploid Festuca pratensis suggested that the altitudinal distribution of the two cytotypes could be explained by their contrasting seed physiology, seedling cold tolerance and growth rhythm.

Collection and characterisation of forage genetic variation has been in progress for over 25 years at the Welsh Plant Breeding Station (WPBS), and although only a small proportion of the collected material has proved valuable, 19 registered varieties have been developed using exotic wild ecotypes.

INTRODUCTION

All breeding activities rely on genetic variation to provide the raw material for varietal construction. Cereal and horticultural crop breeders rely largely on land races and old varieties as their source of genetic variation and only use putative ancestors and wild relatives as a last resort. However, forage grass breeders have the opportunity to utilise naturally occurring ecotypes and relatives of cultivated species, as these are often little differentiated from, and almost always cross fertile with, the relatively unsophisticated forage cultivars. This provides a seemingly limitless genepool of variation on which the breeder can draw. In the past 25 years staff of the WPBS have sampled this genepool and after characterisation have utilised a number of these exotic populations in cultivar construction.

The temperate forage grasses are mostly outbreeders, with the major agronomic characters controlled by large numbers of additive genes. Adaptation to environmental pressures have resulted in the evolution of contrasting ecotypes within a species and these display physiological variation expressed as differences in flowering date, growth rhythm and resistance to stresses such as low temperature, drought and disease. Adequate characterisation of this variation is difficult as the expression of genetic variation often varies with the environment, leading to a large genotype/environment interaction. Practically, however, we have studied these ecotypes both under field conditions as spaced plants or small plots for agronomic characters, and in controlled environments for reaction to stress. Where data have been collected over a number of years comparisons are made with relevant standard cultivars that are included every year. Thus our evaluation data may only be applicable to our own environment and extrapolation to other situations would appear to be difficult. This is illustrated by data (not shown) from a 1983 multi-site trial on seasonal growth of *Lolium perenne* landraces, where none of the populations had consistent spring growth characterisation over the four environments. Pamir, the Italian landrace, for example was characterised as only intermediate to good in UK and Belgium, good in Ireland but good-very good in the Netherlands. Similar contrasting expression of variation has been shown for data on mean stem length and mean emergence date (TYLER 1985).

From this we may conclude that the degree of expression of genetic variation is dependent on the environment of evaluation. However, if data are compared from one environment only some useful indications of the extent of genetic variation should be possible.

VARIATION IN QUALITATIVE CHARACTERS

Although most characters in forage grasses are quantitatively inherited one of considerable importance, the chromosome number, is qualitative. Characterisation of all our *Festuca* collections for chromosome number has made it possible to construct a distribution map, which provides considerable information on the location of the various chromosome races.

Figure 1 shows that the hexaploid form of *F.arundinacea* and diploid *F.pratensis* are the common forms throughout lowland N.W. Europe. *F.pratensis* extends further north and *F.arundinacea* further south; a rhizomatous form of the hexaploid being confined to the N.W. Iberian peninsular. Tetraploid *arundinacea* subsp. *fenas* appears to be confined to mid and high altitudes in a more or less continuous band from the Cantabrian mountains in N.Spain through the Pyrenees to the French, Swiss and Italian alps. The tetraploid subsp. *F.pratensis* subsp. *apennina*, again a mountain cytotype,has been recorded over all the Alpine massive, the Appennines and Carpathians. In N.Africa a softer leaved form of the hexaploid occurs in Tunisia and Algeria but not to our knowledge in Morocco, where the octoploid var. *atlantigena* and the decaploid *letourneuxiana* is found at all altitudes along stream sides in the Atlas mountains.

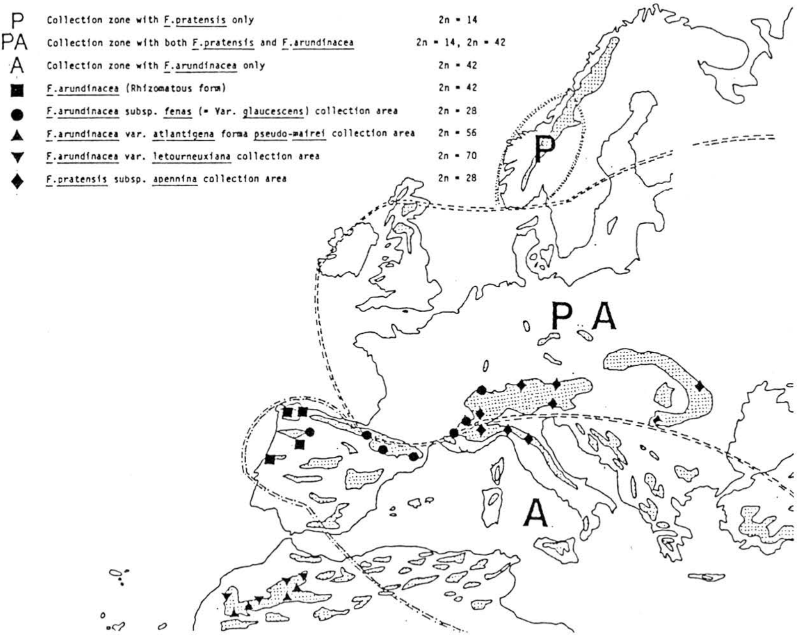

FIG. 1. Distribution of chromosome races of Festuca section Bovinae

There are a number of obvious gaps in our data, firstly the easterly limit of subsp. *fenas* and secondly the westerly distribution of subsp. *apennina*. The mountains of Yugoslavia, Greece and Bulgaria have not been explored and would appear to be fertile areas for these cytotypes.

VARIATION IN QUANTITATIVE CHARACTERS

Clinal variation

The initial search for variation was concerned with major differences in growth rhythm and it was considered that this was likely to be found in the major climatic zones of Europe. The main determinant of growth rhythm differences is the time of onset of reproductive activity and is usually measured as mean emergence date. It is a generally held opinion that flowering is earlier from material originating from the South and later from northern latitutes. This is illustrated in Table 1 which shows data from 173 populations of *Festuca arundinacea* and it can be seen in general that the above does hold true and a cline exists from North to South. However, high altitude populations from low latitude are considerably later than populations from low altitudes at the same latitude. Also considerable variation exists within regions, suggesting that factors other than macroclimate are influencial. Of some interest is the average coefficient of variation of individuals within populations for the different zones. Again, a cline appears to exist from North to South with considerably lower within population variation being shown in northerly than southerly populations. It is suggested that in the harsher environments of the north and at high altitudes, the less well adapted genotypes have been selectively eliminated resulting in populations with low variability, in contrast to populations from the more benign climate of southern Europe which retain greater genetic variability. Thus for maximising the collection of genetic variation, we might expect more success in the south than the north.

TABLE 1. Time of inflorescence emergence and within population variation of Festuca arundinacea from different origins

	Latitude (degrees)	No. of populations	Mean emergence date (days from 1 April)	Average within population coeft. of variation (%)	Range between populations (days from 1 April)
Scandinavia	60	21	74.9	4.4	78.3-69.7
High altitude FRA/ITA	45	20	64.7	6.2	75.1-50.1
N.E. Europe	53	10	64.3	5.9	70.8-47.4
N.W. France	49	11	56.1	9.4	71.5-36.8
Hungary	47	6	53.1	14.1	68.0-41.3
N.W. Spain	43	9	53.4	13.3	59.1-47.0
N.E. Spain	43	5	47.2	14.4	52.7-43.8
S. France	43	26	36.6	17.2	52.5-24.4
C. Spain	42	3	40.9	26.4	47.9-37.1
Tunisia	36	39	39.8	14.8	58.3-23.5
Morocco (2n=56)	33	7	44.4	19.6	47.6-38.7
Morocco (2n=70)	33	16	36.5	26.6	57.2-24.8

The association between latitude, and thus macroclimate, and flowering time is reflected in the growth rhythm of populations. Scandinavian and North and East European populations generally display poor spring growth, good summer growth but a rapid decline in autumn and winter. At the other extreme, populations in close proximity to the Mediterranean have good spring growth followed by a summer depression and good autumn and winter growth. Between these two extremes there is a clinal situation, with small differences being found associated with gradual changes in climate. Although this general relationship of growth rhythm and climate is valuable, prediction of specific growth characters often involves more detailed climatic consideration. Spring growth data obtained (not shown) in Aberystwyth for a range of *Dactylis* ecotypes showed no relationship with spring temperature at the origin, as might have been anticipated. However, if winter temperature of origin was also considered some pattern could be discerned. Populations originating from regions either with extremely cold or warm winters gave poor spring yields even though spring temperatures of origin were high. This is probably due to winter senescence in the former and winter growth, causing depletion of food reserves, in regions of warm winters. Populations from regions with intermediate winters showed a linear relationship between spring yield and spring temperature of origin.

Using this information, in 1971 an expedition was planned to collect variation in spring growth of L.perenne by making a north to south transect from Belgium to Switzerland, which represented an intermediate winter climate, avoiding the milder winters of S.W. France and Iberia and the relatively extreme winter temperatures of Central and Eastern Europe. Altitudinal transects were made along this latitudinal transect to increase the variation in spring temperatures. The results of characterisation showed a similar association to that found in the intermediate zone with Dactylis but with one notable exception. The populations collected in the Zürich uplands all had considerably more spring growth than would be anticipated from their spring temperature of origin. In fact they gave the amount of growth that would be expected from regions such as the north Italian plains (TYLER & CHORLTON 1979). These anomolous populations illustrate a commonly found occurrence; that general overall relationships between macroclimate and growth attributes are usually found but that exceptions to this rule are by no means uncommon. Such abrupt deviations from the clinal pattern suggest that features of the environment, other than climatic, can have considerable influence.

Discontinuous variation

In the course of plant collection more or less sharp environmental differences are often observed within a short distance. These may be contrasting managements in closely adjacent fields, abnormally high environmental pressure in restricted areas within a field, for example a traversing path, field entrances or drinking troughs, or other distinct micro-habitats. Obviously all observed environmental variation does not necessarily indicate genetic dissimilarity, and only by restricted sampling, separate multiplication and characterisation can genetic differences be confirmed. In a number of instances considerable variation has been observed in ecologically differentiated situations.

During collection in the plains of northern Italy in 1967 two contrasting managements in the same macro-environment were sampled; one from a non-irrigated and the other an irrigated Italian ryegrass meadow. When compared in a common field environment in the UK the non-irrigated collection reflected more nearly the prevailing macroenvironment, good spring growth followed by a dramatic reduction in summer regrowth - typical of the growth rhythm observed from populations with summer water stress and high temperatures. However, material collected from the irrigated meadow gave a lower spring peak but showed a plateau of high production in the summer. It appeared that centuries of summer irrigation under high light and temperature conditions had led to the evolution of an ecotype with very high summer growth rates. Other apparently associated disadvantageous features such as disease susceptibility and poor seed retention were observed in this material and considerable breeding effort was required before this valuable summer regrowth character could be utilised in a commercially viable cultivar (TYLER 1970).

At an even spatially closer level a hay meadow population in the Valtellina was compared with a sample collected from a path that traversed the same meadow. The results in Table 2 show differences of the magnitude one would expect from populations drawn from the north and south of Europe. Thus within one meadow genetic variation was present that covered a considerable range of the variation that exists in the species as a whole.

TABLE 2. Characteristics of perennial ryegrass progeny obtained from plants collected from a hay meadow in N.Italy

	Date of flowering	Spring growth (kg/ha/day)	Seedling height (cm)	% Survival after freezing
Path	31 May	3.7	6.5	92
Meadow	1 May	20.5	11.4	57

Two main conclusions can be drawn from these and a number of other similar examples of microhabit differentiation (TYLER & CHORLTON 1976). Firstly, that sampling in areas with extreme contrasts in environment even within the same general habitat is likely to provide a wealth of genetic variation, and that grassland management can be a major environmental force in contributing to this variation. Sampling in microhabitats is therefore a major feature of our collection strategy. Secondly, if this type of variation is ignored and sampling covers the whole range of variation considerable problems are likely to be encountered at the seed multiplication stage. For example in the previous example the 30 day range in flowering date would at best lead to assortative mating between early and late genotypes and at worst lead to the elimination of probably the late flowering genotypes resulting in considerable genetic change even over one generation of multiplication. This was observed in the regeneration of a group of L.perenne populations collected in Romania, when the original collected seed was compared with seed derived from only one seed generation. Although the majority of populations which were derived from one seed generation showed no difference to plants derived from original seed, one population with much the highest variance in the original sample showed a 5-day shift in flowering date and a consequent narrowing of variability.

These are just a few examples of the extent of genetic variation encountered in over 25 years of plant collection and evaluation. The most dominant feeling is that the extent of variation in natural and semi-natural ecotypes far exceeds that encountered in both old and modern varieties. Secondly, the variation is often, but certainly not always associated with and can be explained by, considerations of climate and local management. Many of the anomolies are unresolved, and would require considerable detailed investigation to find a plausible explanation.

This is illustrated by an attempt to explain the distribution of a naturally occurring cultivated diploid forage species *F.pratensis* and a related wild tetraploid relative *F.pratensis* subsp. *apennina* found in the Swiss and Italian Alps (TYLER *et al.* 1978). As indicated on the distribution map (Fig. 1), both cytotypes occur in the Alps, however their ecological and altitudinal distribution are somewhat different. Ecologically *apennina* was found in habitats with high moisture conditions conferred by a combination of northerly or westerly aspects, shade, damp soils or standing water. The diploid appeared to have a much wider ecological tolerance. The altitudinal distribution was that only *apennina* was present at altitudes above 1800 m and only diploid *pratensis* below 1100 m, with a wide overlap zone between the two altitudes where both were found including a number of sympatric populations. The material was characterised in an attempt to explain the altitudinal distribution.

The germination behaviour of seed collected from these populations showed that tetraploid *apennina* had a low temperature pre-treatment requirement for optimum germination, whereas the diploid responded normally. Constituents of mixed seed collections could be separated by the differential germination responses. Further work on germination using pre-treated seed, on a temperature gradient bar showed considerably different responses to temperature (LINNINGTON *et al.* 1979). The diploid showed a typically broad response curve over the 20-30°C range, with a rapid fall off in germination over 30°C. The optimum temperature was 26°C. The tetraploid had a higher optimum temperature, 29°C, and a much narrower germination curve, showing higher sensitivity to temperatures below the optimum. Examination of seedling cold tolerance using three increasingly severe tests showed that *apennina* seedlings were consistently and considerably less cold tolerant than the diploid.

Finally, growth rhythm studies on the two cytotypes showed *apennina* to have a strongly summer orientated rhythm with 70% of its D.M. production occurring in the June-August period compared with less than 50% in the diploid. This was followed by complete senescence in *apennina* from October to May whereas diploid *pratensis* retained a proportion of green leaf and gave some D.M. production (TYLER & CHORLTON 1974).

Using the above characterisation, the following hypothesis was made to explain the distribution of the two cytotypes. Relevant features of the alpine climate were obtained from ROLLER (1967). In January, temperature decreases with altitude from approximately -3.0°C at 900 m to approximately -8.5°C at 2000 m, snow cover is present from mid December to early March at low altitudes and increases with altitude from late October to late May at high altitude. Early frosts, on average, are expected in mid-October at low altitudes but are prevalent in late August at high altitudes. Late frosts do not occur on average later than the end of April at low altitudes but can continue into June and July at high altitude.

From the characterisation data it is concluded that the diploid has no cold requirement for germination and is therefore an autumn germinator, seed will germinate shortly after shedding in mid August. At low altitudes seedlings could be 9 weeks old by the first frost and 3-4 months old by the first snowfall. Seedling cold tests suggest that survival should be possible at low and intermediate altitudes but unlikely at very high altitudes.

In contrast the tetraploid has seeds with a cold requirement and will remain dormant overwinter and germinate in spring in the rather open habitats of high altitude. Seed dormancy in the population will be favoured by the death of any seedlings germinating in autumn. Mature apennina plants senesce during late autumn and winter thus escaping winter frost.

These alternative life-styles: spring versus autumn germination, winter growth versus winter senescence offer reasons for the presence of tetraploids and absence of diploids at high altitudes. Furthermore, the high optimum germination temperature of apennina together with its relatively narrow temperature range for germination would tend to delay germination until the warmer weather arrives in early summer after the departure of late frosts.

The absence of the tetraploid at altitudes below 1000 m may be explained by its very late spring germination, putting it at a competitive disadvantage in closed mixed communities prevalent at low altitudes. Survivors of the seedling stage could also be at a competitive disadvantage here as adult plants, since they are winter dormant and growth starts some 6 wks later than the diploid in spring.

The two cytotypes meet at mid-altitude, again in rather open habitats where the survival strategies are maintained, and it is likely that a dynamic situation exists between the two forms depending on the severity of the previous winters. After hard winters the tetraploids are likely to be favoured and after mild the diploid. This can only be a hypothesis and would require detailed field observations to confirm, or otherwise, our explanation.

Since this work was completed it has been found that many of the Festuca populations at mid-altitude are triploid - presumably apennina x pratensis hybrids. This was only revealed by a change in sampling technique from seed to vegetative, and illustrates that the degree of genetic variation detected can often be limited by sampling methods. Of interest is the possibility that backcrossing between partially female fertile triploids and either diploid or tetraploid plants may occur, followed by chromosomal stabilisation to either 14 or 28. There would then be the possibility of gene flow between the two cytotypes leading to increased genetic variation in both pratensis and apennina in the neighbourhood of triploid populations.

Utilisation of genetic variation

Identification, collection and characterisation of genetic resources are the necessary precursors to utilisation. After characterisation, 'elite' ecotypes are identified and utilised by the breeders by whatever breeding technique appears appropriate. The proportion of collected ecotypes that have been utilised are of course very low. Approximately 2000 forage grass and legume ecotypes have been collected between 1964 and 1981 and have contributed to 19 registered varieties, so that approximately only 1% of the collections have proved valuable as breeding material. This is probably the sort of utilisation percentage found by most forage genetic resources workers and raises the question of what happens to the other 99%.

As forages exhibit considerable genotype/environment interaction it is likely that certain populations, unacceptable in one environment, may prove valuable in another. Consequently, the WPBS has been active in seed exchange activities for many years providing a seed exchange list as the means of communication. Recently, however, the ECP/IBPGR has taken the initiative to encourage institutes to collaborate in the construction of an European inventory of forage genetic resources. The aim is to provide a catalogue of all resources available for exchange, and also to indicate deficiencies in resources and where they exist, to encourage plant collecting expeditions.

RÉSUMÉ

Les fourrages sont uniques en ce que les écotypes sauvages ont des caractères morpho-physiologiques semblables aux cultivars de récolte plus avancés et offrent ainsi un vaste potentiel de variation génétique qui peut être utilisé sans gros problèmes pour la sélection. Cette variation sous forme de différences dans le rythme de croissance et la résistance est attestée dans les macro-climats contrastés d'Europe et de façon discontinue dans des micro-habitats.

On peut souvent tenter d'expliquer les modèles pratiques de distribution et ainsi être en mesure de prévoir l'emplacement de variations spécifiques. De nombreuses anomalies, demeurent inexpliquées, certaines peuvent être résolues par une caractérisation plus intensive de l'environnement de collectage et de l'écotype même.

Une caractérisation détaillée de Festuca pratensis diploïde et tetraploïde existant naturellement suggère que la distribution altitudinale des deux cytotypes pourrait être expliquée par la physiologie contrastée des graines, la tolérance des semis au froid, et le rythme de croissance.

Depuis plus de 25 ans, la Welsh Plant Breeding Station (WPBS), s'intéresse au collectage et à la caractérisation de variation génétique pour le fourrage et 19 variétés déposées ont été développées en utilisant des écotypes sauvages exotiques, bien que seule une petite proportion du matériel collecté se soit révélée utile.

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EVALUATION OF AN ITALIAN GERMPLASM COLLECTION OF *LOLIUM PERENNE* L. THROUGH A MULTIVARIATE APPROACH (*)

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SUMMARY

Fifty-eight accessions of perennial ryegrass (*Lolium perenne* L.) collected from North to South Italy, were analyzed using a multivariate approach applied to a set of 8 quantitative traits. Two principal components were found to explain more than 60% of the total variability in the dependence structure. Heading time, forage dry matter yield and plant height appeared to be the major sources of diversity among populations. On the basis of the two principal components, similar populations were clustered according to the UPGMA method; three clusters were identified. The results of cluster analysis confirmed the presence of a remarkable diversity within the germplasm collection and, on the whole, the multivariate approach appeared to be a valid system for perennial ryegrass germplasm evaluation.

INTRODUCTION

Perennial ryegrass (*Loliun perenne* L.) is an outbreeding diploid species (2n=2x=14) belonging to the tribe *Festuceae* which, as other herbage grasses, comes from Europe and Western Asia (Borrill, 1976). *L. perenne* is usually referred to as a cool-season grass and it is considered typical of moist, cool environments without severe winter and summer temperatures; nevertheless, it appears well adapted and commonly spontaneous in the Mediterranean region (Neal-Smith, 1965). As a consequence, Italian breeders are interested in perennial ryegrass with the aim to synthetize, starting from local materials, varieties to be

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used both for pasture and turf establishment in a wide range of climatic and edaphic environments, from low to high altitude, provided that soils with adequate moisture content are available. This is not the only reason to collect, maintain, catalogue and evaluate the Italian germplasm of *L. perenne*. Besides, as recently stressed by a working group of IBPGR on forages (1984), the danger of genetic erosion and the need for germplasm conservation is very present for perennial ryegrass of Italian origin probably as a consequence of the large amount of seed of non-adapted, North-European varieties which are commercialized in Italy every year (Lorenzetti and Falcinelli, 1987).

On the basis of the above remarks, the objective of this study was to evaluate and to classify perennial ryegrass populations collected all over the Italian peninsula. Results recently reported by Veronesi and Falcinelli (*in press*) showed the multivariate analysis to be a valid system to evaluate an Italian germplasm collection of *Festuca arundinacea* Schreb., a species related to *L. perenne*. The same approach was used to deal with the set of morpho-physiological and agronomical data available on *L. perenne*.

MATERIALS AND METHODS

Fifty-eight perennial ryegrass natural populations were collected at sites ranging from North (45°58' North Latitude) to South (39°05' North Latitude) in the Italian peninsula in 1982; 16 populations will be referred as Northern, 25 as Central and 17 as Southern populations (Fig. 1). The division of the Italian peninsula into North, Central and South is not an artificial one; as a matter of fact, from a climatic point of view, in general, the Northern part of Italy, up to Po valley, is characterized by less severe summer drought stress and winter minimum temperatures lower than those typical of Central and Southern Italy. In turn, the climate of Central Italy can be considered to be sub-Mediterranean while only from Rome Southward is a real Mediterranean climate approached. Sampling techniques recommended by Hawkes (1980) were applied. All populations were tested at Perugia (43°05' North Latitude). Two hundred seeds of each population were germinated in Petri dishes in Autumn 1982. Seedlings were planted in Jiffy pots and grown in a glasshouse. Eighty randomly chosen plants were transplanted in the field (60x40 cm) in March 1983 using a completely randomized design. The following characters were recorded during 1984-85 on a single plant basis:

- heading time (days from April 1, 1984 and 1985);
- plant height (cm) at heading time, 1984 and 1985;
- length and width of flag leaf (cm) at heading time, 1984 and 1985;
- 1984 dry matter yield (g/plant) at heading time;
- 1985 seed yield (g/plant) and 1000 seed weight (g).

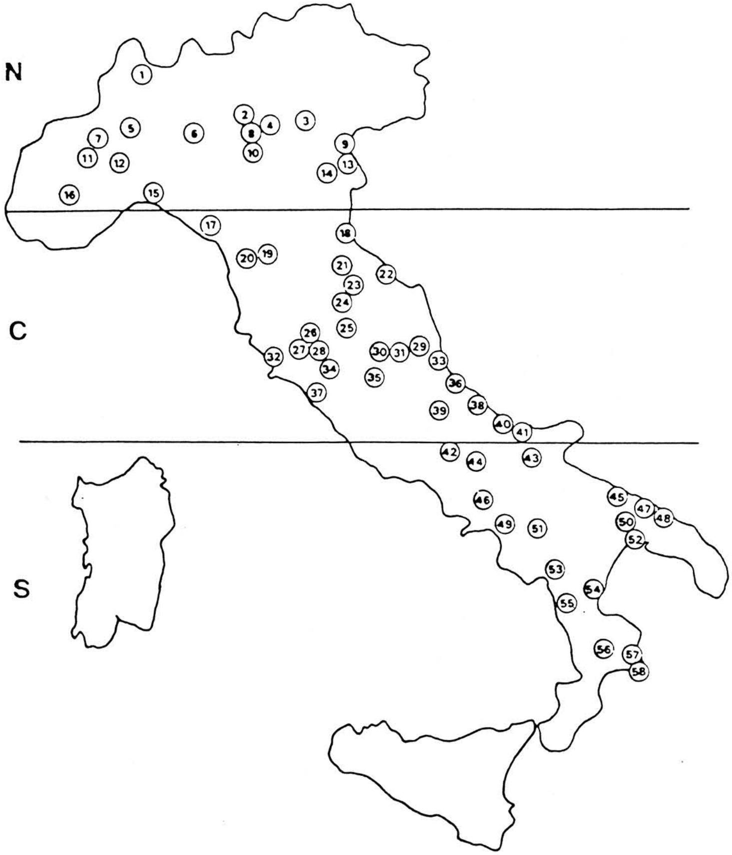

Figure 1. - Collection sites of the 58 examined populations. Numbers 1 to 16 refers to Northern populations, numbers 17 to 41 to Central populations, and numbers 42 to 58 to Southern populations.

For the purpose of statistical analysis, data relative to heading time, plant height, length and width of flag leaf, in which differences were not significant between 1984 and 1985, were averaged over years.

Data relative to 60 plants per population, randomly chosen among survivors at the end of the experiment, were checked across the populations for homogeneity of within population variances and normality within the populations. They were then submitted to a nested model of analysis of variance (Sokal and Rohlf, 1981) with origins (North, Central and South Italy), populations within origins and plants within populations as sources of variation. After this preliminary analyses, average values of each population for each trait, standardized to zero mean and unit variance, were subjected to principal component analysis (PCA). Two rotated orthogonal components (varimax method of rotation) were extracted and the relative scores determined. From these scores, squared euclydean distances were established, cluster analysis (UPGMA method) performed and a tree diagram drawn.

RESULTS AND DISCUSSION

Average values and range of variation of population means for each character are reported in Table 1 for Northern, Central and Southern populations together with ranges of variation relative to latitude, altitude and climatic characteristics of collection sites. A wide range of variation was present for all analyzed characters. In particular, Northern populations appeared to be the most variable for seed yield and 1000 seed weight; Central populations for heading time and plant height and Southern populations for dry matter yield, while length and width of flag leaf did not show clear differences in variability among origins.

Results of the analysis of variance are reported in Table 2. The source of variation "Populations within origins" was always significant while the mean square relative to "Origins" was significantly higher than the mean square relative to "Populations within origins" for plant height and length of flag leaf. Therefore, only for 2 out of the 7 analyzed characters was it possible to differentiate perennial ryegrass accessions on the basis of their Northern, Central and Southern origin.

Variation was further studied by means of PCA. A principal component matrix, after orthogonal rotation for the 7 biological variables, is given in Table 3. Using PCA these 7 variables were grouped into 2 components with eigenvalues greater than 1 which explained 60.53% of the total variation. For the purpose of interpretation, only those component coefficients greater than 0.5 were empirically considered important. Component 1 was strongly associated with plant height, length and width of flag leaf, seed yield and 1000 seed weight, accounting for

Table *1* - Average values ($\bar{x}$) and range of variation of populations means for each character evaluated in Northern, Central and Southern populations, followed by ranges of variation relative to latitude, altitude and climatic characteristics of the collection sites.

CHARACTERS	POPULATIONS					
	NORTHERN		CENTRAL		SOUTHERN	
	$\bar{x}$	RANGE	$\bar{x}$	RANGE	$\bar{x}$	RANGE
HEADING TIME	31.80	23.10 - 42.40	35.30	11.30 - 42.80	35.20	28.40 - 43.00
PLANT HEIGHT	55.90	51.30 - 60.10	51.30	47.10 - 61.40	49.10	44.50 - 52.40
LENGTH OF FLAG LEAF	15.00	12.50 - 18.00	12.80	10.70 - 15.70	12.50	9.80 - 15.40
WIDTH OF FLAG LEAF	0.53	0.45 - 0.60	0.51	0.45 - 0.58	0.50	0.40 - 0.56
DMY	145.00	99.00 -186.00	158.00	93.00 -235.00	130.00	47.00 -223.00
SEED YIELD	12.60	3.10 - 18.00	11.20	5.80 - 16.30	10.40	6.30 - 18.20
1000 SEED WEIGTH	1.55	1.27 - 2.12	1.44	1.16 - 1.90	1.39	1.13 - 1.65
NORTH LATITUDE (°')	-	45°58'- 44°18'	-	44°06'- 42°00'	-	41°48'- 39°05'
ALTITUDE (m a.s.l.)	-	10 - 640	-	10 - 1450	-	10 - 1050
RAINFALL (mm year)	-	576 - 1146	-	526 - 1375	-	464 - 2051
AVERAGE MIN. T. (°C)	-	-4.0 ; +0.9	-	-3.5 ; +5.5	-	-0.3 ; +6.0
AVERAGE MAX. T. (°C)	-	+28.1 ; +30.4	-	+20.6 ; +32.5	-	+26.9 ; +33.7

Table 2 - Mean squares for 7 characters in Northern, Central and Southern populations.

CHARACTERS	MEAN SQUARES		
	ORIGINS (df=2)	POPULATIONS WITHIN ORIGINS (df=55)	PLANTS WITHIN POPULATIONS (df=3422)
HEADING TIME	4144.90 ns	2297.80 **	24.80
PLANT HEIGHT	11880.50 **	249.00 **	49.00
LENGTH OF FLAG LEAF	1889.50 **	171.20 **	8.50
WIDTH OF FLAG LEAF	0.30 ns	0.10 **	0.01
DMY	241505.80 ns	113486.50 **	4672.40
SEED YIELD	1189.50 ns	640.40 **	25.40
1000 SEED WEIGHT	6.00 ns	2.14 **	0.02

ns = not significant;
** = significant at 0.01 probability level.

Table 3 - Principal component matrix after orthogonal rotation for 7 characters of the 58 examined *L. perenne* populations.

CHARACTERS	COMMON COMPONENT COEFFICIENTS		COMMUNALITY
	COMPONENT 1	COMPONENT 2	
HEADING TIME	-0.42588	0.80126	0.82339
PLANT HEIGHT	0.83979	0.02142	0.70571
LENGTH OF FLAG LEAF	0.71025	0.33357	0.61571
WIDTH OF FLAG LEAF	0.74533	0.25253	0.61928
DMY	0.00190	0.85659	0.73375
SEED YIELD	0.55620	-0.01573	0.30960
1000 SEED WEIGHT	0.63831	-0.14844	0.42947
EIGENVALUES	2.66338	1.57353	
PCT. OF VARIANCE	38.05	22.48	
CUM. PCT.	38.05	60.53	

38.05% of the total variation. Populations with high scores in component 1 were characterized by tallness, wide and long flag leaves, high seed yield and 1000 seed weight. Component 2 was made up of heading time and dry matter yield, accounting for 22.48% of the total variation. Increasing values of this component are shown by early heading and high forage yielding populations. On the whole, considering the loadings relative to the 1st and 2nd component, it can be inferred that heading time, dry matter yield and plant height are the major sources of diversity among the analyzed L. perenne populations.

In order to establish a taxonomic connection among germplasm accessions, similar populations were clustered according to the UPGMA method on the basis of the mean values of the 2 principal components. The phenogram obtained for the 58 perennial ryegrass populations is reported in Figure 2. In correspondence to 50% phenon (in taxonomic context, a 50% phenon connotes a group of populations affiliated at no lower than 50% on the similarity scale used in analysis, Sneath and Sokal, 1973), 3 clusters represented homogeneous groups. The first one was formed by populations 5, 10, 17, 18, 24, 26, 27, 28, 30, 31, 33, 34, 39, 41, 46, 49, 53 and 56 (cluster A), the second by populations 22, 23, 32, 36, 40, 43, 44, 47, 48, 50, 51, 52, 54 and 58 (cluster B) and the third by populations 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14, 15, 20, 21, 25, 29, 35, 37, 38, 42, 45, 55 and 57 (cluster C). Two populations, 16 and 19, did not belong to any cluster.

In figures 3, 4 and 5 clusters A, B and C are shown superimposed on the map of collection sites; average values and range of variation of population means for each cluster are reported in Table 4. Cluster A (Fig. 3) includes mainly Central Italian types coming from collection sites characterized by an average of 500-550 m a.s.l. and rainfall higher than 1000 mm per year. These populations show lateness in heading time ($\bar{x}$=39 days from April 1), high dry matter yield ($\bar{x}$=173 g/plant) and low seed yield ($\bar{x}$=9.7 g/plant). Cluster B (Fig. 4) includes Central and Southern Italian populations coming from collection sites with altitudes and rainfalls lower than those typical of cluster A (on the average 220-270 m a.s.l. and 700-800 mm per year, respectively). These accessions are characterized by early heading time ($\bar{x}$=31.9 days from April 1), low dry matter yield ($\bar{x}$= 108 g/plant) and low seed yield ($\bar{x}$=9.6 g/plant). Cluster C (Fig. 5) includes 13 Northern, 7 Central and 4 Southern populations, coming from collection sites similar in altitude to those of cluster B with rainfall close to 900 mm per year. Populations belonging to cluster C are intermediate with respect to those of clusters A and B for heading time ($\bar{x}$=33.6 days from April 1) and dry matter yield ($\bar{x}$=154 g/plant) while they show the highest seed yield ($\bar{x}$=13.6 g/plant).

On the whole, from Tables 1 and 4, it is possible to note that the multivariate approach proved to be efficient in that it produced 3 clusters much more differentiated compared to the initial Northern,

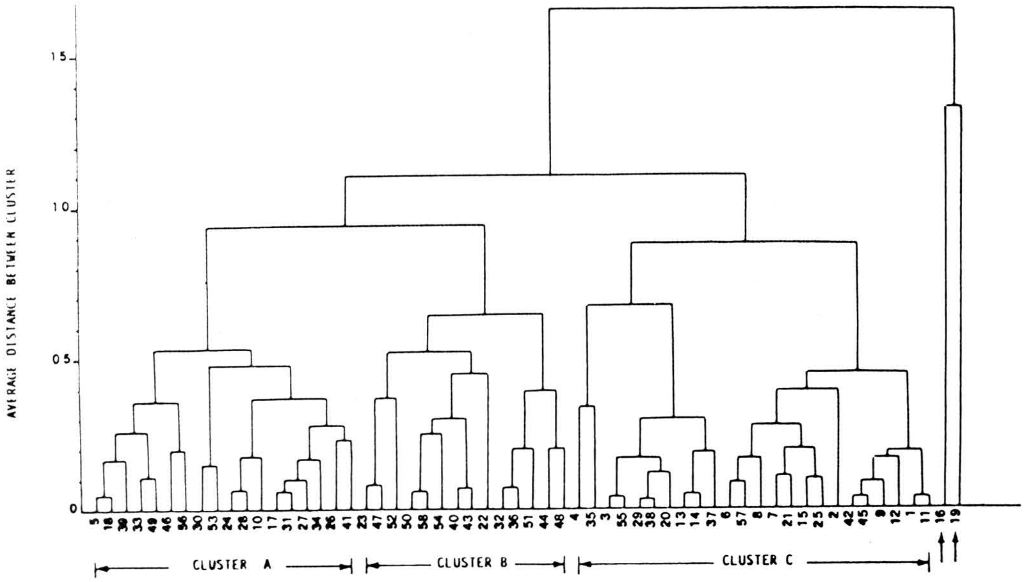

Figure 2. - Phenogram of the 58 examined populations after cluster analysis on the basis of the 2 principal component mean values. Arrows indicate populations which do not belong to any cluster in correspondence to 50% phenon.

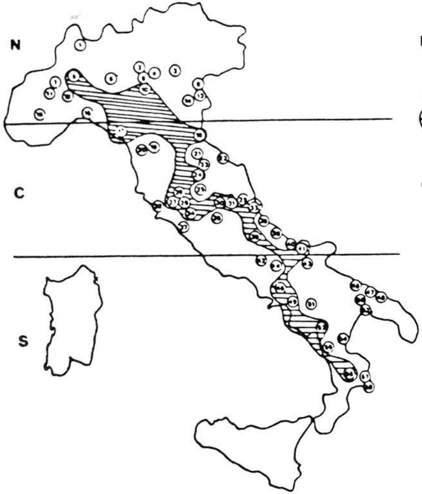

Figure 3. - Plot of the 58 examined populations according to collection sites. The continuous line indicates populations belonging to cluster A.

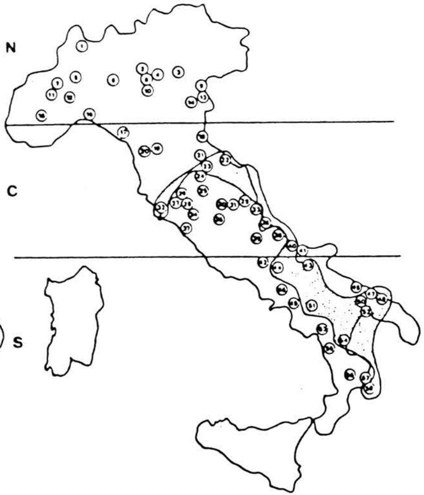

Figure 4. - Plot of the 58 examined populations according to collection sites. The continuous line indicates populations belonging to cluster B.

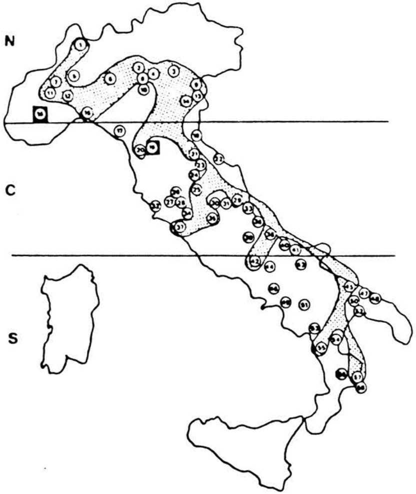

Figure 5. - Plot of the 58 examined populations according to collection sites. The continuous line indicates populations belonging to cluster C. Populations 14 and 19, ◘ , do not belong to any cluster.

Table 4 - Average values ($\bar{x}$) and range of variation of populations means for each character evaluated in populations belonging to clusters A, B and C.

CHARACTERS	CLUSTERS					
	A		B		C	
	$\bar{x}$	RANGE	$\bar{x}$	RANGE	$\bar{x}$	RANGE
HEADING TIME	39.00	33.00 - 43.00	31.90	28.40 - 39.30	33.60	25.00 - 40.80
PLANT HEIGHT	50.40	44.50 - 56.40	47.70	44.80 - 53.10	55.70	45.00 - 61.40
LENGTH OF FLAG LEAF	12.60	11.20 - 15.70	12.00	9.80 - 13.60	14.90	12.50 - 18.00
WIDTH OF FLAG LEAF	0.49	0.47 - 0.54	0.48	0.40 - 0.53	0.54	0.46 - 0.60
DMY	173.00	139.00 - 235.00	108.00	47.00 - 148.00	154.00	117.00 - 220.00
SEED YIELD	9.70	3.10 - 16.30	9.60	6.40 - 13.90	13.60	7.20 - 18.20
1000 SEED WEIGTH	1.34	1.13 - 1.54	1.36	1.16 - 1.71	1.53	1.27 - 1.90

Central and Southern groups. Furthermore, the clustering process made it possible to discriminate between populations that, according to traditional procedures, would be considered quite similar (for example accessions 8 and 10, 23 and 24, 29 and 31, which are geographically close but fall into different clusters). Some clear discrepancies in the general correlation between clusters and locations, should be more precisely investigated, as for example populations 18, 33 and 41 of cluster A which came from collection sites at low altitudes and close to the sea. At the same time, populations 16 and 19 which are highly differentiated and do not find a place in any cluster, may be regarded as cases of peculiar adaptation or genetical introgression.

As already reported for *F. arundinacea* (Veronesi and Falcinelli, *in press*), the multivariate approach appeared to be a valid system to illuminate the intraspecific variation of perennial ryegrass, allowing a detailed characterization of populations in terms of adaptation and productivity, with a higher discriminant capacity in comparison to single trait analysis. Last but not least, the results seem to show that, even in the presence of large amounts of non-adapted varieties on the Italian seed market, *L. perenne* Italian germplasm does not seem to have already undergone a heavy process of genetic introgression similar to that clearly shown by *F. arundinacea* in North Italy (Veronesi and Falcinelli, *in press*).

This phenomenon is probably a consequence of:
a) *L. perenne* imported seed is utilized in Italy, prevalently for turf establishment while *F. arundinacea* seed is exclusively utilized in forage production;
b) results of previous research (Lorenzetti and Falcinelli, 1976; Falcinelli and Ceccarelli, 1977; Falcinelli and Lorenzetti, 1980) clearly show that the persistence of foreign varieties of *L. perenne* does not exceed 2 years while *F. arundinacea* varieties of foreign origin, as well as Italian ecotypes under the usual forage management techniques, persist for several years.

On the basis of these results and considerations, and because this species could be more utilized in pasture establishment, it seems useful to increase efforts in collecting and maintaining the Italian germplasm of *L. perenne* in an attempt to avoid the danger of genetic erosion which could take place in the near future.

ACKNOWLEDGMENTS

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RÉSUMÉ

Cinquante-huit accessions de _Lolium perenne_ L. collectionés du nord au sud d'Italie sont analysés à travers une analyse multivariée, appliquée à un groupe de huit caractères quantitatifs. Deux composantes principales qui expliquent plus du 60 p. 100 de la variabilité totale dans la structure de dépendance, sont trouvées. L'épiaison, la production de matière sèche et l'hauteur des plantes résultent etre la fonte principale de diversité des popolations. Sur la base des deux composantes principales, les memes popolations sont regroupées en accord à la métode UPGMA; trois groupes sont identifiés. Les résultats de la Cluster Analysis confirment la présence d'une diversité remarquable au sein de la collection de matériel génétique et, l'approche multivariée semble être un sistème efficace pour l'évaluation du matériel génétique de _L. perenne_.

GENETIC VARIATION AMONG FRENCH WILD POPULATIONS OF PERENNIAL RYEGRASS

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Summary :

A set of 226 wild populations of perennial ryegrass was collected in France in 1983 and studied from 1984 to 1986 in 9 nurseries of various locations. Agronomic traits were scored on a 1-9 scale and submitted to a multivariate variance analysis to obtain the main population effects. These were used for a multivariate hierarchical clustering adding a geographical contiguity constraint.

This method yields 14 classes each of them grouping populations having similar agronomic behaviour and a common origin. They are presented on France maps and shortly described on the main factorial axes. The usefulness of such a method for genetic resources management is discussed.

INTRODUCTION :

226 seed samples of wild population of perennial ryegrass (Lolium perenne L.) were collected from various regions in France in 1983. This work was part of a cooperative program between 3 plant Breeding Stations of the National Institute for Agronomic Research (INRA) and 8 breeders companies.

Thus 11 different teams participated to the collection following a same protocol : information about locality, soil, environment was collected and will be used as illustrative variates. The populations were implanted in 1984 as spaced plant nurseries in nine locations (3 INRA and 6 private breeders) and as turf plots in three other locations. Data from spaced plant nurseries only will be considered here. Each population was represented by a single plot of 30 plants disposed in an incomplete block design (6 commercial varieties being replicated three times as control). Several traits of agronomic value were recorded, most of them on a 1-9 visual scale, the lower values being given to the desired characteristics : unalternative, frost and disease resistant, vigourous plants. Only the heading date was expressed in the rank of the day.

Data were submitted to a multivariate variance analysis using the following model :

$$y = Mu + l_i + l \times bl_{ij} + POP_k + eps_{ijk}$$

where l_i is the effect of the i^{th} location lx bl_{ij} the effect of the j^{th} bloc within the i^{th} location and POP_k the main effect of the k^{th} population. Estimation of these effects were obtained through the BLUP (Best Linear Unbiaised Predictor) method (MANGIN et al, 1986).

Main population effects (i.e. the average behaviour in all locations) for each trait were used for multivariate statistics (SPAD, 1985).

- A principal component analysis using normalized data.
- A hierarchical clustering method using the euclidian distance on the factorial variates.
- A geographical contiguity constraint allowing two populations to belong to the same cluster only in they are declared to be contiguous : A primary contiguity matrix is built by joining the populations not so far from each other than 50 km, then a secondary matrix is calculated by relating each element of the network to its second nearest neighbour.

RESULTS AND DISCUSSION :

Wild populations of perennial ryegrass generally have higher values than controls, indicating that unselected material appears to be poorly adapted to agronomic management. Some populations however do present some interesting characteristics as regards natural adaptation : frost or disease tolerance.

The principal components summarize this overall information from agronomic traits. Correlations between the first two new components and the initial variates are illustrated on figure 1.
The first component is positively correlated with frost resistance, spring growth, summer aspect and persistency. According to the scale, the interesting populations are thus on the negative side of axis 1. The second component is positively correlated with reheading, autumn growth and alternativity and negatively with heading date. Again the more interesting features are on the negative side of axis 2 : late, unalternative populations.

These two components explain 55 % of the total inertie. The following components are much less explicative and will not be presented here. Illustrative variates exhibit much lower correlations with the principal components than active variates do and must be considered with care. The most significant ones are along the axis 1 : altitude, grassland and pasture management on the negative side, roadside and sandy soil on the positive one. (Figure 1).

From hierarchical clustering with contiguity constrainst, we have chosen a partition into 14 classes. The barycentres of each class on the plan defined by the first 2 main axes have been reported on figure 1.

In fact, most classes largely overlap on each others, but the segregation appears more clearly when considering separately the groups of populations from a same region. This is shown on Figures 2-6.

In the south west region (figure 2), three classes are cohabiting : 1, 2 and 3. These three classes are clearly distinct in the main plan 1-2 :

(1) is characterised by alternativity, low frost resistance and spring growth, but on the other hand by a quite high level of disease tolerance.

(2) groups alternative, very early heading populations having a poor autumn regrowth and persistency.

Populations belonging to the class (3) are persistant early heading, green in summer but disease susceptible.

Geographically, populations from class (1) are mostly located in the west part and those from class (2) in the east part of this subpyranean region. The average altitude for (2) and (3) is 366 m and 294 m respectively whereas it is 679 m for populations belonging to (3).

In the middle west region of France (figure 3), there are three classes of populations too :

class (4) gathers populations having quite average values for all traits, except relatively good disease resistance and tillering.

Populations from class (5) behave relatively bad in summer and autumn, show multiple heading and poor tillering.

We found in class (6) only 5 populations showing intermediate heading date and disease resistance but bad summer aspect, frost susceptibility and short persistency.

No ecological variate significantly differentiates one class from each other : (6) is located in the west wetlands and (4) more south whereas (5) is widespread upon the region.

Two classes only have been built with populations from the Massif Central (figure 4) class (7) is made of semi late heading, disease susceptible populations, whereas class (8) comprises intermediate heading, persistant, frost resistant, spring and autumn vigourous, remaining green in summer, but disease susceptible populations.

Altitude and the type of living area are quite discriminant factors between these two sympatric classes. Populations of class (7) mainly come from roadsides and an average altitude of 490 m whereas populations of class (8) are found in grassland at an average height of 800m.

In north-west of France (figure 5), there are again three classes of populations which pratically do not overlap on the map.

Class (9) is found in middle Brittany and Anjou : their populations show intermediate values for heading date and other traits except a nearly bad persistency and summer appearance.

Populations of class (10) coming from west and south Brittany, are intermediate to late for heading, quite vigourous in autumn but show a poor tillering.

The last one, class (11) is located in Normandy and associates early heading, non alternative nor re-heading, frost resistant populations showing a good vigour in spring and summer.

North-East France around Paris also hosts three classes (figure 6).

Class (12) is made of early, non alternative populations showing interesting values for frost resistance, spring growth, summer aspect and persistency.

Populations of class (13) are late and unalternative but are less vigourous in summer and autumn.

Finally, Class (14) gathers late, unalternative, persistant populations showing a good autumn regrowth.

Ecological factors can be involved in differentiating classes (12) and (14): the populations of the former are mainly found in pasture grassland on chalky soil whereas those of the latter grow on loamy and silty soils.

DISCUSSION AND CONCLUSION :

What could be the usefulness of such a partition using both agronomic traits and map coordinates ?

Firstly, the constitution of our initial germplasm for genetic resources conservation can be supported by this classification. It is possible to multiply, separately or in bulk, populations sampled in each class. Thus the chance of preserving various sources of genes in enhanced.

Indeed the introduction of the geographical contiguity constraint prevents us, in the eventuality of a convergent evolution, to miss an original source of genes leading to a same ideotype than another gene pool. The plant breeder can therefore use various independant entries in crosses and thus find interesting transgressions.

In addition, the results of this method may be helpful in the understanding of evolution an differentiation within the species Lolium perenne :

- Are the various ideotypes of ryegrass randomly distributed or in relation to specific ecological factors ?

Although the 226 populations studied do not cover the totality of France, we tried to search for the existence of clinal variation among french populations of ryegrass. For this purpose we mapped the values of the first and second principal components, each being divided into four classes. Remind that the first component summarize the general vigour throughout the year, the lower values denoting the most vigourous populations and the secund component is a precocity axis.

Figures 7 and 8 present the maps for components 1 and 2 respectively. No simple clinal figure appears for both components. Vigourous populations (low values for the first component) can be found in at least three different locations : Normandy Auvergne and Bassin Parisien. A trend to later heading date of populations from south to north of France could be seen, with the exception of Normandy as an island of precocity.

Thus no single variate or even principal component can give a satisfactory image of the differentiation of Lolium perenne. The method we used artificially cut classes which are located in restricted areas. But there is no proof that this coincidence is really the consequence of a specific evolution and diversification. This would imply the study of genes themselves in state of quantitative characters (for example those encoding for isozymes).

When particular ideotypes can be related to specific ecological factors, they could be called "ecotypes". This may be the case for the classes (3) in the Pyrennées and (8) in Auvergne as "altitude ecotypes".

But many other ecological variates have to be taken into account, like climatic or edaphic data ... This will be done in further studies.

As a conclusion, the method we used on an incomplete sample of french populations may be of interest for the plant breeder as an aid for genetic resources management and for the identification of the most valuable classes having separate origins.

Aknowledgements :

The authors are very grateful to the French Association of Forage grass Breeders for its participation to the collect and multilocal study of wild populations.

Résumé : 226 populations naturelles de ray grass perenne ont été collectées en France en 1983 et étudiées de 1984 à 1986 en pépinières dans 9 lieux. Des caractères d'intérêt agronomique furent notés selon une échelle de 1 à 9 et les données soumises à une analyse de variance pour obtenir des effets principaux populations. Ceux-ci furent utilisés pour une classification hiérarchique ascendante sous contrainte de contiguité géographique.

Cette méthode aboutit à une partition en 14 classes, chacune regroupant des populations ayant des comportements agronomiques similaires et une origine commune. Ces classes sont présentées sur des cartes de France et brièvement décrites sur le plan factorial principal. L'apport d'une telle méthode pour la gestion des ressources génétiques est discuté.

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FIGURE 1

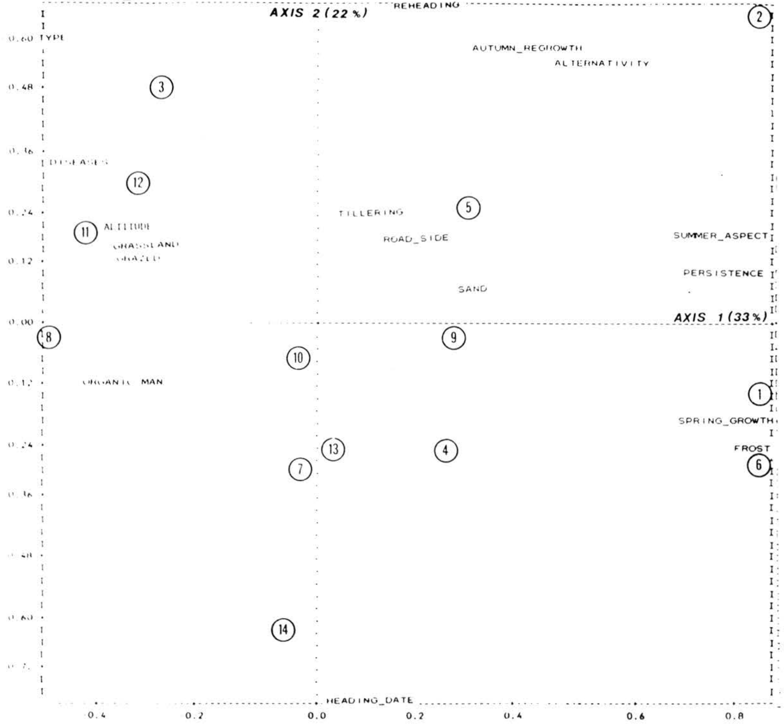


FIGURE 2

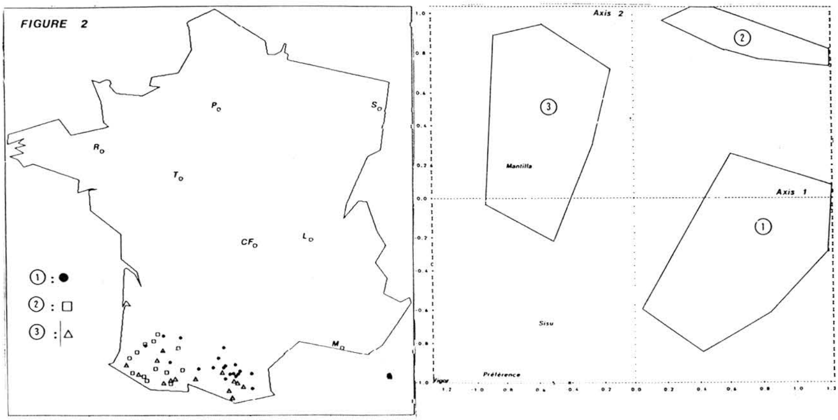


FIGURE 3

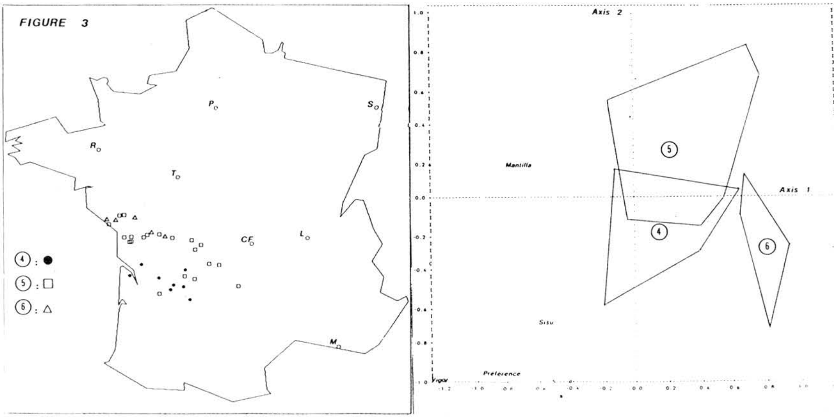


FIGURE 4

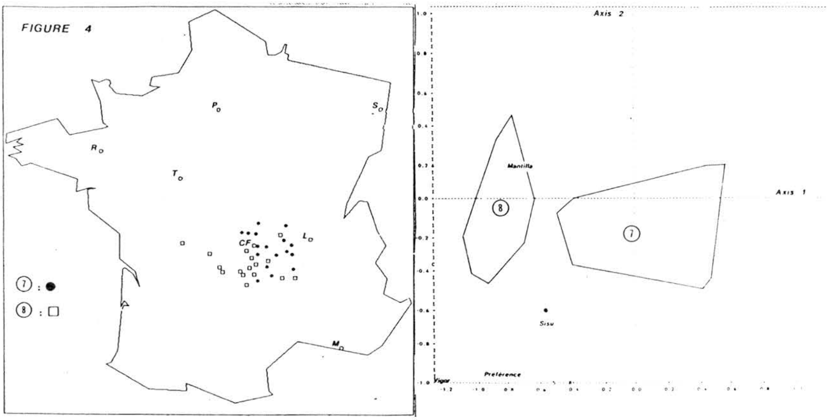


FIGURE 5

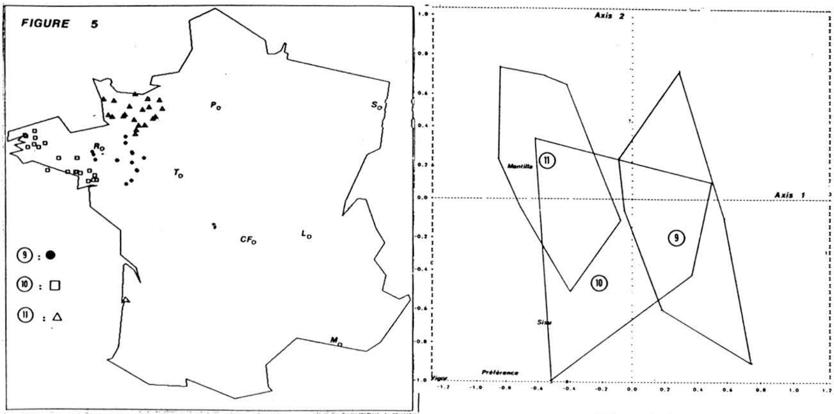


FIGURE 6

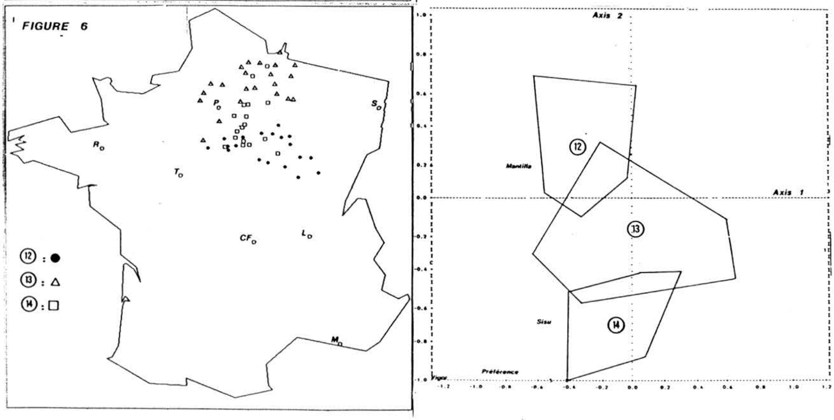


FIGURE 7

FIRST PRINCIPAL COMPONENT

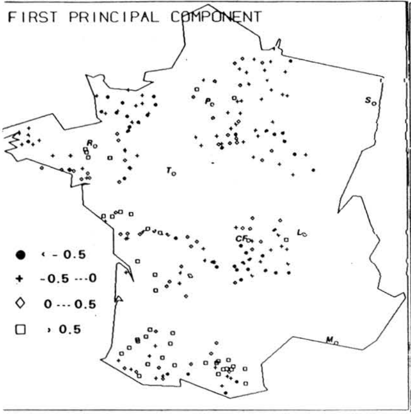
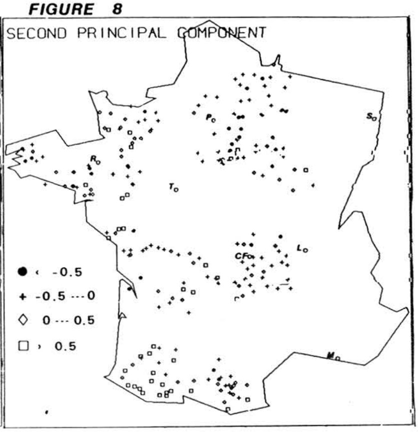


FIGURE 8

SECOND PRINCIPAL COMPONENT



Aspects of the variability of subterranean clover populations and their implications in breeding. The environmental influence on flowering time.

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SUMMARY

Two collections of Sardinian native strains of subterranean clover, previously examined for flowering time at Perth (Australia), were also examined for flowering time in Sardinia (Italy). The first, at one location, and the second at four climatically differentiated locations.

The results obtained indicated that the overall variability in flowering time was largely an expression of phenotypic plasticity. This plasticity was likely to be promoted by differences in temperatures and the length of the cold season among the environments in which the strains were examined.

The average flowering time of the collections was progressively retarded on increasing the length of the cold season. The range of variability observed at Perth was remarkably shortened in Sardinia, as early lines were much more retarded than late lines.

The variability among strains related to the climatical variability of their collection sites was better discriminated at Perth than in Sardinia. The implications on selection models framed for selecting cultivars suited to Sardinian environments are discussed.

INTRODUCTION

The time of flowering is a characteristic of key adaptative significance in annual self-reseeding legumes such as subterranean clover (*Trifolium subterraneum* L. sensu lato). The survival of these plants, and, as consequence, the annual regeneration of their pastures, is primarily associated to the seed yield, and this is largely influenced by the time of flowering. As regards to persistence from year to year and productivity, the essential requirement is that a variety to be grown in a given environment must flower early enough for adequate seed to be produced before the advent of the dry and hot season, but not too early to imply a marked loss in the potential for dry matter yield allowed by the environment. Ultimately, the maturity grading of the varieties must be "adjusted" to the climatical conditions of the environment in which they will be grown.

Consideration of the maturity characteristics of the genotypes in relation to the length of the growing season of the environments, is therefore of critical importance in any breeding program aimed at developing high yielding and persistent cultivars. A similar program, based on selection of native populations, has been established in Sardinia recently (PIANO, 1984). For the success of this program, selection models are required on the most suitable flowering time of the varieties to be developed for the different climatic environments in Sardinia.

This kind of knowledge can be obtained in studying the relationships of the maturity characteristics of the native populations with environmental characteristics of their collection sites related to the length of the growing season, such as annual rainfall, temperature regimes, etc.. Due to the obvious implications on fitness, we can in fact assume that time of flowering of the native strains is largely "adjusted" to the environments in which they grow naturally.

However, time of flowering of subterranean clover strains is certainly characterized by some level of plasticity. In fact, even in an uniform edaphic environment, a plastic flowering behaviour should be of considerable adaptative value, because of the marked fluctuations of climatic conditions from year to year. The reliability and the successful utilization of a selection model obtained in relating the maturity characteristics of the native populations, with the climatical characteristics of their collection sites, is therefore expected to be influenced by the environment in which flowering time is observed.

On the basis of the above considerations, the question arises to what extent the maturity grading observed in one environment can reflect the maturity grading in another, and to what extent the variability of the strains in relation to the climatical variability of their natural habitats is better expressed in one environment rather than in another.

The aim of this work is to study the environmental influence on "flowering time expression" of subterranean clover genotypes and its implications on breeding programs specifically designed to develop cultivars adapted to Sardinian conditions.

MATERIALS AND METHODS

A wide collection of natural populations of subterranean clover originating from 144 sites in Sardinia was examined by the author at Perth (Western Australia) during 1981-82. From these populations 1260 pure lines were singled out, 723 of which were classified as belonging to the ssp. ***subterraneum*** L. sensu stricto (PIANO, 1984). All the lines were also characterized for flowering time, which in Perth conditions ranged from 83 to 190 days (days from sowing to first flower).

During 1982-83, the lines singled out were examined at Ussana, near Cagliari (South Sardinia); time of flowering of each line was recorded. In both Ussana and Perth the materials were grown in single rows 2m apart and approximately 2m long. Considering the inversion of the seasons in the two localities, the sowing took place at similar dates: early May in Perth and early November in Ussana.

During 1982-83, an examination of 140 lines of ssp. ***subterraneum*** was also carried out in 4 climatically differentiated localities in Sardinia: Abbasanta, Enas, Planu Sanguni and Ussana. These lines were partly provided by the Western Australian Department of Agriculture (but originated from Sardinia), and the rest chosen from the main collection. In this experiment the lines were grown in small plots of 4 rows, 1m long and 20 cm apart in a complete randomized block with 2 replications. The sowings took place during the last 10 days of October 1982, at approximately the same dates for the four localities.

An analysis of variance was conducted for each experiment and a subsequent combined analysis for the four experiments which involved the collection of 140 lines. By a linear regression analysis the relationship between flowering time at the different Sardinian localities and flowering time at Perth was studied for the two collections examined. Furthermore, for the lines of the main collection, the flowering times observed at Perth and Ussana were regressed on the average annual rainfall of the collection sites. As annual rainfall is in Sardinia positively correlated with the length of the growing season, this kind of relationship can provide useful guidelines in selecting cultivars with a maturity grading suited to the different environments.

The results reported here refer exclusively to the lines of the ssp. *subterraneum*, the only ones which were examined in all the 5 localities.

RESULTS AND DISCUSSION

For the main collection of lines the frequency distribution of flowering time at Ussana and Perth is reported in figure 1.

The difference between early and late lines was much less at Ussana than at Perth. In fact, the wide range of variability observed in Western Australian conditions (105 days) was shortened to only 55 days in Southern Sardinia. Furthermore, the average flowering time of the collection was delayed by about 13 days at Ussana.

These results indicate a considerable environmental influence on flowering time expression. However, it is also evident that the changes induced by the environment are not homogeneous for the lines of different maturity grading registered at Perth. Since, if the delay had affected equally all the lines, the extent of the range of variation should have been similar in the two localities. This is clearly shown in figure 2a. The flowering delay which occurs at Ussana does not involve all the lines. It is magnified in very early lines (up to 35 days), but progressively

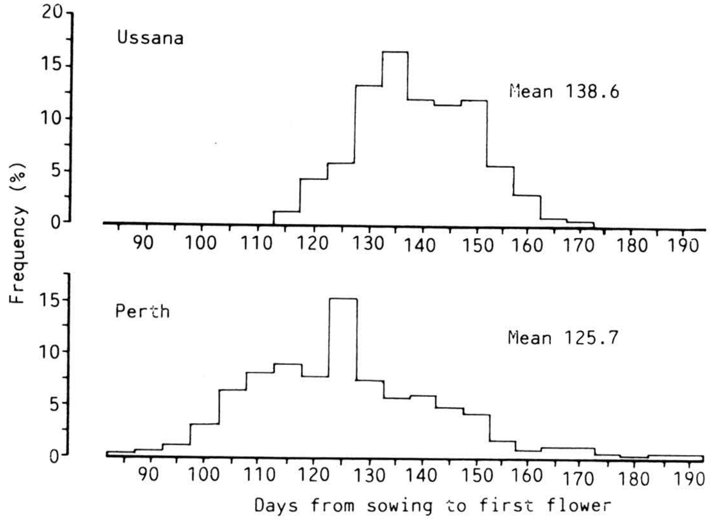

Figure 1 - The flowering time frequency distribution in a Sardinian collection of subterranean clover lines when grown at Ussana (Sardinia) and Perth (Western Australia).

shortens on increasing the maturity grading of the lines at Perth, so that the latest genotypes flower earlier than in Western Australia.

Considering the main environmental factors involved in flowering time expression (AITKEN, 1955; MORLEY and DAVERN, 1956; EVANS, 1959; MORLEY and EVANS, 1959), these results might be related to differences in temperature during the growing season between the two sites. These differences are likely to affect both the stages from sowing to flower initiation, and from flower initiation to first flower appearance (MORLEY and DAVERN, 1956). Due to the colder and longer Sardinian winter season, early genotypes, whose cold requirements for flower initiation are generally limited, are strongly delayed in flowering by the slowing effect of the cold in the post-initiation phase. On the other hand, very late genotypes can be hastened, presumably because their normally higher cold requirements for flower initiation are satisfied earlier in Sardinia and, as the season advances, the temperatures which occur during their post-initiation phase become progressively suited for a rapid flower development.

If this interpretation is correct, on increasing the length of the cold season, early lines should be further retarded in flowering, and the slowing effect of the cold in the post-initiation phase should involve a progressively increasing proportion of lines. This assumption has been corroborated by the results obtained in evaluating the collection of 140 lines at four Sardinian sites. In these sites, the temperatures during the growing season decreased according to the order: Ussana >Enas >

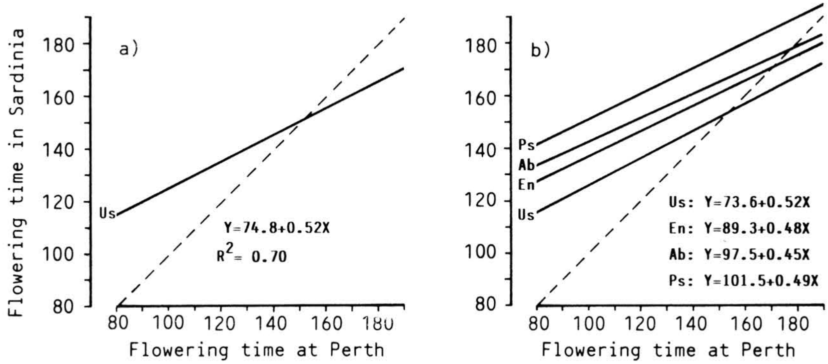

Fig. 2 - Relationship between flowering times in Sardinia and Western Australia for: a) a collection of 723 lines examined at Perth (W.Australia) and Ussana (Sardinia); b) a collection of 140 lines examined at Perth and at four Sardinian localities (Us = Ussana; En = Enas; Ab = Abbasanta; Ps = Planu Sanguni).

Abbasanta > Planu Sanguni. For istance, during the months of March and April the mean montly temperatures were 9.6 and 13.1 at Ussana, while at Planu Sanguni the means were 6.1 and 9.2 respectively.

As expected, on lengthening the cold season the average flowering time of the collection was progressively retarded, the proportion of lines which delayed in respect to Perth increased and, at the coldest environment, also the latest lines flowered later than in Perth (table 1, fig. 2b). In Sardinia, also this collection halved the variability observed at Perth. However, the range of variation registered at Ussana (the warmest locality) was not significantly shortened at the colder localities as it remained around 45 days (table 1). This derives from the fact that, as the environments became colder and colder, the increasing flowering delay of the early limits was accompanied by an increasing delay of the late limits.

The results of the combined analysis of variance indicated that all the sources, "Lines", "Environments", and "Lines x Environments" were higly significant ($P < 0.005$), but only a minor part of the total variance was attributable to the component "Lines x Environments".

From the results of the regression analysis given in table 2, can be clearly deduced that the "deviation" was negligible for all the regressions examined. In effect, the response of lines with a similar maturity grading at Perth was quite homogeneous within each environment in Sardinia. Furthermore, we never found lines with flowering time relatively "stable" on varying the environmental conditions.

The results obtained indicate that a date of flowering in one

Site	Mean	Limits	Range	C.V. %
Perth	137	83-178	95	16.4
Ussana	144	115-164	49	8.1
Enas	154	128-173	45	7.1
Abbasanta	159	133-179	46	6.6
P.Sanguni	168	140-189	43	6.7

Table 1 - Mean flowering time (days) and parameters of variability in the collection of 140 lines examined at different sites.

Site	Regression	Deviation	R^2
	(Mean squares)		
Ussana	18784.9**	2.7	0.96
Enas	15934.4**	6.9	0.94
Abbasanta	14054.7**	8.7	0.92
P.Sanguni	16819.0**	7.7	0.94

Table 2 - Results of the regression analysis of flowering time at four Sardinian localities on flowering time at Perth. *(** $P < 0.005$)*

location may be an inexact index of the date in another. For the lines examined, a clear relationship could be, however, drawn on the behaviour of a genotype in one environment when grown in another.

At the first stages of a selection program, when hundreds of lines must be screaned, flowering time data are necessarily recorded at one central location, although, in doing so, "genotype x environment" interaction is neglected. But, for the Sardinian strains data recorded in two environments are available. The question arises if either the data of Perth or Ussana must be considered in framing selection models for the maturity requirements of subterranean clover cultivars in Sardinia.

The relationship between flowering time of the lines and annual rainfall of their collection sites, is shown for both data from Perth and Ussana, in figure 3. In both cases, the limits of the regression curves (which indicate the average flowering time of the lines originating from the driest and wetest environments) are far away from the limits observed in the flowering time frequency distribution, which were about 85-190 days at Perth and 115-170 days at Ussana (fig. 1). This derives from the fact that early lines and late lines do not all necessarily originate from dry and wet environments, respectively. In particular, very early lines are often found in very wet environments and, anyway, the variability among sympatric lines is considerable (PIANO, in press).

Nevertheless, it is clear from figure 3 that the relationship based on Perth data allows a better discrimination of the variability in flowering time related to the rainfall variability of the "habitats". In fact, the range between the two limits of the regression curve is 30 days using flowering time data from Ussana, and 45 days using data from Perth. Therefore, Perth environment allows us to better evidentiate the variability among lines expressed by their phenotypic plasticity.

This latter aspect may be of relevance when selecting for appropriate earliness. As already shown, the delaying effect of Sardinian climate declines on increasing the maturity grading of the lines (fig. 2). In respect to Perth, the differences in flowering time among genotypes are consequently more shortened in the early range of maturity than in the late. Within the early range, strains which at Perth vary by about

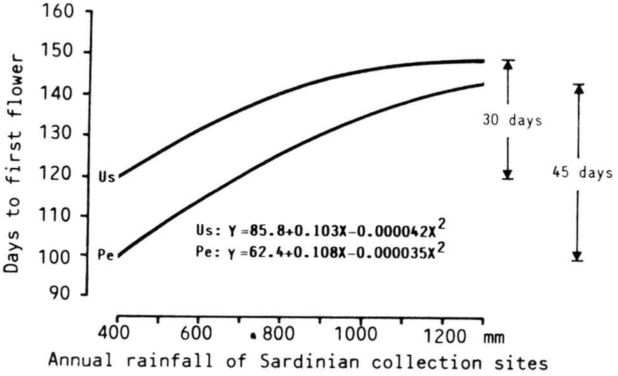

Figure 3 - Relationship between flowering time and annual rainfall at the collection sites when examined at Ussana and Perth.

30 days (similar to the cultivars "Dwalganup" and "Seaton Park") vary by about 12 days only at Ussana. Therefore, remarkable differences may be neglected if the selection for earliness is based on data from Sardinia.

However, whether or not excessive enphasis on earliness must be put in selecting cultivars for short season environments in Sardinia, is a matter of discussion, considering that even in Ussana which experiences one of the shortest and warmest growing seasons, very early lines are strongly retarded in flowering. Nevertheless, these lines, which better expressed their earliness in Perth, mainly originated from similar environments.

Long term survival of subterranean clover strains greatly depends on their ability to escape prevention of seed setting in occasional adverse seasons, and not only to produce adequate seed in ordinary seasons. Earliness is, therefore, important. As long term persistence is an essential requirement for the agronomical utilization of the species, this also applies to the cultivated varieties.

The results obtained have indicated that subterranean clover strains have a high plastic flowering behaviour, no matter of their maturity grading. The ability to hasten or delay flowering time is a property of clear adaptative significance to avoid limitations of seed setting when the hot season comes early, or the cold season is prolonged. As regards the implications in breeding, what emerges from our results is that the variability in flowering time is largely expressed in terms of phenotypic plasticity and that this must be adequately considered in planning selection programs.

ACKNOWLEDGEMENTS

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RESUME'

Aspects de la variabilité des populations de la tréfle souterrain et ses conséquences sur le travail d'amélioration. L'influence du milieu ambiant sur l'époque de floraison.

Un vaste ensemble de génotypes sardes de tréfle souterrain a été examiné en ce qui concerne sa date de floraison à Perth (Australie) et à Ussana (Sardaigne - Italie) ainsi qu'un ensemble plus restreint à Perth et dans 4 localités différentes de la Sardaigne.

Les résultats obtenus ont indiqué que la variabilité quant à l'époque de floraison est grandement liée à la plasticité phénotipique et que cette plasticité est determinée par les différences de température des milieux dans lesquels les génotypes sont examinés.

L'époque de floraison moyenne de l'ensemble a été progressivement retardée suivant l'augmentation de la durée de la saison floide. De plus, le "range" de variabilité observé à Perth a été fortement raccourci en Sardaigne car les lignes précoces étaient beaucoup plus en retard que les lignes tardive.

La variabilité parmi les génotypes associée à la variabilité climatique de leurs milieux d'origine est apparue plus distinctement à Perth qu'en Sardaigne.

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Levels of variability in cocksfoot (Dactylis glomerata L.)

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SUMMARY

Some aspects of the available genetic resources are presented, with examples of the structuration of diversities in the Dactylis complex. The use of genetic markers at the molecular level is described .

Introduction

In a preceding meeting of the Fodder Crop Section (JACQUARD, 1982), we have presented some aspects of the available genetic resources in cocksfoot . In the present paper, we intend to up date the knowledge of variability at the light of new results or interpretations (JACQUARD, 1986).

The variability or heritable variation can be observed at 3 levels :

1. molecular, variation of isolated genes as revealed by electrophoresis (allozymes) or other biochemical technic, like chromatography (i.e. flavonoids), or with the aid of technics of molecular biology (analysis of aminoacid sequences in proteins or base sequencies in DNA, as in c-DNA; at this level the determinism is mono or oligogenic ;

2. phenotypic, variation for physiological or morphological characteristics, mainly under the control of a large number of genes, its genetic basis has been revealed by artificial selection and quantitative genetics ;

3. fitness, of which the variation is the ultimate parameter of population genetics, expressed in terms of fecundity and survival, that is to say the last judgement which determines the selective forces acting on phenotypes

and genes .

Some authors (SCHARLOO et al., 1977 ; SCHARLOO, 1984) have combined these levels in the " Magic Triangle " of evolutionary genetics, including the interactions with the environment. The environment may acts on the molecule-phenotype connexion , but influences mainly the expression of trait in terms of fitness, and strongly acts on the fitness-molecule connexion if the variants are not neutral .

These levels of variability are observed though genetic, physiological and demographic approaches. The description of the hereditary variability or genetic diversity at the several levels allows to study the structure of the biological samples, at contrasting scales of space (from the microsite to the continent) and time (from one generation to geological dimension) in view to integrate the two point of view : biogeography and evolution.

The most used markers of molecular variation are still the enzymes revealed by monodimensional electrophoresis. This technics gives a valuable information on the diversity of structural genes to be used in the study of mating systems and of the gene flows But, the number of accessible loci is however limited, and the attempt to characterize the heterozygosity of the populations on the basis of very few loci are sometimes of doubtful value, then the interest on the bi-dimensional electrophoresis that reveals the diversity of regulatory genes. The flavonoids allow an another type of access to the polymorphism at a more advanced stage of the metabolism. Another type of markers of very great interest are the restriction fragments (RFLP's). It seems that the majority of structural markers are neutral, and, in fact , we need to use neutral markors if we want to know the evolutionary history and the relationships within and between biological entities .

Examples of the structuration of diversities in the Dactylis glomerata L. complex)

The diversity of cocksfoot has been studied on a large number of samples and at several scales by R. LUMARET and co-workers. These studies include analysis of populations along clines .

1. Molecular variation

The polymorphism of many systems of enzymes has been studied :

Glutamate-oxaloacetate-transaminase (1 locus) : GOT

Acid phosphatase (1) : AcPH

Peroxydase (1) : PX
Malate dehydrogenase (1) : MDH
Tetrazolium-oxydase (1) : TO
Phosphoglucose-isomerase (2) : PGI
Alcool dehydrogenase (2) : ADH

ROY and LUMARET (1987) have studied the variability for 4 systems (GOT, PX, PGI, AcPH) for 9 populations collected along a water stress cline of hundreds of kilometers The geographic location of the populations genetically analysed ranges from Scotland to Morocco . The allelic distribution for the 4 enzymatic systems have been related to an aridity index of the place of origin . Two to four alleles per population were found at the GOT 1 locus . The proportion of allele 1.00 increased sharply with decreasing aridity and was partly compensated by a decrease in allele 0.72 . A more extensive study of the polymorphism at this locus in nearly 100 populations confirms this result (LUMARET, 1984) . As an example, we give a comparison in three pairs of closed localities (Table 1) .

Table 1

Comparison of allelic distributions at the GOT 1 locus in three pairs of close localities near Montpellier (South of France). The first site is the driest and the second the wettest. Allelic frequencies are in parenthesis (Adapted from LUMARET, 1984).

Locality and site	Nb plants	alleles				chi square
		1.00	0.72	0.38	0.10	
Col de Mourèze						
(South face)	39	28(18)	90(58)	22(14)	16(10)	33.16
(North face)	29	57(49)	49(42)	06(05)	04(04)	**
Aumelas						
(shallow soil)	59	14(06)	99(42)	97(41)	26(11)	8.72
(deep soil)	47	26(14)	64(34)	79(42)	19(10)	*
Causse de la Selle						
(open habitat)	36	20(14)	58(40)	50(35)	16(11)	11.86
(closed habitat)	39	36(23)	37(24)	69(44)	14(09)	*

Levels of significance: * = P 0.05 ; ** = P 0.01 .

The polyphenolic compounds (flavonoids) have been also used as chemical markers of genetical diversity between and within natural populations (ARDOUIN et al. , 1985). In the ordinations obtained by F.A.C. of H.P.L.C. flavonoid data concerning 120 individuals of 20 diploid populations and 94 individuals of 12 tetraploid populations of D. glomerata the axes 1 and 2 account for 33 % of the total variation , for the 2 samples .

2. Phenotypic variation

Phenotypic variation described in terms of leaf tissue water relations, turgor pressure or potential against total water potential (ROY & LUMARET, 1987), for individuals sampled in a contrasting habitat along a 40 m cline (dry and wet sub-site) reveals a discrimination as strong as for a cline of 1300 km between Morocco and a mountain site in the Massif Central (France) .

In that case, simultaneous variation has been established between a clinal variation in leaf tissue water relations and allozyme polymorphism of some populations. Very few examples of such a research have been presented before. But no link between the enzyme function and the physiological parameters was established. Proving such links is necessary to increase our understanding of the processes of plant evolution by joined physiological and allozyme studies .

Variability of other physiological or morphological characteristics can be exemplified in cocksfoot (ROY, 1982):

a. seasonality of leaf demography in Mediterranean natural population from a xeromorphic sward in the French "garrigue " (D'HERBES , 1979) and Septentrional natural population of Northern France,

b. growth rate of the seedling leaves, in relationship with the latitude of origin of the populations (BROUE et KAWANABE , 1967),

c. relative growth rate (EAGLES , 1971).

d. morphogenesis, using the modular and metameric model of plant growth : the pattern of morphogenesis is entirely determined by the phyllochrone, the number of phyllochrone at a given time t,the number of tillers (modules) and the number of leaves (metameres) per tiller. This type of variation has been notably studied for individuals from populations growing in contrasting habitats (Table 2) (GARNIER & ROY , 1987), and the resulting consequences in terms of photosynthetic area. The genetic variability of the dynamics of modular growth in cocksfoot has been also studied for the progenies of 3 crosses between 3 individuals of the hispanica form. The general pattern of variation has been described using the ordination, obtained by F.A.C. of morphogenesis and growth data concerning 98 individuals of the 3 progenies . The 2 axes account for 64.8 % of the variation. Three contrasting types of morphogenesis can be defined using the main contributing variables : slow, medium and fast growing type. The respective characteristics of the 3 types are :

- for the slow, a long mean duration of the phyllochrone (PHY) , from 142 to 230 °C ,a number of phyllochrones on the main stem (NTY) of 10 or minus, a number of tlllers/phyllochrone (TAL) of 1.7 to 3.0 ;
- for the medium , PHY between 139 °C to 142 °C, NFY of 11, TAL between 3.0 et 6.1 ;
- for the fast, PHY under 139 °C, NFY of 12.0, TAL upper 6.1.

Table 2

Morphogenesis variation between 2 habitats
(Mean from October to August)

		Open sward	Under story
Number of modules/plant	D.g.	10.5	7.4
	B.e.	12.9	9.8
Number of metamere/modules	D.g.	1.8	2.3
	B.e.	2.3	4.4
Green area/leaf (cm^2)	D.g.	1.0	1.4
	B.e.	1.9	2.8

D.g. = Dactylis glomerata ; B.e. = Bromus erectus

In the same study, despite the fact that 2 parents are closely related, contrasting responses between 2 crosses have been observed in the allocation of resources : for a given level of nitrogen, and 2 levels of water supply, the progenies from one cross invest preferentially in the size of the metameres (mean weight of a leaf) and progenies from an other cross invest preferentially in the number of modules (tillers).

3.Fitness variation

In terms of ressource allocation, populations from abandoned and controled environment have been compared (JACQUARD & HEIM, 1983). The partitionning of the dry matter harvested from aerial parts at flowering , between leaves, stems and panicles differs according to the origin. The reproductive effort is higher in the natural population than in the " domesticated " population .

For fecundity and survival, it seems that there is a positive relationship between the performance and the diversity status at the autotetraploid level. The plants that possessed the highest level of heterozygosity (3 or 4 alleles per locus) have statistically the greatest longevity and the highest annual production of seeds (LUMARET, 1984). Examples of such results are presented in table 3 (unpublished data from TOMEKPE).

Conclusions

We have now at our disposal a wide range of genetic markers at the molecular level , in majority neutral. They can be used to :

1. study the genetic structure of the hierarchical units of evolution and selection : neighbourhood , population , metapopulation , species, complex of species ;

2. to check the gene flow in natural conditions or in controled conditions (family , synthetic) or to check the mating (crosses, selfing, polycrosses).

At the phenotypic and fitness level, considering a grass cover as a " population " of tillers and leaves , there is a variation, of potential use, for : modular parameters (as Integrated Physiological Unit), metameric parameter (mainly for the phyllochrone duration), biomass allocation at the whole plant level , and survival.

But it is still very risky to use a low number of structural loci to estimate the heterozygosity and the supposed associated " vigour " , despite the fact that there is some evidence of statistical connexion between the three levels of variability .

RESUME

Quelques aspects des ressources génétiques sont présentés , avec des exemples de srtucturation de la diversité dans le complexe Dactylis . L'utilisation de marqueurs génétiques au niveau moléculaire est décrite .

Table 3

Average number of panicles/plant and average leaf weight/plant according to the genotype at 3 loci (M : monogenic ; SD : simplex-digenic ; DD : duplex-digenic ; TRI: trigenic ; TET : tetragenic) in a Dactylis population from the south of France.

	Genotypic classes					Significant differences
	M	DS	DD	TRI	TET	
			GOT 1			
Number of plants(N)	46	161	124	116	5	
Number of panicles/ plant (P)	17.76	19.54	20.99	23.81	26.50	M-TRI S-TRI D-TRI M-TET S-TET
Leaf weight/ plant(L)	0.95	1.28	1.33	1.35	1.54	M-D M-TRI M-S M-TET
			PX 1			
N	25	129	95	192	11	
P	18.81	18.83	20.94	22.52	21.90	M-TRI S-TRI
L	0.89	1.22	1.21	1.41	1.30	M-TRI S-TRI D-TRI
			AcPH 1			
N	239	154	59			
P	20.71	20.23	23.52			M-D S-D
L	1.26	1.32	1.25			NS

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La germination des graines dans la gousse : facteur de "pérennité" des populations naturelles des espèces annuelles du genre medicago L.

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Résumé

La gousse des espèces du genre Medicago L. représente l'élément essentiel de la propagation de ces espèces. Dans les conditions naturelles, les graines germent à l'intérieur de la gousse qui reste fixée au collet de la plante. Toutefois la germination n'intéresse pas la totalité des graines de la gousse ; celles qui n'ont pas germé, génétiquement apparentées aux premières, conservent leur prouvoir germinatif. Des conditions favorables à la germination, suivies de température ou d'humidité non satisfaisantes pour la croissance de la plantule, ainsi que le surpâturage peuvent être à l'origine de l'élimination des individus issus de la première vague de germination. Les graines non germées constituent donc une réserve de semences aptes à la réinstallation de la population dès le retour à des conditions favorables. Ce mécanisme permet de conserver le stock de graines à un niveau suffisant dans le sol et favorise le maintien "in situ" des populations naturelles.

Introduction

De nombreuses espèces de Medicago annuelles existent en Tunisie à l'état spontané dans divers milieux édaphiques et tous les étages bioclimatiques. A chaque cycle de végétation les plantes de luzernes annuelles produisent individuellement une grande quantité de gousses et de graines dures L'existence des graines dures permet à ces espèces de survivre à certains phénomènes catastrophiques tel que le feu et le gel, de même cette qualité leur confère une bonne résistance à l'ingestion par les animaux.
FRIEDMAN et ORSHAN (1972) ont observé que 85 % de graines de Medicago laciniata agées de vingt ans germent après quarante huit heures. Cette observation révèle que les graines de Medicago annuelles peuvent avoir une grande longévité. L'élément de dissémination des Medicago annuelles est la gousse : elle est indéhiscente et dans les conditions naturelles une

graine germe à l'intérieur de la gousse qui reste fixée au collet de la plante. Dans certains cas on observe deux ou trois plantes à des stades de développement différents émergeant d'une même gousse. Une germination des graines d'une même gousse échelonnée dans le temps éviterait la compétition spatiale ainsi que l'élimination possible, sous l'action de diverses contraintes particulièrement climatiques, des plantules qui en émergeraient simultanément.
Ce mode d'installation des différentes espèces de *Medicago* annuelles dans une aire donnée a des répercussions sur la génétique et la dynamique des populations naturelles. Les graines d'une même gousse sont au moins demi-frères sinon pleins frères sachant que l'ensemble des espèces annuelles est réputé autogame.
Si, après des conditions favorables à la germination et à un début de développement de la plantule, une période de sécheresse s'installe, les individus issus de la première vague de germination sont éliminés. Les graines non germées appartenant aux mêmes gousses peuvent prendre le relais en germant dès le retour à des conditions favorables (fig.1)

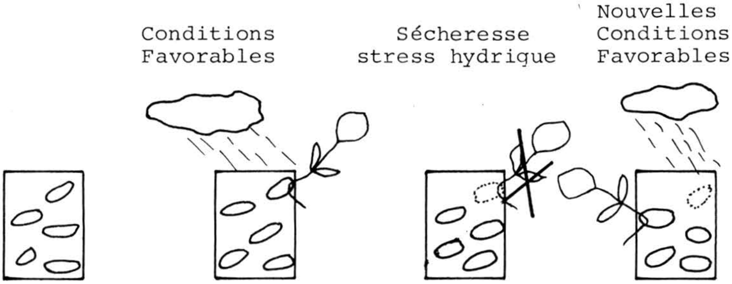

Fig.1 - Mode de germination des graines dans la gousse en fonction de l'alternance conditions favorables - stress hydrique.

Ce mode de germination échelonné dans le temps permet donc de maintenir relativement stable la structure génétique des populations naturelles.
L'objet de cette note est d'apporter quelques arguments en faveur de l'hypothèse d'un système d'installation des populations naturelles de Medicago annuelles adapté à des régions caractérisées par l'irrégularité des précipitations. (particulièrement le climat méditerranéen).

Matériel et Méthode

Quatre espèces, largement répandues en Tunisie, ont été retenues pour cette expérience. Chaque espèce est représentée par deux populations d'origines différentes :

Medicago truncatula, 01, se retrouve dans tous les étages bioclimatiques allant de l'humide au saharien. Elle est représentée par les populations :
- 009.01 : Etage sub-humide à hiver doux avec une pluviométrie P > 500 mm.
- 321.01 : Etage semi-aride supérieur à hiver tempéré avec une pluviométrie 400 < p < 500 mm.

Medicago polymorpha, 03 , s'adapte à tous les milieux. Elle est représentée par les populations :
- 005.03 : Etage sub-humide à hiver doux avec une pluviométrie P > 500 mm.
- 321.03 : Etage semi-aride supérieur à hiver tempéré avec une pluviométrie 400 < P < 500 mm.

Medicago orbicularis, 04, est une espèce inerme dont l'aire de répartition s'étend de l'humide au semi-aride. Elle est représentée par les populations :
- 010.04 : Etage semi-aride supérieur à hiver tempéré avec une pluviométrie 400 < P < 500 mm.
- 326.04 : Etage semi-aride supérieur à hiver doux avec une pluviométrie 500 < P < 600 mm.

Medicago minima, 06 , est localisée entre les étages semi-aride et saharien. Elle est représentée par les populations :
- 113.06 : Etage aride supérieur à hiver tempéré avec une pluviométrie 200 < P < 250 mm.
- 321.06 : Etage semi-aride supérieur à hiver tempéré avec une pluviométrie 400 < P < 500 mm.

En Tunisie, le semi-aride ainsi que l'aride supérieur représentent les zones où les *Medicago* annuelles peuvent être utilisées pour l'amélioration des parcours et la valorisation des jachères.

Les huit populations différentes proviennent de six sites. Le sol des stations numérotées 009, 113, 321, 326 est défini, selon la classification française, comme peu évolué. Ce sont des sols d'apports modaux sur sable fluviatile. Les stations numérotées 005 et 010 ont des sols calcomagnésimorphes.

Remarque : une station est définie par les trois premiers chiffres ; le 4ème et le 5ème chiffres représentent l'indicatif de l'espèce. Ainsi ce code permet de distinguer une population en indiquant sa position géographique et l'espèce à laquelle elle appartient.

Dans chaque population un échantillon de quatre vingt huit gousses de l'année est tiré au hasard. Toutes les gousses proviennent de la collecte effectuée au cours de l'été précédent l'expérimentation.

Cet échantillon est réparti en 3 lots au hasard.
- Un lot de 22 gousses sert de témoin.
- Un second lot de 22 gousses subit un traitement à l'azote liquide. Les gousses sont immergées pendant 5 minutes dans l'azote liquide afin de réaliser la

scarification des graines à l'intérieur des gousses.Cette opération est nécessaire pour lever l'inhibition tégumentaire des graines dures et faciliter leur germination.

- Un troisième lot de 44 gousses est destiné à subir un stress hydrique sous certaines conditions. Les gousses qui ont donné au moins une plantule sont soumises à un stress hydrique par arrêt de l'arrosage pendant deux semaines.

Chaque gousse est enfouie dans un Jyffipot plein de terreau à un centimètre de la surface. L'arrosage par aspersion est quotidien. L'expérience est menée dans un compartiment d'une serre en verre.

Discussion

Après traitement à l'azote liquide 50 à 100 % des gousses, ayant subi cette scarification par le froid, donnent trois à dix plantules qui émergent par gousse selon les espèces considérées Tableau 1.

N° des populations	Témoin		Traitement à l'azote liquide		Stress Hydrique			
					Avant Stress		Après Stress	
	1	2	1	2	1	2	1	2
TRU : 00901	0,32	2,29	0,82	3	0,27	1,08	0,66	1
TRU : 32101	0.27	1,83	0,55	2,33	0,39	1,24	0,29	1
POL : 00503	0,23	1,20	0.73	3,62	0,39	1,88	0,06	1
POL : 32103	0,36	1,63	0,59	2,92	0,52	1,87	0,13	1
ORB : 01004	0	0	0,91	10,35	0,05	1	0	0
ORB : 32604	0,09	1	1	11,64	0.05	1	0	0
MIN : 11306	0,14	1	0,86	3,63	0,05	1,5	0,5	0,5
MIN : 32106	0,05	1	0,86	3,16	0,05	1	0	0

Tableau 1 : germination des graines dans la gousse.

N.B : 1 = Fréquence des gousses ayant donné une plantule au moins.
2 = Nombre moyen des plantules par gousse.

Si l'ensemble des graines d'une même gousse est scarifié correctement celles-ci peuvent germer simultanément sans préjuger des phénomènes de compétition entre plantes qui pourraient apparaître ultérieurement.

Dans la nature la scarification peut-être obtenue par les grandes amplitudes thermiques (Nuit - jour)de la saison chaude.

Les graines d'une même gousse ont subi en principe un même traitement indispensable à leur levée. Toutefois une seule graine germe par gousse, rarement deux ou trois graines et exceptionnellement plus de trois graines germent par gousse. Or le nombre moyen de graines par gousse, quelque soit l'espèce à laquelle on s'adresse, est nettement supérieur à celui des graines qui germent par gousse à une saison donnée (sauf pour Medicago lupulina et Medicago rugosa) Tableau 2.

Espèce	Nombre moyen de gr/G	Espèce	Nombre moyen de gr/G
M.truncatula	5 - 8	M. minima	3 - 6
M.polymorpha	4 - 7	M.orbicularis	10 -24
M.lupulina	1	M.rugosa	1 - 2

Tableau 2 - Nombre moyen de graines par gousse chez différentes espèces de Medicago annuelles

C'est pourquoi nous avons vérifié l'existence de graines viables à l'intérieur des gousses prélevées dans la nature et ayant donné in situ au moins une plante. Au cours de cycle végétatif (au printemps) des plants de Medicago annuelles ont été prélevés dans des populations naturelles en prenant soin d'arracher la plante avec sa gousse accrochée au collet.
Le dénombrement des graines dans ces gousses ainsi que le nombre moyen de plantes qui en émergent, pour les deux espèces Médicago truncatula et Medicago ciliaris sont présentés au Tableau 3.

Espèce	Nombre moyen plante par G	Nombre moyen graine par G
M. ciliaris	$\frac{98}{67} = 1,46$	$\frac{200}{67} = 2,98$
M. truncatula	$\frac{140}{100} = 1,40$	$\frac{343}{100} = 3,43$

Tableau 3 - Nombres moyens de plantes et de graines observés dans les gousses, ayant donné au moins une plante, prélevées dans la nature

Les graines extraites de ces gousses sont scarifiées mécaniquement puis placées dans des boites de pétri sur du papier filtre imbibé d'eau distillée. L'ensemble est installé dans une étuve réglée à 20° C ± 1°C. Au bout de 48 heures, 90 % de ces graines germent. Il apparait évident que les graines, provenant de gousses ayant donné au moins une plante, gardent leur pouvoir germinatif et restent viables.
Par ailleurs nous avons constaté chez la plupart des espèces

de Medicago annuelles que le taux de germination des graines non scarifiées atteint rarement 20 % après 10 jours d'incubation, alors que les graines scarifiées ayant le même âge germent à 95 % au bout de 48 heures.

La scarification, en levant l'inhibition tégumentaire, est indispensable pour la germination des graines dures.

Le troisième lot, formé par les gousses n'ayant pas subi le traitement à l'azote liquide, fournit les résultats présentés au tableau 1.

Entre 30 % et 50 % des gousses de Medicago polymorpha et de Medicago truncatula donnent entre une et deux plantules par gousse ; par contre seul 5 % des gousses de Medicago orbicularis et Medicago minima fournissent une plantule par gousse.

Certains auteurs,Mc COMB et ANDREWS 1974, IBN TATOU 1981, se sont intéressés au rang de la graine qui germe à l'intérieur de la gousse. En orientant la gousse par rapport à son plan équatorial on peut définir deux extrémités distales et proximales. Ils ont pu montrer que le poucentage de graines germées d'un rang donné est plus élevé aux deux extrémités et diminue graduellement lorsqu'on s'éloigne de celles-ci.Ils qualifient ce mode de germination de séquentielle.

Les gousses qui ont donné au moins une plantule sont soumises à un stress hydrique par arrêt de l'arrosage pendant deux semaines et et cela quatre mois après leur mise en place dans les Jyffipots. Après ce stress hydrique les arrosages réguliers sont repris. Par cette opération nous voulons savoir si des graines, appartenant aux mêmes gousses, peuvent germer successivement et donner des plantules.

Medicago truncatula et à un moindre degré Medicago polymorpha donnent des réponses (Tableau 1) qui semblent aller dans le sens d'un mode d'installation adapté à un climat caractérisé par les irrégularités des précipitations et de longues périodes de sécheresse. Le cas de Medicago minima et Medicago orbicularis nécessite un temps d'observation plus long, c'est pourquoi il est important de poursuivre l'expérience pour donner une assise correcte à notre hypothèse.

Conclusion

La dureté des graines et la germination échelonnée dans le temps des graines d'une même gousse forment deux caractéristiques qui, associées au régime de reproduction autogame des Medicago annuelles ainsi que la tolérance de certaines espèces à la sécheresse, constituent un ensemble de facteurs leur conférant la qualité d'espèces pionnières et en tant que telles elles peuvent coloniser des habitats variés et plus ou moins perturbés.

Summary

The spiral pods, spiny or not, of Medicago species are not dehiscent. They represent the main propagatory element of the species. In natural conditions, part of the seeds germinate within their pods attached to the mother plant. Yet,not all pod's seeds germinate. The other seeds that remain in the pod without germination are genetically related to the first ones, these latter keep their germination power. Favourable conditions on the one hand followed by nasty ones such temperature and humidity and over - grazing (a quite natural phenomenon in arid areas) might be the cause of the elimination of the plants issued from the first wave of germination. Ungerminated seeds constitute a valuable stock of seeds ready to be reused as soon as favourable conditions are met. This mechanism enables to keep the stock of seeds at a good level in the soil and contributes to the in situ conservation of natural populations.

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ESTIMATION OF GENETIC VARIATION AND STABILITY OF SEED PRODUCTION CHARACTERS BY MEANS OF CLONAL EXPERIMENTS WITH NATURAL GRASS POPULATIONS.

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Summary

The paper describes an efficient experimental design with blocks within replicates, and the statistical model for genotype-location-year (GxLxY) experiments using this design. Results from a large scale clonal experiment with natural populations (6) and cultivars (2) of timothy (*Phleum pratense* L.) and red fescue (*Festuca rubra* L.), originating from the northernmost part of Norway (69-70°N), are also presented. Genotypic variation for seed production is large both within cultivars and natural grass populations, and the variation is equally large within cultivars and populations. Heritabilities for seed yield characters are high. Estimates of variance components and correlations vary a lot both with test site and plant material. GxY-interactions are generally much smaller than GxL-interactions, while GxLxY-interactions are pronounced. Genotype x environment-interactions are much larger in timothy than in red fescue. Variance component methods (i.e. genetic correlations and ecovalence) and cluster analysis for studying stability and specific adaptation facilitate the ranking of the stability of individual genotypes.

Introduction

Why do we conduct experiments with clones? Although some form of progeny test normally is used nowadays for the identification of superior parents for synthetic varieties, there will always be a need for selecting genotypes by means of clonal tests, especially in the beginning of a breeding program. Since outlined by BURTON & deVANE (1953), clone tests have been extensively used in the breeding of forage grasses. Based on the assumption that the variation we observe between clones is essentially genotypic in origin, some of the objectives of a clonal test are: 1) to identify phenotypically superior genotypes, 2) to compare the average effects of populations, 3) to estimate components of the genotypic and environmental variation, *with as high precision as possible*.

Experimental design

In conventional replicated trials with a large number of randomized entries it will often be difficult to control the random error variance within large replicates. Incomplete blocks have been used widely to cope with this problem. A special type of incomplete block design has been developed and used at our department (AASTVEIT 1977). Contrary to a conventional incomplete block design, the blocks contain the same genotypes in all replicates and at all locations. In an experiment with r replicates, each replicate is divided into b number of blocks. Each population must be represented with an equal number of genotypes in each block. Sequences of n genotypes are drawn randomly from each of p populations and put together in each block. Each block is uniq and made up of $p \cdot n$ genotypes. Genotypes are randomized within each block, and the blocks are randomized within replicates at each location. It has been shown theorethically that, if the number of genotypes in the test is large, this design will give better estimates of the genetic variation than similar randomized, complete block designs (AASTVEIT 1977).

Statistical model

Data from experiments with clones of perennial species are correlated over years because observations are taken on the same plot each year. A combined analysis over years can be done by assuming a split-plot in time model with years as subplots (STEEL & TORRIE 1980). Table 1 presents the expectations from a combined analysis of variance of a split-plot in time design with blocks within replicates. The model is a mixed nested model. Blocks are nested within replications and locations, and genotypes are nested within blocks and populations. All effects, except the population effect were considered random. Mean square expectations are presented only for the genotypic effects.

Plant material

Three of the most promising natural populations, collected in the northern part of Norway as a part of a national herbage breeding program, and in additon one cultivar of timothy and red fescue have been used in the present investigation on the genetics of seed production. The origin of the populations and the cultivars are shown in Table 2. Except form the red fescue cultivar 'Leik', they all originate at 69-70°N latitude. 100 genotypes from each of the populations and the cultivars were laid out in block designs as described earlier, at the following locations in Norway: Southern - Ås (60°N), Intermediate - Kvithamar (63°30'N) and Northern - Bardu (69°N). Seed yield and other characters were observed for 2 years.

RESULTS AND DISCUSSION

1. Genotypic variation

Variation between genotypes (G/B/P), and the genotype x location (G/B/PxL) and genotype x year x location effect were highly significant. Genotype x year interaction (G/B/PxY) was significant only in timothy, but much smaller than the GxL-interaction.

Table 1. Combined analysis of variance over populations, years and locations of a split-plot in time design with years as subplots and blocks within replicates.

SOURCE OF VARIATION	df	EXPECTATIONS OF MEAN SQUARES
LOCATION (L)	2	
REP/L	3	
YEARS (Y)	1	
Y x L	2	
Y x REP/L	3	
BLOCKS/REP/L (B/REP/L)	24	
Y x B/REP/L	24	
POPULATIONS (P)	3	
P x L	6	
P x REP/L	9	
P x B/REP/L	72	
P x Y	3	
P x L x Y	6	
P x Y x REP/L	9	
P x Y x B/REP/L	72	
GENOTYPES/BLOCKS/POP(G/B/P)	180	$\sigma^2_E + y\sigma^2_{GR} + r\sigma^2_{GLY} + ry\sigma^2_{GL} + rl\sigma^2_{GY} + rly\sigma^2$
G/B/P x L	360	$\sigma^2_E + y\sigma^2_{GR} + r\sigma^2_{GLY} + ry\sigma^2_{GL}$
G/B/P x REP/L	540	$\sigma^2_E + y\sigma^2_{GR}$
G/B/P x Y	180	$\sigma^2_E + r\sigma^2_{GLY} + rl\sigma^2_{GY}$
G/B/P x L x Y	360	$\sigma^2_E + r\sigma^2_{GLY}$
G/B/P x Y x REP/L	540	σ^2_E
TOTAL	2399	

(l=3 locations, y=2 years, r=2 replications with 5 blocks within each replicate, 4 populations, and 50 genotypes within each population).

Table 2. Origin of plant material.

Timothy (Phleum pratense L.)

POPULATION	ORIGIN	LATITUDE
Lakselv	Finnmark, N-Norway	70°N
Kautokeino	Finnmark, N-Norway	69°N
Karasjok	Finnmark, N-Norway	69°30'N
cv.'Engmo'	Troms, N-Norway	69°N

Red fescue (Festuca rubra L.)

POPULATION	ORIGIN	LATITUDE
Alta	Finnmark, N-Norway	70°N
Gibostad	Troms, N-Norway	69°N
Storfjord	Troms, N-Norway	69°N
cv.'Leik'	Oppland, S-Norway	61°N

Table 3 presents estimates of variance components for each of the three natural timothy- and red fescue populations and the cultivars.

Table 3. Estimates of variance components for seed yield in Norwegian timothy and red fescue populations.

SPECIES/ POPULATION	S^2_G	S^2_{GL}	S^2_{GY}	S^2_{GLY}	Error^1	Error^2
Timothy:						
Karasjok	127.5***	94.0***	10.7	55.9***	126.6	120.3***
Kautokeino	151.8***	78.3***	16.6	35.5***	171.1	95.7***
Lakselv	186.8***	75.9**	21.6	90.4***	175.1	193.6***
cv.'Engmo'	110.4***	90.1***	17.4*	48.9***	92.5	149.5***
$\chi_{(3)}$	6.40 ns	7.63 ns	7.81*	29.6***	24.6***	26.0***
Red fescue:						
Alta	115.1***	20.9	62.6*	114.3***	344.1	10.1
Gibostad	152.7***	-	-	224.6***	309.5	-
Storfjord	116.7***	39.5*	17.2	76.8**	240.2	-
cv.'Leik'	176.7***	45.8*	-	165.7***	289.8	-
$\chi_{(3)}$	2.98 ns			27.95***	4.51 ns	

Error^1 = Whole-plot error (Genotypes x year x reps/loc)
Error^2 = Sub-plot error (Genotypes x reps/loc)

Except from the GxY-variance, all components are highly significant in timothy. The genotypic variation for seed production is equally large within both the natural populations and the cultivar. Both the error variances and the three-factor interaction are significantly different. A more thourough discussion of the genetic variation for seed production in timothy can be found elsewhere (ROGNLI 1987a). In red fescue, highly significant variance components were only recorded for genotypes and the three-factor interaction. The genotypic variation for seed yield was not different within the red fescue populations either.

Measured by the genotypic coefficient of variation (GCV%), the character seed yield/plot showed on average the largest genotypic variation (GCV%=24.8 in timothy and 33.0 in red fescue). The heritability in broad sense was also high for this character (0.64 in timothy and 0.72 in red fescue). Date of ear emergence has a higher heritability in timothy (0.86) than in red fescue (0.59). The characters seed yield/panicle, fertility and 1000-seed weight, measured in red fescue, are also highly heritable (h^2_b=0.65-0.89) and genetically variable.

Table 4 presents mean values, heritabilities and genotypic coefficients of variation for each population at each location. In addition the combined estimates over populations are also presented.

Table 4. Mean values,. broad sense heritabilities and genotypic coefficients of variation (GCV%) for seed yield in red fescue, estimated at different locations.

	LOCATIONS								
	SOUTHERN			INTERMEDIATE			NORTHERN		
POPULATION	MEAN	H^2_B	GCV%	MEAN	H^2_B	GCV%	MEAN	H^2_B	GCV%
Alta	31.8	0.30	34.4	29.5	0.51	38.9	34.6	0.53	36.7
Gibostad	34.8	0.26	28.2	27.0	0.60	42.2	29.5	0.55	42.3
Storfjord	35.4	0.42	29.4	29.8	0.49	33.8	34.7	0.78	46.5
cv.'Leik'	57.4	0.55	30.2	51.2	0.72	33.7	30.2	0.49	27.6
Combined estimate	39.9	0.40	31.3	34.4	0.61	37.3	32.2	0.61	39.5

On average the genotypic coefficients of variation and the heritability are lower at the southern location than at the intermediate and the northern location. There is a tendency that the genetic variation in the populations are expressed to the greatest extent in or near their site of origin. This means that they have a high heritability and a large GCV% in environments near their origin. The intermediate location seems to possess a climate that is well balanced for the expression of a large genotypic variability in both species, both in populations from the north and from the south.

Fertile tillers, which were scored visually, had the highest genotypic correlation with seed yield. Seed yield/plot could be best predicted by a regression model including only fertile tillers as the independent variable.

2) Phenotypic stability

Why is stability important in relation to seed production? Breeding and seed multiplication is difficult in northern marginal regions of Europe due to a wet and short growing season. Genetic shifts may occur if the breeding of northern plant material is located in regions further south. By studying the environmental stability of the reproductive system one may be able to identify breeding sites that minimize the risk of genetic shifts.

It has been stated that it is very difficult to use any of the most useful stability parameters for selection because they give very different rankings in different years (HEINE & WEBER 1982). Norway has a very variable climate, and it is necessary to breed for wide adaptation in order to keep the number of cultivars at a reasonable level. At our department we have adopted the following strategy (see AASTVEIT & AASTVEIT 1984): If GxY- and GxL-interactions are mainly caused by the same factors of climatic nature, could we select for stability by conducting a one year test at two or three locations representing maximal climatic differences within the geographic region of interest? There are results that support this hypothesis (AASTVEIT & AASTVEIT 1984; ROGNLI 1987b). The variance component approach makes the ranking of the stability responses fairly easy. A special form of this approach, that has been very little used, is to consider a character estimated at three locations as three diffferent characters, and estimate the genotypic correlation between them by using the genotypic variance components (FALCONER 1981).

Table 5. Genotypic correlations between environments for the character seed yield in timothy.

ENVIRONMENT OF ORIGIN AND:	POPULATION			
	KARASJOK	KAUTOKEINO	LAKSELV	cv. 'ENGMO'
Intermediate environment (63°30'N)	0.78	0.64	1.00	0.70
Southern environment (60°N)	0.31	0.89	0.63	0.28

Table 5 shows the results of the correlation between the environment of origin, which is the northern location, and the other environments for the character seed yield in timothy. Except for one population, 'Kautokeino', the correlation is higher between the northern and intermediate location than between the northern and the southern. This pattern was the same for all characters. A high genetic correlation between the character in different environments means that the character is determined by nearly the same genes in these environments. The risk of genetic shifts will probably be lowest if the plant material is transfered between such environments.

Ecovalence (WRICKE 1962), is defined as the relative contribution of each genotype to the total genotype x environment-interaction sum of squares. Estimation of the GxL-interaction by means of ecovalence has shown that a large majority of the genotypes, both within the natural populations and the cultivars are phenotypically stable, while a small part of them are responsible for the interaction, both in timothy (ROGNLI 1987b) and in red fescue.

HAYWARD et al. (1982) and CLEMENTS et al. (1983) used pattern analysis in the study of stability in perennial ryegrass and cocksfoot, respectively. In order to study the specific adaptation of genotypes, a pattern analysis has been run on the GXL-matrix of residuals for each genotype at each location (see Table 6.)

Table 6. Cluster analysis on the GxL-interactions in red fescue.

CLUSTER GROUP	ENVIRONMENTS SOUTHERN (60°N)	 INTERMEDIATE (63°30′N)	 NORTHERN (69°N)	PROPORTION OF GENOT. (%)	AVERAGE SS_{GxL}
I	-39.2	30.4	8.8	3.8	254
II	21.5	- 2.5	-19.5	12.3	830
III	- 5.6	-34.1	39.7	3.0	2770
IV	8.3	21.8	13.5	10.8	726
V	-44.6	- 3.7	48.3	1.0	4336
VI	-19.4	0.8	18.7	11.5	731
VII	- 1.0	3.9	- 2.9	29.5	25
VIII	37.5	-24.9	-12.6	8.5	2185
IX	-17.2	25.1	- 7.9	17.8	988
X	48.0	-49.8	1.8	2.0	4787

Residuals = $(Y_{ij}-Y_{i.}-Y_{.j}+Y_{..})$
i=genotypes j=environments

Table 6 presents the means of cluster groups after clustering on the residuals of seed yield of red fescue in 1983. The total interaction sum of squares are the squared residuals, summed over all genotypes and locations, which also form the basis for the estimation of ecovalence. Therefore there is a direct connection between this cluster groups and the ecovalence concept (see ROGNLI 1987b). The cluster group with the smallest average residual at every location simultaneously will comprise the most stable genotypes. In this case it is cluster group IIV which also is the largest group with 29.5% of the genotypes. The number of cluster groups have arbitrarily been set to 10, which was found to give a good separation of the groups. Genotypes in for excample group 5 have a low phenotypic stability, and they are high yielding in the northern environment, while about average yielding at the intermediate location and very low yielding in the south. Except from a few of the groups, all populations are represented within the groups. This means that within the populations there are large variation in the environmental responses.

Résumé

Une modele experimentale avec des blocs an dedans des replications pour l'analyse statistique des interactions genotypes x locations x années, est descrit dans cette papier. Les resultats des experiences d'une grande echelle avec des clones de Phleum pratense L. et Festuca rubra L. des populations d'origine naturelle (6) ou cultivèe (2) de la Norvege du nord (69-70°N) est presentèe. Sur la base des donnèes de 100 genotypes (clones) par population et 4 ramets/case dans 2 replications et 3 locations diverses en Norvege (60°, 63°30', 69°N) pendant 2 annèes, la variation genotypique et la stabilitè phenotypique de la rendement des semences est estimèe.

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Agronomic and feeding value of Italian Ryegrass (Lolium multiflorum L.) populations collected in the Po valley

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SUMMARY

In the irrigated plains of Lombardy the continuous elimination of permanent meadows and their replacement with maize imposes the collection and conservation of Lolium multiflorum. Thirty populations were collected in the Milano-Lodi-Crema area. All the collected material was studied in greenhouse, in concrete boxes with the useful sizes of 150 x 40 x 25 cm, at the density of 350 plants per square metre. The study was divided into three experiments, conducted under the same methodology. Dry matter yield, earliness, mortality and crude protein content were evaluated. In the first and third experiments the material was studied during 25 cuts (three years) while in the second one it was studied during 17 cuts (two years). If compared with the tester cultivars Asso and Barmultra, the collected populations showed a greater stability, a good yielding capacity and a higher persistency. Some collected populations showed also a good alternativity. These characteristics, combined with a good forage quality make this material a suitable genetic resource for the creation of cultivars to be exploited in the Northern and Central Italy plains. The plants selected on the basis of yielding capacity were cloned and submitted to selfing and to crossing. Progenies S_0 and S_1 were studied in greenhouse under the same conditions of parental populations.

INTRODUCTION

In the irrigated plains of Lombardy (Italy) permanent meadows are continuously eliminated and replaced with maize. This imposes the collection and conservation of Lolium multiflorum and other species typical of these meadows (dactylis, bromus spp. etc.). Many expeditions were made by the Forage Crops Institute of Lodi, together with English colleagues as well as colleagues from Belgium, Holland, Japan and France. Beside the objective of contrasting the disappearence of the genetic source, our collection has a breeding objective, chiefly concerning stability and persistence. At present, Lolium multiflorum is extensively used as catch crop, sown in autumn and harvested in spring, before sowing maize. But we consider this plant useful for artificial meadows, able to give a full production for two years and one spring harvest in the third year, before a new maize sowing. For that purpose, persistence and yielding stability through the seasons are the most important traits. Moreover, we think that the materials selected from the collected populations are very useful for binary mixtures with lucerne and white clover because of their high persistence.

MATERIALS AND METHODS

Three collection groups were made. The first one includes 10 populations collected in 1982 in the Milano-Lodi-Crema area, 5 of which were from permanent meadows and 5 from "marcite", more than 80 years old. The second consisted of 17 populations collected in 1983 in the Crema region only. The third one consists of 8 populations collected in 1983 in the Lodi-Crema district. In the said area, (about 40 Km x 25 Km) either permanent meadows, summer irrigated, or "marcite", summer and winter irrigated, have complex systems of irrigation with water supplies every 15 days over the growing season. The summer temperatures are rather high, the mean of maximum temperatures in the second part of July being 30°C. Usually the number of cuttings per year is 5-6 for the meadows and 9-10 for the "marcite". About 100 inflorescences were collected from each population inside an area of 300-400 m^2. The collected populations were studied in greenhouse, in concrete boxes 150 cm long, 40 cm high and 25 cm wide. The mean of maximum temperatures in greenhouse were, on average, 5-7 degrees higher than the corresponding field values, in the spring and summer months, while the average minimum values were about the same in both situations.

The sowing was made in Petri dishes in January, and the seedlings were transplanted in the boxes at the first leaf appearance, at the distance of 2.5 cm on the row, that means 350 plants/m^2. Individual plants were identified by means of plastic separators. Concerning fertilization, K_2O = 200 Kg/Ha, P_2O_5 = 100 Kg/Ha were supplied before transplanting. As for N, 60 Kg/Ha at transplanting and 30 Kg/Ha after each cut were supplied too.

Experimental design: randomized blocks, with plots of 2 rows including 54 plants (27 + 27). Replicates: 13 for the first group, 4 for the 2nd and 3rd group.

Dry matter yield, no. of inflorescences at differents cuttings, and mortality were recorded. Samples from one replicate of some harvests were analyzed for crude protein and total sugar content in leaves and stems. The three groups of collected populations were studied in three different experiments but with the same technical procedures. The tester cultivars were Asso for all the three groups, and Barmultra for the second and third group only. The first group was studied for three years (25 cuts), the second one for two years (17 cuts) and the third group for three years (25 cuts). At the end of the experiments the plants resulting the most vigorous with a good yielding stability through the seasons, were chosen, cloned and transplanted in pots. Each selected plant was submitted to selfing and to crossing. The cross was realized as a general polycross of all the selected materials. The progenies, either of the polycross or of the selfing, were studied in the same technical conditions as the parental populations. In total, 48 polycross progenies and 10 selfing progenies were studied. All this material was established in three replicates. Another series of cross and selfing was realized in the 1987. On 119 plants submitted to selfing, 60% were autosterile, 24% gave from 10 to 50 seeds, 8% from 50 to 100 seeds and 8% more than 100 seeds.

RESULTS

Dry matter yield

In all the three experiments the collected populations show a stability, through the seasons and years, much higher than the tester cultivars Asso and Barmultra. In each experiment, a very poor variation was detected among the collected populations, while there was a highly significant difference between the collected populations and the tester cultivars. This difference was in favour of Asso at the first cut of each year, while at the following cuts, and chiefly between June and September, the difference was in favour of the collected populations. Barmultra is similar to the collected populations at the first cut of each year, while it performs like Asso at the subsequent cuts. The dry matter yield trend of polycross progenies and tester cultivars shows that, at each cut, some progenies yield more than control cultivars. On the average of 7 cuts, 6 out of 48 progenies overyielded Asso by more than 10%, while 11 progenies show to be higher yielding than Barmultra by 20 to 30%. Beside the advantage concerning productivity, an improvement was reached on the stability of production through the seasons. In fact, while the coefficient of variation through the cuts was in the collected populations between 40 and 60%, in the progenies it was between 15 and 25%.

Number of inflorescences at differents cuttings

The number of inflorescences was measured per plot at the second and third cut of first and second year. At first, we observe the ability of cv Asso to give a high number of inflorescences/plot at each cut. On the contrary, cv Barmultra shows a low heading ability, even with lower values than the minimum ones of the collected populations. Among these last, 2 populations in Group 2 and one in Group 3 show, at the third cut, values which are not different from cv Asso. The statistical analysis shows significant differences among the collected populations and, at the same time, an interaction with the season. The same situation was observed in the polycross progenies. The maximum values for progenies are similar or superior to those of Asso, while Barmultra confirms its lower heading ability trough the different cuttings.

Mortality

The percentage of dead plants was registered at the end of the first year for all the three groups. For the collected populations, mortality after 8 cuttings was on average 5%, while for Asso and Barmultra was 36% and 31% rispectively. All the five "marcite" populations are better persistent and higher yielding.

Crude protein and total sugar content

The analysis of crude protein and total sugar content was made on the collected populations and tester cultivars, separately on leaves and stems, in two different seasonal situations: July and October. In the autumn no stems were present of course. Both analyses were made with an Autotechnicon analyser, II generation A fairly high variability was observed in leaf crude protein content, either in July or in October, while the variability was very poor in the stems, in which the average content was very poor too. So it seems possible to select positively for leaf crude protein content, but its interaction with seasons reduces this possibility. In July, stems show a greater sugar content and a greater variability than leaves. The leaf sugar content increases, in average and in variability, from July to October. The variability of total sugar content in stems suggests the possibility of positive results in selection. The correlation between crude protein and total sugar content was always negative when a same part of the plant (leaves or stems) was considered: highly significant for leaves (r=-0.70**) but not significant for stems (r=-0.28)

CONCLUSION

The continuous elimination of very old permanent meadows imposes the collection and conservation of *Lolium multiflorum*

and other species typical of these meadows. The presented results show an opportunity for breeding purposes. In fact, it seems possible to obtain an improvement in forage yield, as well as in persistence and in stability of yield through the seasons. With regard to crude protein content, the good variability recorded in the leaves could not give successful results in a selection work, because its interaction with season. As to total sugars content, the presented results suggest that selection may be effective when working on stems. This fact is important for the creation of varieties rich in energy to be adopted in binary grass-legume associations, in view of a better utilisation of legumes by the ruminant animals. Finally it must be underlined that some of the selfed families don't seem to show an inbreeding depression. For this reason two ways of varieties creation should be tried:
1) synthetic varieties and 2) "semi-hybrid" varieties, by exploiting pollen competition. By the second way it should be possible to cumulate vigour linked to the maximum heterozigosity with vigour linked to the quality of genes, gene combinations and "linkats".

RESUME

L'élimination des prairies permanentes en faveur du mais, de plus en plus frequente, démande la collecte e la conservation du Lolium multiflorum qui est une des espèces dominantes des prairies des plaines irriguées de la Lombardie. Trente populations on été collettées dans les territoires qui forment le triangle Milano-Lodi-Crema. Le materiel a été étudié en serre sur parcelles ayantes les dimensions utiles 150x25x40 cm. Densité de la parcelle: 350 plantes par mètre carré. Les caractères mesurés étaient les suivants: matière sèche, précocité, mortalité, teneur en proteines et en glucides. Les coupes ont été 25 pour le premier et le troisième expériment (trois ans) tandis que le second expériment a été etudié pendant 17 coupes (deux ans). En comparaison avec les cultivars Asso (Institut de Lodi) et Barmultra, les populations collectées résultent, en moyenne, superieures pour le rendement, la persistence et la stabilité de rendement. Ces résultats indiquent que à partir de ce materiel on peut constituer de nouvelles varietés performantes surtout en mélanges binaires avec une legumineuse (luzerne, trèfle violet, trèfle blanc). Les plantes choisies ont été croisées et autofecondées. Il est rémarquable qu'on a trouvé des individus dont les familles autofécondées n'accusaint pas la chute de rigueur.

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Table 1. Dry forage yield (g/plot). First group: 9 cuts 1983 + 8 cuts 1984 (1st cut not collected) + 8 cuts 1985 (5th, 6th, 7th cuts 1985 not collected)

Day		18	17	8	29	19	11	7	3	12	**X**
Month		A	M	Ju	Ju	J	A	S	O	D	**1983**
Collected Populations	max	98	95	53	39	25	27	42	38	34	**48**
	x	92	89	51	35	24	25	39	35	30	**47**
	min	83	78	46	31	23	22	37	32	27	**44**
cv	Asso	120	99	43	18	17	10	27	27	36	**44**
			15	14	12	6	6	5	13		**X**
			M	Ju	J	A	S	O	N		**1984**
	max		76	58	49	33	44	31	18		**43**
	x		70	52	46	31	42	29	17		**41**
	min		63	50	43	30	38	25	15		**39**
cv	Asso		76	48	36	21	29	20	13		**35**
			19	17	6	26			22		**X**
			A	M	Ju	Ju			O		**1985**
	max		42	40	36	19			68		**40**
	x		39	38	31	14			59		**36**
	min		34	34	26	10			48		**33**
cv	Asso		40	37	24	9			44		**31**

Table 2. Dry forage yield (g/Plot). Second group: 8 cuts 1984 + 9 cuts 1985

Day		30	29	19	13	6	6	5	13	**$\bar{X}$**	19	17	6	26	19	22	12	18	5	**$\bar{X}$**
Month		A	M	Ju	J	A	S	O	N	**1984**	A	M	Ju	Ju	J	A	S	O	D	**1985**
Collected population	max	55	58	50	35	30	42	29	19	**38**	42	43	40	22	23	28	35	37	18	**30**
	$\bar{x}$	48	55	46	32	28	40	26	16	**36**	35	37	36	20	19	24	31	31	15	**28**
	min	42	49	42	28	25	38	24	14	**34**	33	32	32	19	16	20	28	27	12	**24**
cv.	Asso	75	64	34	19	9	26	16	14	**32**	46	43	24	9	8	10	16	23	13	**21**
cv.	Barmultra	48	52	27	22	17	32	18	10	**28**	33	29	13	5	6	10	14	15	9	**15**

Table 3. Dry forage yield (g/plot). Third group: 9 cuts 1984 + 8 cuts 1985 + 8 cuts 1986

Day		10	18	8	25	13	6	3	5	13	**$\bar{X}$**	17	6	26	19	13	12	18	5	**$\bar{X}$**	10	8	26	16	9	1	25	25	**$\bar{X}$**
Month		A	M	Ju	Ju	J	A	S	O	N	**1984**	M	Ju	Ju	J	A	S	O	D	**1985**	A	M	M	Ju	J	A	A	S	**1986**
Collected population	max	77	103	94	86	56	47	62	58	27	**64**	175	57	35	47	55	59	69	28	**60**	93	30	48	40	36	38	40	48	**42**
	$\bar{x}$	57	96	83	80	49	41	58	52	25	**60**	130	52	32	43	48	54	63	26	**56**	73	29	43	35	30	33	34	45	**40**
	min	40	90	78	72	34	26	55	48	22	**57**	120	49	25	32	38	39	53	22	**52**	60	24	38	26	20	20	24	36	**36**
cv.	Asso	81	112	76	62	25	22	51	52	31	**57**	180	50	20	28	30	28	46	25	**51**	134	28	36	24	13	14	20	27	**37**
cv.	Barmultra	56	115	65	67	25	31	59	50	23	**55**	178	37	21	30	39	45	60	27	**53**	91	24	34	23	16	20	22	29	**32**

Table 4. Dry forage yield (g/plot). Polycross progenies: 7 cuts 1987

Day		18	10	30	29	22	17	10	$\bar{X}$
Month		M	A	A	M	Ju	J	A	**1987**
Polycross Progenies	max	42	37	45	55	47	35	28	**37**
	$\bar{x}$	29	31	36	44	38	29	24	**33**
	min	21	26	30	38	31	24	20	**30**
cv Asso		40	35	35	47	34	27	12	**33**
cv Barmultra		26	30	36	43	27	21	18	**29**

Table 5. Percent content of crude protein and total sugars in collected populations and testers

		CRUDE PROTEIN (% of D.M.Y.)				TOTAL SUGARS (% of D.M.Y.)			
		LEAVES		STEMS		LEAVES		STEMS	
		July	October	July	October	July	October	July	October
Collected population	max	22	25	7	=	6	13	10	=
	$\bar{x}$	17	20	4	=	3	7	5	=
	min	13	16	3	=	2	5	1	=
cv Asso		20	20	6	=	2	7	4	=
cv Barmultra		20	24	6	=	2	5	3	=

DESCRIPTION OF NATURAL VARIABILITY AND MANAGEMENT OF GENETIC RESOURCES

WORKSHOPS

Workshops were organized for the first time during an annual meeting of the Eucarpia Fodder Crops Section. A few years ago, in Freising-Weihenstephan, the idea of workshops was put forward by section members who wished to encourage livelier discusions amongst the participants. In fact, the informal exchange of experiences and critical observations may be very fruitful for practical plant breeders as well as for those who are engaged in theroretical research.

For practical reasons, the four workshops had to be organised simultaneously but in different rooms. The groups were formed more or less according to the field of interest of the participants. Consequently the approach to the problem of genetic variability which was the central point of the discussion, was very different in each group.

The group leaders offer below a short summary of these discussions. This is followed by a more general conclusion.

S. BADOUX

GENETIC RESOURCES FOR MEDITERRANEAN AREA

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1. Reasons for a workshop on genetic resources for the Mediterranean area :

a) The Mediterranean environment has peculiar climatic conditions, from an agronomic point of view, which can be summarized as follows (ASCHMANN, 1973) :

1) at least 65 % of the rainfall occurb between November and April ; little or no rainfall in June/September.

2) the total number of hours of sub-zero temperature is less than 262 (3 %) each year.

b) The Mediterranean area has a sequence of favourable and unfavourable periods for plant growing, which is not found anywhere else and which calls for tailor-made improvement programmes capable of matching crop requirements to the climate.

c) In such a situation, it is particularly true that the "best adapted ecotype within a given species may be better than the least adapted variety".

d) In the Mediterranean area there is a unique richness of variability in fodder grasses and legumes, which is not found in other areas with a Mediterranean climate (Australia, California, Chile, South-Africa).

2. In some Mediterranean areas, Italy for example, landraces of red clover, lucerne, sainfoin, white clover, are in danger of extinction. Urgent steps are necessary to locate, collect and store these landraces (IBPGR, 1984).

3. Most of the seed of annual forage species (vetches, crimson clover, Egyptian clover, Persian clover) used in Mediterranean rotations is of unspecified origin ; these species should receive more careful attention in order to provide specified varieties of known agronomic value to the farmers.

4. In many Mediterranean countries, most pastures are based on natural flora. These pastures are a very important source of genetic variability which can be preserved with planned grazing.

5. Sowing new pastures (e.g. in abandoned land) requires a careful choice of species and varieties, fitting a well defined farming system, which is often based on using high productive pastures and marginal land. The knowledge of the system in which the species will be used is a prerequisite for the collection strategies and breeding work, helping to reduce the number of species on which to focus attention.

6. Grass species useful for the less severe Mediterranean environments are Festuca arundinacea, Dactylis glomerata and Lolium perenne. For these species, there is a risk of genetic erosion due to massive introduction of seed of Northern varieties and there has been a claim for genetic conservation by IBPGR (1984). The main characters required for Mediterranean conditions are : 1) the ability to grow in the winter and, 2) to be dormant in the summer (i.e. the exact opposite of the varieties presently available on the market). Some warm-season grasses and self-reseeding annuals (Lolium rigidum) should receive major consideration.

7. In Mediterranean pastures, the relative importance of legumes is much higher than in more Northern European pastures ; it has been spoken of as **legumeland** in contrast with **grassland**.

a) The possibility offered by perennial species (lucerne, birdsfoot trefoil, white clover, sainfoin, sulla) are well known.

b) Annual clovers and medics (Trifolium campestre, T. subterraneum, T. resupinatum, T. echinatum, T. balancae, Medicago arabica, M. polymorpha, M. orbicularis, M. rigidula, M. truncatula, M. hispida, etc.) are very common in Mediterranean natural pastures but their role in sown pastures is not so clear as it is, for example, in the Australian ley-farming system. A careful study of their possible contribution to forage production and distribution is necessary if an effort of collection, breeding and seed production has to be done.

INTRODUCTION OF NEW VARIABILITY, ESPECIALLY FOR VIGOUR, INTO A HIGH LEVEL GENETIC MATERIAL

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The concept of vigour referred to forage plants was the starting point for discussion. Many different definitions were proposed. This led to the following remarks :

1. One unrestrictedly valid definition of vigour cannot be found. Each definition is sound according to the adopted landmarks. Its validity is measured by its effectiveness in the breeding work. One must consider that forage plants may be either annual or perennial and may be submitted to one or more cuts per year.

2. The definition of vigour cannot leave the way the vigour is measured out of consideration. The influence of the considered cut, the plant biological stage, the plant density and other factors affecting the measurement of vigour in many experiments were recalled. The need to measure the vigour in conditions as close as possible to the ones of the target environment of the future variety was also stressed.

3. Dry forage yield is a good expression of vigour in classic forage crops (legumes and grasses). Taking into account the reported remarks, the following definition of vigour was accepted : **Vigour is the capability of the plant to draw the available environmental resources and to transform these resources into biomass within a range of time which is defined according to farm practices.** Finally, a reflection upon the concepts of potential vigour and actual vigour was suggested. The following discussion pointed out that selection is more effective when the breeder is working on the actual vigour.

The debate upon the variability for vigour can be resumed as it follows :

1) In forage plants, a high variability either for vigour or for the main morpho-physiological characters is still available nowadays. Because of that, at present priority must be given to the study of better methods and techniques for the exploitation of the existing variability.

2) The values of the variability for vigour vary in relation to the adopted methods and techniques.

3) Creating new variability for vigour is certainly important even though it is not a priority. The main guidelines are the following :

a) exploitation of material already improved

b) introduction of new characters by crossing between different origines

c) utilisation of vitro culture techniques

d) utilisation of selfing phase.

Experimental results in alfalfa show that the mean of some single cross families increased with inbreeding. This increase was transmitted to Syn 2 generation of synthetic varieties, indicating that selection for vigorous plants within vigorous progenies during the selfing phase was successful in competitive conditions. Finally, it must be underlined that in some grass species some of the selfed families do not seem to show any inbreeding depression.

NATURAL VARIABILITY IN STRESS TOLERANCE

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Outline plan/agenda

The following plan was adopted to form the basis of the workshop :

Stress factors :

Physical	Light	
	Temperature	high
		low (freezing)
	Water	drought
		waterlogging
Biotic	Pests	
	Diseases	
	Grazing animals	
Edaphic	Minerals (salinity)	
	pH	
	Nitrogen	
Management		

Sources of variation : Species
Ecotypes

Relationships between stress tolerance and yielding ability - total and seasonal.

The role of polyploidy in stress tolerance.

Discussion

Only two main stress factors could be considered in the time available. These were : winter cold
summer drought

In general species ranked as follows in terms of high-low general stress tolerance : Dactylis
Festuca arundinacea
Poa pratensis

Ryegrasses were generally poor while white clover was widely adapted.

Within species or between closely related species there were often very distinct types. For example, summer versus winter growth forms in Dactylis or the range from perennial to annual types in the Lolium complex (perenne-multiflorum-rigidum).

Winter hardiness

When growth is not required extreme adaptation to winter conditions is achieved by lack of growth - complete senescence - dormancy. Material from high latitude/altitude slows growth in the autumn - it may be very disease susceptible in milder climates.

The major problem is to combine winterhardiness with potential for growth in late autumn/early spring especially in a fluctuating climate. A plastic response is required which allows growth at low temperatures but not in mid-winter when dehardening effects can be a problem - a photoperiod response may be useful in this situation. It was agreed that **it is very necessary to definie limits in terms of amounts of growth compatible with winter hardiness**. Light (daylength and intensity) also has an important role in hardening. Transfer of material from extreme Northern and Southern latitudes to intermediate areas may produce undesirable changes in hardening response.

The spatial and temporal variation found in the Alps at intermediate latitude should be a source of useful breeding material both for grasses and white clover

There appears to be no direct genetic relationship between freezing tolerance and tolerance of winter diseases although there is evidence of co-adaptation in some material. Some kind of hardening response appears necessary in both cases which involves many physiological changes including changes in cell membranes, which also have a role in conferring drought tolerance.

Drought tolerance

Again where stress is severe, as in parts of Southern Europe or North Africa, adaptation is extreme in terms of summer dormancy or an annual life cycle. In less severe conditions growth may occur but at a reduced rate. Thus again it is necessary to define limits in terms of growth compatible with survival. In order to maintain yield under drought stress it is necessary to maintain leaf area though leaf expansion which requires maintenance of leaf water content/turgor. Leaf adaptations (ridging, folding, etc.) which affect stomatal behaviour provide useful selection criteria. The relative water content of leaves is also a simple but effective trait for selection.

Root systems are also important and wide species differences exist. Tall fescue has deep roots which can avoid water stress (and also waterlogging problems) although this can result in low nutrient uptake and hence yield reductions. Dactylis has an extensive but shallow rooting system which assists in drought survival without loss of nutrient uptake capacity. The situation in white clover is complicated by initial production of a tap root followed later by a number of shallow roots.

The role of polyploidy

Great care must be taken not to generalize too broadly. For example, tetraploid ryegrasses appear less frost tolerant than diploids but have better resistance to low temperature fungi. However they do have the potential to build up high levels of heterozygosity which may be important in conferring a high degree of general adaptability.

Topics not discussed but considered important :

Effects on quality
Nitrogen deficiency
Genetic stress

In general, high levels of stress tolerance require some reduction in yield potential. It is considered desirable to discuss with Testing Authorities how they may give due recognition to this in their trials and recommendations.

MANAGEMENT OF GENETIC VARIABILITY

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1. The evaluation and description of the created genetic variability

1.1 Description of plant accessions for inclusion in gene banks. Most important information :

- species
- place of origin with precise description (altitude, latitude, etc.)
- reliability of collection (how many plants collected ?). Other information is welcome but is not a necessity.

1.2 Breeder's evaluation of plant collections

Mostly desired : evaluation under sward conditions, but this requires seed multiplication. During multiplication, there is the risk of loosing genetic variability (loss of extremes), but this fear might be academic.

An alternative to sward testing is the testing in row which requires less seed. Evaluation as spaced plants is of little value. Time of ear emergence and chromosome number are considered to be the most relevant registration characteristics at this early stage of the breeding programme. Thousand grain weight is also of value as a characteristic determining establishment (important in warmer climates). Agronomic data should always include control varieties in order to express the performance of the collected material relative to these.

2. Maintenance of the created genetic variability

There were two opinions :

- Not all material should be conserved. Only maintain the best material. This material has gone into the created varieties. It is thought that the existing varieties possess sufficient variation within themselves. Conservation of these is therefore a secure source of new genetic variability. By recombination gene blocks can be broken up.

- Therefore material should be collected as much as possible and maintained in order to cope with any eventualities in future.

There was complete agreement as to the need for conserving genetic variability in natural sites in the regions of origin (representative areas and selection pressure). Eucarpia members should actively lobby their relevant political bodies with a view to setting up such conservation areas.

3. New advances in breeding methods

Some of the workshop participants felt the need for co-operative efforts to study and improve current breeding methods. The majority of the participants were (more or less) satisfied with methods currently available.

CONCLUSIONS

S. BADOUX

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Fodder plant breeders are fortunate enough to still be able to directly utilize variation from natural ecotypes. However this variation is only easily available for breeding purpose if it is correctly described. The characterization of populations is now less difficult due to the general use of computerization and powerful methods such as multivariate analysis.

Breeders are usually not in a position to maintain large seed collections. This is to a greater extent the task of governmental agencies which, however, are not just acting as seed museums, but have to co-ordinate collection, regeneration and registration of the material as well as to identify eventual gaps. They have to insist on the correct and complete registration of passport data which should include the description of the collection site and its environmental factors.

Due to its peculiar climatic conditions, the Mediterranean area is characterized by a very large variability of its grasses and legumes genetic resources. For this reason, it needs special consideration. Two different factors may contribute to the genetic erosion in this area :

- several landraces, especially annual and perennial legumes, are in danger of extinction

- genetic pollution may be due to the massive introduction of seeds from other regions which contribute to spoil the original populations which are mostly winter growing but summer dormant.

It was concluded that an effort of collection, description but also of breeding and seed multiplication has to be made in favor of the Mediterranean area.

Breeders are mainly interested in the agronomic data of new accessions. Time of ear emergence is considered as the most important characteristic in this connection but other morphological and physiological characters may also be relevant.

It is interesting to note that evaluation of single plants is in general not considered as satisfactory ; therefore testing in rows or sward condition may be necessary. To facilitate the exchange of information between breeders, the necessity of comparing new accessions with control varieties (such as the reference varieties proposed by the IBPGR, EPC/GR forage working group) was emphasized.

Variability is necessary to improve yield, quality and also hardiness of plants. As a consequence, a workshop was devoted to the introduction of new variability, especially for vigour, into high level genetic material, and another one to the natural variability in stress tolerance.

Unfortunately it is not easy to have a simple definition of vigour, but it is clear that it can only be described in relation with the environmental and management conditions in which the future variety will be grown. Variability is sufficient to breed high yielding varieties. The problem is to a greater extent to find methods suitable to exploit it and to introduce it in new varieties : this is in fact the art of breeding.

As the question of the utilization of natural variability when breeding for stress tolerance is very broad, only winter hardiness and drought tolerance could be considered during the workshop. Ecotypes are usually well adapted to their natural conditions, but they may be stressed when they grow outside their original area.

Frequently the breeder wishes to combine characters which are opposite from the physiological point of view, e.g. winterhardiness and early spring growth or drought tolerance and summer production. In such cases, it is necessary to definie limits in terms of growth compatible with plant survival.

BREEDING FOR ADAPTATION TO NATURAL ENVIRONMENT AND TO MANAGEMENT

PAPERS

Ecophysiological approach to plant growth. Consequences for breeding strategies for forage species in contrasting conditions and different managements.

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Introduction.

Models of plant growth can be used either as a predictor of yield of forage crop in a given situation (descriptive model) or as a tool to understand how the environmental factors interact with the plant population (explanatory model) as defined by TORSSELL (1984). In fact, very often, the models used by agronomists are intermediate between these two types. Predictive models may be based only on statistical relationships between environmental factors and components of yield. Such a model may be used as predictor only in the range of conditions in which it has been established. Explanatory models are based on series of processes integrated in a mechanistic system and they can used with more accuracy as a predictor of crop growth in a wider range of situations.

So for breeding strategies which aim to adapt genotypes to very different conditions of environment and different types of management, these models can help the breeder to define more closely the ideotype of plant he must select.

I. A general approach to plant growth modelling

A general explanatory model of crop growth can be used for analysing the effect of environmental parameters. This model can be divided in two submodels :

. a trophic model which involves all processes of light interception, CO_2 assimilation and respiration. These processes have been mathematically described by several authors (MONTEITH, 1972 ; GOUDRIAAN, 1982 ; JOHNSON and THORNLEY, 1984). The first variable to consider is the L.A.I. which determines the quantity of energy absorbed by the crop and susceptible to be converted in organic matter.

. a morphogenetic model of aerial parts which involves all processes of growth and developpment of the different organs : leaf appearance rate, leaf elongation rate, tillering or branching, stem elongation etc ... The main output which can be estimated by this model is the leaf area expansion rate.

The connexion of these two submodels implies hypothethis on the partitioning of assimilates in the different organs of the plant. This problem has been studied in different ways. BROUWER (1983) developped the concept of "functional equilibrium" between shoot and root. JOHNSON (1985) has proposed a partitioning coefficient based on N and C concentration in each compartment. GILLET et al. (1984) have proposed for perennial forage crops a more general hypothesis to account for the effect of different environmental factors like temperature, nitrogen, water stress or light on shoot/root ratio. This hypothesis implies the existence of hierarchy for assimilate use in favour of aerial parts. A programme of morphogenesis of aerial parts can be determined by internal factors (like floral induction) and by external factors (temperature, water deficit, quality of light) and expressed in terms of leaf elongation rate, leaf appearance rate, stem elongation rate etc ... Each day, such a programme defines a demand in carbo-hydrates and proteins for its realization. With this concept C and N can be only considered as material for morphogenesis. It is fundamental to know, in a given situation, if the growth rate is limited by the morphogenetic programme itself or by the material C or N. In the case of non-limiting nitrogen it should be possible to define the maximum demand in C required by the morphogenesis of aerial parts and to compare it with the amount of available C resulting from light interception and photosynthetic processes.

If the offer in C is higher than the demand of aerial parts, the residual assimilates can be used for root growth or synthesis of C reserves. If the morphogenetic programme of the plant is increased by any external factor (like temperature, water status) or by an internal factor (like floral induction) the demand for C rises and if C supply remains unchanged the part devoted to root decreases. Conversely if the morphogenesis of aerial parts is limited by nitrogen deficiency or by drought, for the same amount of available C the part devoted to root system increases and carbo-hydrates can be eventually stored as reserves.

With this very simple hypothesis it is possible to account for some apparently contradictory results on the effect of nitrogen supply, irrigation or temperature on the shoot/root ratio because these effects are mainly dependant on the level of available C. GASTAL and SAUGIER (1986) showed that nitrogen deficiency promotes root growth only at high light intensity.

This general scheme indicates that the effect of any environmental factor (N, water, temperature) must be studied simultaneously on the morphogenetic processes (C demand) and on the photosynthetic processes (C supply). The effect of these factors on leaf area expansion must be particularly studied because an increase of L.A.I. leads to a higher C supply. So it is necessary to discriminate the direct effect of environmental factors on conversion efficiency of light from the indirect effect on interception efficiency. The major difficulty in building and validating such a model is the determination of the C demand.

Morphogenetic processes are usually described as the rate of increase of plant size expressed in unit of length or area or volume. The C demand would be determined by the quantity of C required by the plant to build one volume expansion unit. This demand includes both structural carbon and the respiratory cost associated with new plant tissues elaboration. Some estimations have been made on a dry weight basis (PENNING de VRIES, 1974) but we have little information about these costs expressed in term of unit of size.

II. A simple model for crop growth analysis

1) POTENTIAL GROWTH IN NON-LIMITING CONDITIONS

By applying the approach of light conversion efficiency developped by MONTEITH (1972) it is possible to relate total dry matter growth to the quantity of intercepted radiation. The slope of the linear relationship obtained is the conversion efficiency of intercepted energy in organic matter. The difficulty to estimate changes in root biomass in field conditions leads to consideration solely of the aerial biomass. In this case the slope of the regression is affected by the partitioning coefficient of assimilates between shoots and roots. As shown by GOSSE et al (1986) low variability exists between species for a given metabolic pathway (table 1).

Species	Number of measure	Regression coefficient	Standard error of regression coefficient
Maize	57	2.49	0.064
Sugar Can	45	2.65	0.061
Panicum sp.	31	2.39	0.049
C_4 species	—	2.51	0.13
Tall fescue	58	1.89	0.071
Phragmites sp.	20	1.88	--
Winter wheat	69	2.00	0.034
Winter rape	30	1.83	--
Spring rape	27	1.95	--
Jerusalem artichok	24	1.94	0.043
Non legume species C_3	—	1.93	0.08
Vigna sp.	31	1.66	0.065
Lucerne	37	1.77	0.057
Vicia faba	7	1.54	--
Legume C_3	—	1.72	0.10

TABLEAU 1

Statistical characteristics of linear regression :
MSa = f (absorbed radiation)

Caractéristiques statistiques des ajustements
MSa = f (PAR absorbé)

So genotypic differences in yield observed at field level in non-limiting conditions would be mainly due to differences in morphogenetic processes and particularly in leaf area expansion which determines the quantity of intercepted radiation. For lucerne and tall fescue the data presented in table 1 have been obtained during the period of highest growth rate. For these species seasonal variability of growth rate can be analysed. For lucerne crop (GOSSE et al., 1984) spring and summer regrowth are described by a common linear regression, but for autumn regrowth the slope is significantly lower (figure 1).

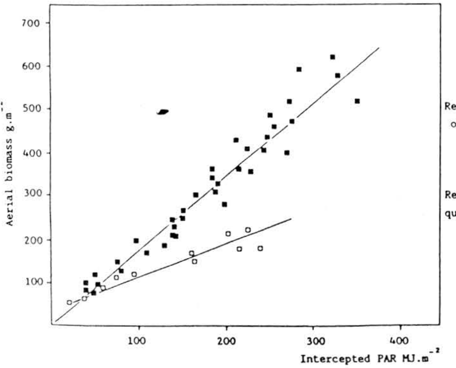

FIGURE 1

Relationship between accumulated aerial biomass and quantity of intercepted photosynthetic active radiation for lucerne crop. (■ spring and summer regrowth : 1.8g DM.MJ^{-1} ; □ autumn regrowth : 0.75g DM.MJ^{-1})

Relation entre la production de matière sèche aérienne et la quantité de rayonnement visible intercepté par une culture de luzerne. (■ printemps et été : 1.8g MS.MJ^{-1} ; □ automne : 0.75g MS.MJ^{-1})

This discrepancy between seasons cannot be explained by a difference in conversion efficiency. If we take into account the changes in root biomass we obtain the same relationship over all seasons and on total biomass basis we found also the same slope, including the growth of lucerne after sowing. The conversion efficiency of intercepted P.A.R. in organic matter is equal to 2.5 g.DM/MJ and very close to that mentioned by CHARLES-EDWARDS (1986) for other species. In autumn the stem elongation is strongly reduced by low night temperature and (or) by photoperiod. So the available carbon largely exceeds the demand of aerial parts and consequently supplemental assimilates can be used for root growth and reserve storage.

A large genetic variability exists for the aptitude of lucerne to elongate internodes in autumn specially when we compare European and mediteranean types. Breeding for this aptitude could be a way to increase the annual yield of the lucerne crop. But the risk of frost sensibility can limit the possibility of use of such a plant material.

The root biomass remains relatively constant during spring and summer. The pattern of evolution of root dry matter during regrowth is always the same during this period, i.e. a large decrease during the first half of regrowth and a recovery of initial biomass at the end. This recovery is achieved at the population level but a large disparity exists between individual plants and high growth rate is associated with high mortality of individual plants which have not recovered their initial root biomass (GOSSE et al., 1987). The higher the growth rate, the higher is the competition for light between individual plants.

This phenomenom is very important to take into account. It implies that we cannot hope to increase the potential yield of lucerne during spring and summer by an increase of shoot/root ratio. In a dense canopy like a lucerne crop, individual plants cannot express their morphogenetic capacity because the quantity of light is physically limited. This fact explains why genetic variability observed on isolated plant is not expressed in dense crop.

The same model applied to a tall fescue sward allows to distinguish reproductive growth in spring from vegetative regrowth in summer (figure 2).

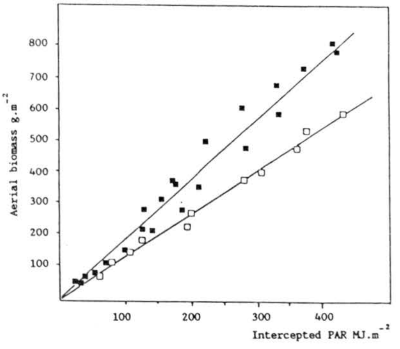

FIGURE 2

Relationship between accumulated aerial dry matter and photosynthetic active radiation intercepted by a tall fescue sward (■ spring regrowth : 1.9g DM.MJ^{-1} ; □ summer regrowth : 1.5g DM.MJ^{-1})

Relation entre la production de matière sèche aérienne et la quantité de rayonnement visible intercepté par un couvert de Fétuque élevée (■ printemps : 1.9g MS.MJ^{-1} ; □ été : 1.5g MS.MJ^{-1})

Similarly to lucerne the seasonal difference of the slope : 1.9 to 1.5 g.DM/MJ cannot be attributed to photosynthetic capacity of leaves (GASTAL, unpublished data). In spring the flowering induction is accompanied by an increase of leaf elongation rate as described on perennial ryegrass by PARSONS and ROBSON (1980) and leads to rapid stem elongation. So the demand for carbon is higher for reproductive growth than for vegetative growth and the difference of the slope between seasons could be a result of the difference in assimilate partitioning. This hypothesis is confirmed by data of ALCOCK and AL JUBOURY (1981) showing an increase of root biomass during summer. One other hypothesis would be a change in canopy geometry between spring (short and erect leaves) and summer (long and bent leaves).

These results on lucerne and tall fescue indicate that during the season of higher growth (spring for grasses, spring and summer for lucerne) the CO_2 assimilation could be a major limiting factor because only a small part of assimilates are allocated to roots. In this case, yield can only be increased by an increase of photosynthetic efficiency or a reduction of respiration losses. Attemps have been made to raise the rates of light saturated photosynthetis by breeding, but without much success (ROBSON and WOOLEDGE, 1981).

For respiration losses of mature living tissues WILSON (1975, 1982) obtained a variation of about 30% between low and high selection lines from *Lolium perenne* cv. S23. ROBSON (1982) showed that half of the extra dry weight produced by the "low" line is explained by a more saving use of C, the rest could be due to a 25% higher number of tillers which enable a faster leaf area expansion and a greater amount of intercepted radiation.

The selection for low respiration rate of mature tissues might be a way to increase the potential yield of species. The problem of using such a criteria in a breeding programme is to know if any penalties such as sensibility to stresses are associated with the low respiration rate (ROBSON, 1982).

During a period of lower growth rate (autumn for lucerne, summer and autumn for grasses) the CO_2 balance could be in excess compared to demand and it might be possible to use more carbon in aerial parts if its morphogenesis programme is enhaced. The response of leaf elongation rate or stem elongation rate to temperature would be a good criteria for earlier growth in spring or later growth in autumn. The use of mediteranean ecotypes of tall fescue or cocksfoot for their aptitudes to maintain high leaf elongation rate at low temperature has been successful. But this genetic progress is limited by the frost sensibility.

2) GROWTH IN LIMITING CONDITIONS

Radiation level and temperature can be considered as the two basic climatic parameters which determine the potential growth of a genotype in a given situation. Other factors like available water or nitrogen and mineral nutrition determine the level of actual growth.

In this paper we only discuss water stress and nitrogen nutrition which are the more limiting factors of pasture production.

2.1) Effect of drought

The general plant growth model can be used for studying the sensibility of a crop to a shortage of water. Figure 3 allows separation of the effect of water stress on lucerne growth in three ways.

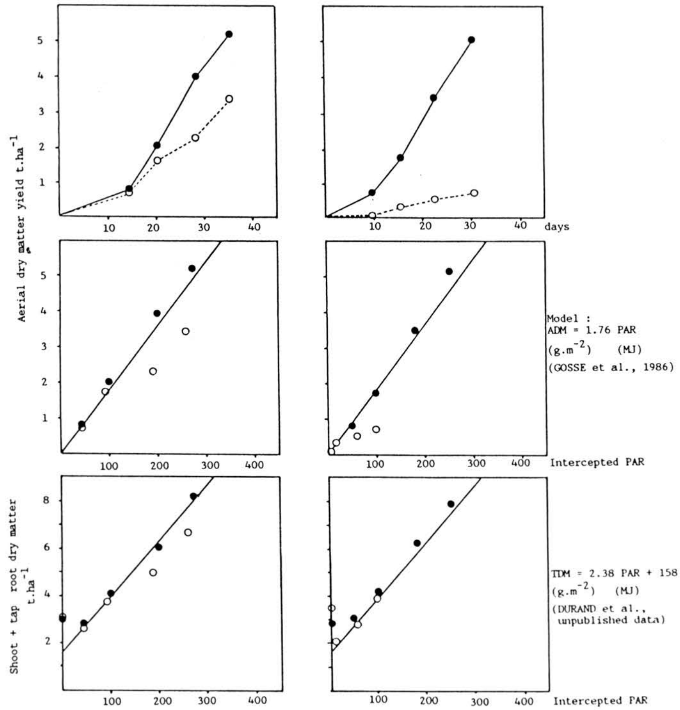

FIGURE 3

Effect of water stress on the evolution of aerial or total biomass of lucerne during two summer regrowth (● irrigated or ○ non irrigated plots).

Effet d'une sécheresse sur la croissance en matière sèche des parties aériennes et de la biomasse totale de la luzerne au cours des repousses estivales.

The first is the reduction of leaf area expansion. For the first summer regrowth water stress occurred 20 days after cutting where the L.A.I. was close to 2.5, so the quantity of intercepted PAR was not affected. By contrast in the second regrowth with an earlier drought, the intercepted PAR was drastically reduced. The second way is the reduction of stem elongation. In condition of water stress C assimilates are produced in excess for the growth of aerial parts and may be used for root growth and reserve storage in the tap root (DURAND, 1987). The third way is the reduction of the conversion efficiency which appeared to have been affected only at the end of the first regrowth.

These results indicate that three ways of improvment of lucerne adaptation to drought must be considered :

- sensibility of leaf area expansion to water stress, especially at the beginning of regrowth when light interception is limited by a low L.A.I.
- sensibility of internode elongation to water stress ;
- sensibility and adaptation of photosynthesis to water stress.

For grasses similar results have been observed. LEAFE et al. (1978) showed that water stress affects more deeply the leaf elongation rate than photosynthetic activity per unit of leaf area. It is the reason why during drought period an increase of carbohydrate reserves can be observed. This storage of sucrose can lead to a supplemental growth after rewatering as shown by HORST and NELSON (1979).

The sensibility of leaf elongation rate to water deficit would be a good criterion for breeding genotypes adapted to moderate drought. Another possibility would be to select plant having high recovery after rewatering as shown by NORRIS and THOMAS (1982).

It is also evident that for grass species a selection for deeper roots and dense hairy roots should be engaged. It is possible to observe differences between species at field level. But the intraspecific variability will be more difficult to observe.

2.2.) Effect of nitrogen deficiency

The effect of shortage of nitrogen on plant growth can be analysed using the same model. So we are able to separate the effect on CO_2 exchange and the effect on morphogenesis.

On figure 4 we observe a large effect of nitrogen nutrition on dry matter growth in spring : 50% of this effect can be explained by a higher quantity of intercepted radiation with high level of nitrogen due to a more rapid leaf area expansion. The rest of the variation could be attributed to an effect of nitrogen on conversion efficiency of intercepted radiation and (or) to an effect on the partitioning of assimilates between roots and shoots.

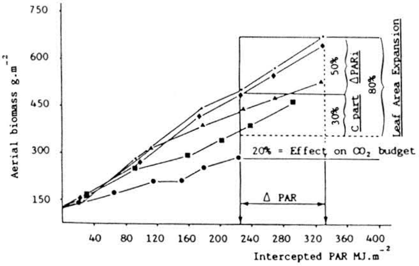

FIGURE 4

Effect of nitrogen of aerial dry matter biomass of tall fescue as resulting of : an increase of intercepted PAR, an increase of percentage C allocated to aerial parts and an increase of CO_2 budget of individual leaf.

● 0kg/ha ; ■ 40kg/ha ; ▲ 80kg/ha ; ◆ 120kg/ha ; * 160kg/ha

Effet de l'azote sur la croissance des parties aériennes d'une Fétuque élevée résultant de l'accroissement : de la quantité de rayonnement intercepté, de la part du C alloué aux parties aériennes et du bilan de CO_2 de l'unité de surface de feuille.

The effect of nitrogen deficiency on photosynthesis must be analysed at leaf level and at canopy level. GASTAL and SAUGIER (1986) found that the light saturated leaf photosynthesis can be reduced by 30% by a nitrogen shortage. But at canopy level these differences are smaller. It is necessary also to take into account an increase of maintenance respiration with higher nitrogen content. So it appears the effect of nitrogen nutrition on the slope of the linear relationship between dry matter and accumulated P.A.R. is probably due in major part to a difference in repartition of assimilate. At lower nitrogen nutrition the morphogenesis of aerial parts is reduced by a lack of N for protein synthesis and the surplus of C can be used in roots. This explanation is in agreement with many results obtained in controlled environment (BROUWER, 1983) but few data are obtained at field level.

This explanation shows that if any genetic variability on the sensibility to nitrogen deficiency exists it must be at the morphogenesis level rather than at photosynthesis level. It is necessary at this stage of research to determine the major component of leaf area expansion of a sward and to study their relationships with the level of nitrogen nutrition.

Leaf elongation rate is very sensitive to the level of nitrogen nutrition as shown in figure 5. So it should be possible to study the relationship between L.E.R. and nitrogen content of expanding leaf and to look for the genetic variability. In other words does any genotype expand leaves at the same rate but with a lower nitrogen requirement ?

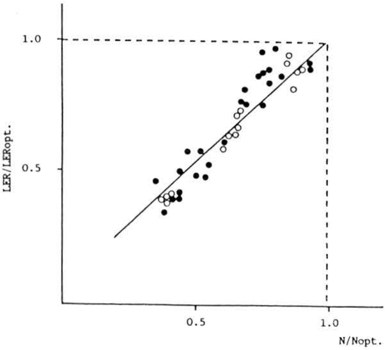

FIGURE 5

Relation between Relative Nitrogen content of leaves (N/Nopt.) and Relative Leaf Elongation rate (LER/LERopt.) of tall fescue.
(○ spring ; ● summer)

Relation entre le rapport des teneurs en azote des feuilles (N/Nopt.) et le rapport des vitesses d'allongement correspondantes (LER/LERopt.) pour la Fétuque élevée.

III. A model for nitrogen use by plants

A simple model of nitrogen uptake by grasses has been devellopped by SALETTE and LEMAIRE (1981). This model is based on a close relationship between N content and dry matter increase of aerial parts during a regrowth :

$$N\% = \alpha(DM)^{-\beta} \qquad (1)$$

This equation represents the "dilution" of nitrogen in the dry matter. It is possible with the equation (1) to deduce the relationship between nitrogen uptake and dry matter growth :

$$\text{Nup. kg.ha}^{-1} = 10\alpha\ (\text{DM t.ha}^{-1})^{1-\beta} \qquad (2)$$

In this equation the coefficient 10 α represents the quantity of nitrogen used to produce the first tonne of dry matter and the coefficient $1-\beta$ is the ratio between relative rate of nitrogen uptake and relative growth rate.

Figure 6 shows each level of nitrogen nutrition is characterized by one particular curve. LEMAIRE and SALETTE (1984a, b) have shown that for a non-limiting level of nitrogen nutrition this relation is stable whatever the site, the year, the season and the genotype. For a large range of species the coefficients 10 α and $1-\beta$ are very close. GREENWOOD et al. (1986) obtained similar relationship on wheat and on very large range of vegetables and it is possible to conclude that for C_3 species no great variability exists for the quantities of nitrogen required for a given yield. For C_4 species as sorghum or maize we obtain lower value for α.

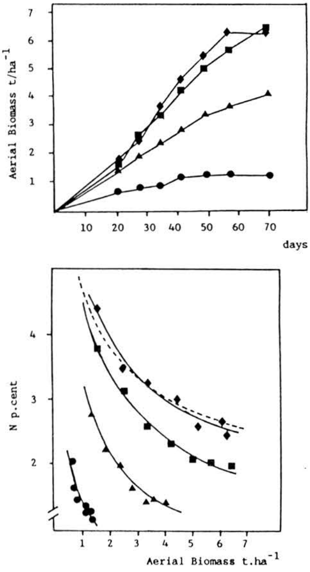

FIGURE 6

Evolution aerial biomass (a) and nitrogen content (b) of tall fescue during summer regrowth according to level of nitrogen supply.

● 0kg/ha ; ▲ 50kg/ha ; ■ 100kg/ha ; ◆ 150kg/ha

---- dilution curve for non limiting N obtained in spring $N = 4.8(DM)^{-0.32}$ (LEMAIRE and SALETTE, 1984)

Evolution de la biomasse aérienne (a) et des teneurs en azote (b) de la Fétuque élevée au cours d'une repousse estivale en fonction de différents niveaux de fertilisation azotée.

---- courbe de dilution pour N non limitant obtenue au printemps (LEMAIRE et SALETTE, 1984)

So the genetic variability in nitrogen requirements to obtain the maximum dry matter growth rate is very small. It appears also that in non-limiting conditions of nitrogen nutrition, nitrogen uptake is mainly determined by the rate of CO_2 assimilation. GASTAL (1984) shows in controlled conditions that nitrogen absorption is closely linked to CO_2 assimilation. Such a result explains the stability of the relationship between N uptake and DM yield in a large range of environmental conditions. The equation (1) explains also why breeding for high nitrogen content of plant leads very often to a negative effect on dry matter yield.

In conditions of nitrogen deficiency it could appear a more important genetic variability. When we compare tall fescue and cocksfoot (figure 7) it appears that at lower nitrogen supply a difference exists between the two genotypes. This difference does not appear at higher nitrogen supply. This result indicates that cocksfoot is more efficient than tall fescue for taking up nitrogen from the soil in case of deficiency.

Objectives for breeders could be to increase the efficiency of nitrogen supply under non-limiting level. This efficiency can be described by the following equation :

$$En = \frac{\Delta DM}{Nf.} = \frac{\Delta\ DM}{\Delta\ Nupt.} \times \frac{\Delta Nupt.}{Nf.}$$

were En is the nitrogen fertilizer efficiency
Δ DM is the supplemental DM yield
Nf is the level of nitrogen supply
Δ Nupt is the supplemental nitrogen uptake.

The global nitrogen efficiency can be subdivided in two components :

$$Em = \frac{\Delta\ DM}{\Delta\ Nupt.}$$ represents the "metabolic efficiency"

and $$Eupt. = \frac{\Delta\ Nupt.}{Nf.}$$ is the "uptake efficiency".

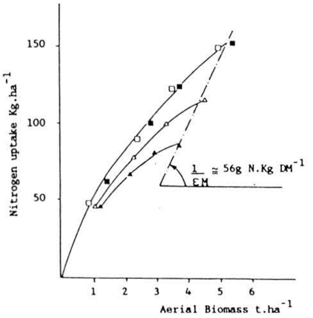

FIGURE 7

Comparison of nitrogen uptake between tall fescue (closed symbols) and cocksfoot (open symbols) for two level of nitrogen supply. □ ■ 120kg/ha (non limiting) and △ ▲ 60kg/ha. EM represent "metabolic efficiency"

Comparaison des prélèvements d'azote de la Fétuque élevée (symboles blancs) et du dactyle (symboles noirs) pour deux niveaux d'apport d'azote. □ ■ 120kg/ha (non limitant) et △ ▲ 60kg/ha

The figure 7 allows to separate differences due to "uptake efficiency" and to "metabolic efficiency". The difference between the two genotypes studied in this example are essentially due to differences in "uptake efficiency". In this experiment "metabolic efficiency" is similar and equal to 18 kgDM/kgN. Such a model could be used to study the variability in a wider range of genotypes. But this work will be very heavy to do in a breeding programme and it would be necessary to use more analytical criteria easier to measure on a great number of genotypes.

For "metabolic efficiency" we have previously indicated that the sensibility of leaf elongation rate to nitrogen deficiency would be a good criterion. For "uptake efficiency" the structure of root system is certainly the main parameter to take into account. In the case presented in figure 7 cocksfoot has a more dense hairy root system in the upperlayer of the soil than tall fescue. The selection for this character is difficult in field conditions but perhaps some correlations could be found with parameters measured in artificial substrates. A breeding programme including such an objective would be able to increase the uptake of other elements like P and K.

IV. Morphogenetic adaptation to different type of management

In practice grasses are used in different ways : cutting for silage or hay, continuous grazing or intermittent grazing. Sometimes the same sward can be successively used by each of different manners. For example in a grazing system, half part of the area devoted to grazing in summer must be harvested for silage in spring because of the difference of growth rate between the two seasons.

So we can wonder if it is necessary to specialize more deeply the genotypes for each type of management. Certainly in practice we must distinguish systems where cutting is predominant and systems where grazing is predominant. In the last case it is also necessary to distinguish between continuously stocked system and rotational grazing.

The general pattern of regrowth after cutting can be described as follows :

. first : exponential growth which corresponds to a limiting L.A.I. for interception of light,

. second : linear growth which corresponds to a full interception of light,

. third : decrease of growth rate due to an increase of respiration losses and the beginning of senescence of the first leaves emerged after cutting.

According to the model we have presented before, the growth rate when full light is intercepted cannot be changed very largely between genotypes. So only the first and the third part of the growth curve can be modified.

For grazing management the speed rate of L.A.I. recovery is the most important parameter. This point mainly depends on the leaf area remaining after grazing, but also on the leaf expansion rate per tiller and the tiller density. These two parameters vary greatly between genotypes but unfortunately there is a negative correlation between them (RYLE, 1964 ; NELSON and ZARROUGH, 1981 ; ZARROUGH et al., 1984). In the case of continuous grazing the results obtained by HODGSON (1981) and BIRCHAM and HODGSON (1983) indicate that the optimum leaf area index at equilibrium must be obtained with 4 or 6 cm of sward height. This structure of the sward corresponds to the best compromise between growth of sward and losses by senescence. On perennial ryegrass such a sward structure leads to a very high tiller density (30 000 to 40 000 tillers.m^{-2}). So for each type of grazing management the aptitude of genotype to maintain a high density of tillers would be a good objective for breeding adapted varieties.

The first genetic control of tillering rate is the rate of production of buds which corresponds in fact to the leaf appearance rate. The leaf appearance rate can be expressed by the phyllochrone interval in degree days. For perennial ryegrass one leaf is produced each 110 degree days (DAVIES and THOMAS, 1983) and on tall fescue 220 degree days (LE-

MAIRE, 1985). We can explain the reason why the tillering density of perennial ryegrass is always higher than tall fescue. It should be very easy to analyse the intra-specific variability on this character in isolated plants. So we could define the potential tillering rate of genotypes. In dense sward tillering rate is lower than potential tillering because of shading of the sheath bases (DAVIES et al., 1983). SIMON and LEMAIRE (1987) showed for Italian and perennial ryegrass and for tall fescue that the tiller production ceases when the L.A.I. is higher than 3. This cessation of tillering is mainly determined by a change of light quality as the shading increases. Perhaps a genetic control of the sensibility of tillering to the quality of light could be detected. So it would be possible to obtain genotypes which maintain high tiller density despite a lower frequency of defoliation.

For silage cutting systems, genotypes must have a delayed senescence to maintain linear growth during a long time. In grasses there is a strict relationship between leaf appearance rate and leaf senescence rate. As result of that, the maximum number of mature living leaves per tiller is more or less constant = about 3 for perennial ryegrass (DAVIES and THOMAS, 1983) about 2 for tall fescue (LEMAIRE, 1985).

So the time between one cut and the beginning of senescence can be estimated for each genotype if we know the phyllochrone interval and the maximum number of living leaves per tiller. For perennial ryegrass the beginning of senescence occurs 3 x 110 = 330 degree days after cutting and for tall fescue we obtain 2 x 220 = 440 degree days. So with a low frequency of cutting (5 or 6 weeks) the yield of tall fescue is higher than perennial, but with a high frequency of cutting (3 or 4 weeks) it does not exist any differences between the two species.

It would be necessary to study the components of morphogenesis for a large range of genotypes and to evaluate both the primary growth and the turnover rate of leaf material. In this aspect, the leaf appearance rate appears to be the key parameter because it defines both the tillering rate, the leaf size and the leaf turnover. So the structure of the sward and the optimum management are mainly determined by this parameter, which is relatively easy to measure.

The problem is to know if the leaf elongation rate is strictely negatively correlated to tillering rate. We can imagine an increase of the leaf elongation rate without decrease the leaf appearance rate and consequently to maintain the same potential tillering rate. But the risk is to obtain a lower site-filling and to decrease the actual tillering rate. ROBSON (1974) showed that the assimilates are preferentialy used for expanding leaf to the detriment of tiller buds. So in low light intensity an increase of leaf expansion rate could decrease the tillering rate. This phenomenom could explain the negative correlation between L.E.R. and tillering. But in a situation of larger quantities of available assimilates this correlation is not observed. So ROBSON (1982) indicates that the economy of C of "low" respiration lines of perennial ryegrass leads to an increase of tillering rate with unchanged leaf elongation rate.

It would be necessary to take into account the variability of lamina width. So for a similar leaf elongation rate a higher lamina width leads to a faster leaf area expansion. For *Dactylis glomerata* a large genetic variability for this parameter exists (MOUSSET, pers. comm.) and further studies are in progress to find out the correlation with other parameters of L.A.I. expansion.

Finally for grazing use it would be necessary to study the variability of lamina sheath ratio. BIRCHAM (1981) reported that in continuously grazed sward only the lamina part is removed by sheep. So genotypes with smaller sheaths could be more adapted to high grazing pressure.

Conclusion

Some major points can be underlined to conclude this paper. The first point is that under non-limiting conditions for nitrogen nutrition and water consumption the potential dry matter growth is directly related to the quantity of intercepted light. No great differences appear between a large range of species. This indicates that the potential yield is physically limited and no significant genetic progress can be considered for the future.

The second point is that the nitrogen requirement for potential growth is the same for a large range of species. As for the first point the genetic variability of nitrogen efficiency is very low when the plants are at optimum level of nitrogen nutrition. These two first conclusions leads to consider that breeding for increased potential yield of forage species could be unsuccessfull for near future.

The third point is the fact that both nitrogen deficiency and drought have a more marked effect on the morphogenesis of the plant than on the photosynthetic activity. Studies on the genetic variability of the sensibility of leaf and stem elongation to water or nitrogen restriction must be more developed in the future to breed varieties adapted to poor conditions.

The fourth point concerned with the adaptation of genotypes to intense defoliation in grazing system or to silage cutting. It appears that more attention should be payed on the "turnover" of leaf material (duration of leaf life).

In conclusion we must encourage a deeper relationship between genetical and ecophysiological approaches to develop more accurate criteria of selection.

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Breeding white clover in relation to biotic and climatic factors

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SUMMARY

White clover forage yield depends on interactions with several other organisms; the companion grass, grazing animal and Rhizobium bacterium. This paper describes how an understanding of the bases of these interactions is essential for the development of effective selection criteria to improve yield and reliability of yield in this species. The implications of the interactions in evaluating white clover breeding material and varieties are discussed.

INTRODUCTION

The herbage yield of white clover is generally regarded as unreliable both within and between years. This unreliability may often be a result of inappropriate management or incorrect choice of variety. However, in realistic agronomic conditions the performance of a white clover variety is subject to interactions with three biotic factors, viz the companion grass, Rhizobium bacterium, and grazing animal as well as environmental factors. All these interactions may contribute to unpredictability and must be taken into account in formulating breeding objectives and developing breeding techniques for white clover.

This paper describes breeding objectives and progress, together with supporting strategic research, designed to develop more productive and reliable varieties of white clover.

COMPANION GRASS

White clover is almost universally grown with a companion grass, most commonly Lolium perenne L. However, traditionally white clover and grasses have been bred separately without regard to the mode of use of clover, and the fact that aggressive grass varieties can severely suppress clover growth.

Recent research has demonstrated that large differences exist in the compatibility of grass and clover cultivars (Table 1).

Using two late flowering L.perenne varieties S.23 and Perma as companion grasses, it was found that Blanca was the most productive clover with Perma and lowest yielder with S.23. With the latter grass Alice was the most productive clover.

TABLE 1 Annual Dry Matter Yield of Clover (t ha^{-1}) grown in mixture with L.perenne varieties

	S.23	Perma
Clover		
S.184	4.4	3.9
Menna	4.6	3.9
S.100	4.3	4.3
Alice	4.8	4.0
Olwen	4.5	4.3
Blanca	4.2	4.8

LSD between clover varieties $P = 0.05$ 0.4

Studies by EVANS, HILL, WILLIAMS & RHODES (1984) have further demonstrated that grass and clover populations which have co-existed together for many years, may develop specific compatibility relationships, such that clover yield and total mixture yield were greater in mixtures of co-adapted grasses and clovers than when an "alien" grass was used.

Interestingly however a grass (Ba 9462) bred for characters which may be associated with compatibility, ie erect habit of growth, permitted a greater yield of all clover populations, and greater total yield than with the "alien" grass S.23. These results indicate that it may be possible to breed for a more general compatibility.

In these latter studies the pairs of grass and clover populations were derived from a wide range of environments.

TABLE 2 D M Yield of white clover populations grown with four companion grasses (R P Collins unpublished data)

t ha^{-1}

		Companion grass		
	'Co-existing'	Aurora	Melle	Talbot
Clover				
Ac 3789	3.44	2.03	2.94	3.07
Ac 3785	3.12	2.86	2.56	1.23
Menna	2.17	3.23	2.17	2.43

One of us (R P Collins) has recently established studies in which clover populations from within a more restricted area (of Switzerland) have been grown with their co-existing grass and three other L.perenne varieties. Strong interactions are evident between grass variety and clover populations. In particular two clover populations of similar leaf size and physiological characteristics (Ac 3785 and Ac 3789) were both productive with their 'co-existing' grasses and with Melle. However whilst one clover Ac 3789 was equally productive with Talbot (approx 3.1 t ha^{-1} annum) the second clover produced a yield on only 1.27 t with Talbot (Table 2). It is of interest to note that Talbot is the variety currently used as a companion grass for National and Recommended List testing in the UK.

These results have important implications for forage breeding. Firstly the large differences in yield which are obtained by mixing compatible grasses and clovers, emphasise the importance of breeding for compatibility in clover and grass breeding programmes. Secondly the evaluation of clover varieties by official bodies for National and Recommended Lists, may be misleading if only one companion grass is used.

To breed effectively for improved compatibility it is essential that strategic research is carried out to identify the plant characters associated with compatibility which may then be used as selection criteria.

In broad terms, two types of compatibility can be identified, viz, spatial and temporal (RHODES 1984). Spatial compatibility arises through the possession (by the components of the mixtures) of characters which permit a greater utilisation of environmental factors such as water, light and nutrients at a given time. By contrast temporal compatibility arises from the components having complementary growth rhythms.

In simple terms it seems likely that a more upright growth habit in the grass may confer spatial compatibility, however many of the competitive effects in grass clover swards cannot be ascribed to competition for light and may occur between root systems. No information exists on root characteristics associated with compatibility. Preliminary work has been commenced by one of us (R P Collins) to quantify the role of root competition in compatibility relationships of grass/clover mixtures, including co-existing combinations.

The bases of temporal compatibility relationships may be much more subtle. It is important to note that the generally accepted seasonal growth curves for white clover in mixtures with ryegrass in UK conditions, ie with peak growth rates in July/August, contrasts with that in monoculture. In the latter the lowest growth rates occur in the July/August period (RHODES & MEE 1984; GLENDINING 1987).

The implications of these contrasting seasonal growth rhythms is that clover yield in a mixture is largely controlled by competition from the grass, rather than as a direct response to environmental factors. Subtle changes in seasonal growth and development (eg flowering date) may therefore be important in controlling compatibility. Competitive ability of grasses declines with the onset of flowering (RHODES 1970) this may account for the increase in clover yields in July/August in mixture with perennial ryegrass.

Recent work (SEWAYA 1987) has shown that no relationship existed between grass monoculture yield and clover yield in mixture with a range of L.perenne breeding material. These results suggest that it will be possible to breed productive grass varieties which are also compatible with white clover.

With the importance of the competitive environment in controlling clover yield, it is clear that breeding for response to any environmental factor should also confer a competitive advantage to the clover to permit full expression of the character. An example of breeding for response to an environmental factor, low temperature, is described in the next section.

CLOVER GROWTH AT LOW TEMPERATURE AND COLD HARDINESS

White clover grows more slowly than ryegrass in spring, and is thus placed at a competitive disadvantage, which may lead to suppression of clover yield not only in spring, but throughout the year. Until recently it has not been possible to obtain genetic resources of white clover which combine good growth at low temperature with cold hardiness. However, material possessing both these characteristics is now being utilised in the WPBS white clover breeding programme. The material has been studied in controlled environments and in mixed and monoculture swards under field conditions. Some of the characteristics of this material are summarised in Table 3. The measure of cold tolerance used is the loss of stolon in mixed swards over the winter period.

It is clear that this improved low temperature growth and spring yield is also associated with substantial improvements in annual yield over the most commonly grown variety, Grasslands Huia.

TABLE 3 Low temperature growth and sward characteristics of some clover populations

	Leaf and Petiole per Unit Stolon length* mg mm^{-1}	Stolon wt g m^{-2} Nov.	March	Spring Sward Yield t ha^{-1} Cut 1	Cut 2	Annual Sward Yield t ha^{-1}
Ac 3785	3.51	136	74	.15	1.10	4.26
Ac 3789	2.50	169	52	.15	0.86	4.14
Grasslands Huia	2.00	103	29	.04	0.64	2.69
LSD P = 0.05	0.40	18		.07	0.20	0.49

*Mean of 6°C and 9°C treatments

GRAZING ANIMAL

Under farm conditions white clover may be cut for conservation and/ or more commonly grazed by cattle and sheep at various intensities. These practices contrast with those used in testing procedures for National List and Recommended List testing in the UK, where only cutting evaluation is used.

Using systems of cutting, lax rotational sheep grazing and continuous sheep grazing EVANS & WILLIAMS (1986) have shown that yield ranking of clover varieties can be reversed under continuous grazing when compared to the other two treatments which gave similar results (Table 4).

That experiment compared clover varieties across the three leaf size categories, but it is now clear that similar interactions can occur within a leaf size category.

It seems unlikely that frequent low cutting treatments can simulate the continuous grazing or intensive rotational grazing by sheep, where stolon is often removed and varietal differences may occur because of variation in amounts and anchorage of stolon.

However, recent results confirm that cutting treatments give a good indication of relative varietal performance under cattle grazing systems (not shown).

TABLE 4 Yield of clover varieties expressed as a percentage of variety S.100

Variety	Leaf Size	Cutting	Continuous Grazing by Sheep
Olwen		115	33
Katrina	Large	112	37
Alice		100	61
Menna		94	103
Ac 3160	Medium	89	91
S.100		100	100
S.184	Small	81	105
Actual yield of S.100 t ha^{-1}		4.8	2.3

RHIZOBIUM

MYTTON (1984) pointed out that full phenotypic expression of nitrogen fixation is a function of complex reactions and interactions between plant genotype, *Rhizobium* genotype and the environment. Superior combinations of plant and *Rhizobium* can be identified *in vitro* but it remains difficult to introduce the *Rhizobium* into soils where strong natural populations exist.

Recently MYTTON (1987) has indicated that when grown with natural populations of *Rhizobium* white clover shows heritable variation in nitrogen fixation. Most clover genotypes are N limited, but some are not. Identification and elimination of non N limited genotypes should facilitate better genetic advance, by ensuring that fixation is not rate limited by demand for its product.

CONCLUSIONS

Genetic resources of white clover are extensive and largely untapped. However in utilising these resources attention must be given to the interactions between the clover plant, companion grass, grazing animal, *Rhizobium* bacterium as well as environmental factors.

Clearly clover performance in a mixture is largely determined by the competitive environment, and an exciting development in breeding will be the production of compatible varieties of grass and clover which are also adapted to various grazing managements.

Our research has shown that attention to these interactions will permit substantial improvements in yield and reliability of white clover. However relevant evaluation techniques must be developed to quantify these improvements under realistic agronomic conditions.

RÉSUMÉ

Le rendement en fourrage du trèfle blanc dépend des interactions avec plusieurs autres organismes. L'herbe environnante, l'animal qui paît et la bactérie Rhizobium. Cet article souligne l'importance d'une bonne compréhension des bases de ces interactions pour le développement de critères de séleçtion efficaces afin d'améliorer le rendement et la fiabilité du rendement dans cette espèce. L'article traite des implications des interactions pour l'évaluation sélection du matériel de et sélection des variétés de trèfle blanc.

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EVOLUTIONNARY GENETICS AND PLANT BREEDING STRATEGIES

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INTRODUCTION

When we asked several scientists ([1]) about their feelings on the eventual relationships that might exist between evolutionnary biology studies and plant breeding strategies, we got very different answers:

1) "No connection !" (this answer should not be taken too seriously)

2) "They are asking similar questions: evolutionnary biologists are trying to explain how so much diversity can be maintained under the force of natural selection, while plant breeders are trying to maintain as much diversity as they can under artificial selection".

3) "The goal of the plant breeder is to undo what natural selection has done; God was a reductionnist, agronomists are holistic".

This paper is a discussion about the usefulness of evolutionnary studies for plant breeding, and why we think that plant breeders should care about the general goals and means of natural selection.

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[1] Chris Gliddon, Pierre-Henri Gouyon, and John Harper

Obviously, natural and artificial selection do share similar mechanisms. In natural selection the selected advantage of a given genotype is measured by its fitness or selective value, that is the number of copies of its genes that it transmits from one generation to the next. It depends on viability (i.e., juvenile survival), fecundity, and adult survival in the case of iteroparous (perennial) species. The theory of evolution by natural selection depends strongly on the concept of fitness. Notice that the fitness value, in population genetics is valid for a given genotype or phenotype, in a given population, in a given environment, at a given time; it is therefore different from the "genotypic value" of plant breeding, which, on the other side, is trying to give an average value, across the maximum number of different environments, of a genotype.

As Roughgarden (1979) stated in his book (writen essentially for graduate students),

> *"Although genes are made of DNA, which is, of course, a chemical, genes cannot simply be stored in bottles on shelves. The only storage place for different genes at this time is living organisms themselves. A reservoir of genetic variation is needed in any species to allow it to adapt to new environments (...). It is important to know how much genetic variation would be good insurance for expected changes in the environment and how perhaps to carry out agriculture in ways that would maintain enough variation in crop plants to meet those expected changes in the environment. (...). Population genetics is sometimes applied to plant and animal breeding to produce a desired phenotype by artificial selection. The theory exists to predict roughly how much change in phenotype will occur in the next generation as a function of the intensity of artificial selection that is applied."*

I - Individual versus Group Selection: ESS and Optimum

In order to show that individual selection may act against the good of the whole, we will take two examples: the sex-ratio and mixtures of genotypes.

1) Sex-ratio

The limiting factor to offspring production is usually not the number of male gametes. On the opposite, the female function must insure not only the production of female gametes, but also that of the zygotes, such as the seeds, which is a real limiting factor to the population growth. Therefore, since the fitness of the population is the total number of offspring it produces, the optimal strategy for a population would be to be constituted with many females and a few males, in necessary and sufficient number to fertilize all the females. This strategy is obviously unstable, since the first rare genotype which would produce more males than the rest of the population would be selected for in such a population because males transmit more their genes than females. The only stable strategy within the population is to produce as many males as females, because this is the only one allowing equal fitnesses of the two sexes. The total fitness of such a population is then far from the maximum, but it is, at least in the general case, the only strategy which can be fixed in natural populations. It is obvious that Nature does not need as many males as it creates: simply consider the fact that most male animals have a "harem"... The sex-ratio is still one at birth, because of sexual selection. Even animal breeders and farmers know that, when they use for instance one bull for many females. It is less well known by plant breeders, although the same process works among hermaphroditic plants for instance. Evolutionnary theory (Charnov 1982) predicts an equal resource allocation towards female and male function, unless the species has a certain selfing rate. In this case, sexual selection is less intense (in the absence of inbreeding depression), and inbreeding species should produce a lower pollen / ovule ratio... which is what is actually found in natural populations.

2) Associations of different genotypes: competition does not maximises total yield

It is a well known fact that the yield of a mixture is not equal to the average yield of both constituents in pure stands, it is often higher. This is in particular true for the dry matter in associated forage crops. It is also often true for the number of seeds per plant in a self-fertilizing species, and is a good argument in favour of composite species in wheat for instance (Allard & Adams 1969). The explanation is very simple, and is related to the fact that inter-genotypic competition might be less intense that intra-genotypic competition, because of niche differences between genotypes (or species) for instance. If this is the case, we are in a case of apostatic selection (i.e., frequency-dependent selection with advantage to the rare genotype). As Cohen (1985) has noticed, the composition of a population made of different varieties of wheat (or different species) evolves like that of a haploid population until, at equilibrium, the proportions of each genotype are such that the mean yield per plant of each variety are equal to each other and therefore to the overall mean yield per plant. In general, the maximum total yield will not be obtained for the same genotypic frequencies, as is shown hereafter in the simple case of two genotypes.

Let w_{ij} be the number of seeds produced by a plant with the genotype i when it is surrounded by plants of the genotype j, and assume that the processus is additive, i.e. that the fitnesses of each genotypes i surrounded by a mixture of the two genotypes, in frequencies p_1 and p_2, is the sum $(p_1w_{i1} + p_2w_{i2})$. Equilibrium frequencies (p_1^* and p_2^*) are obtained for the equality of the fitnesses, that is for

$$p_1^*w_{11} + p_2^*w_{12} = p_1^*w_{21} + p_2^*w_{22}$$

or

$$p_1^* = (w_{12} - w_{22}) / (w_{12} + w_{21} - w_{11} - w_{22})$$

A necessary and sufficient condition for a polymorphism to occur is

$$0 < p_1^* < 1$$

This polymorphism is stable if, and only if, each genotype has a higher fitness in mixture than in pure stand, or if there is total symetry between the two genotypes:

$$w_{12} > w_{22} \text{ and } w_{21} > w_{11}$$
$$\text{or} \quad w_{12} = w_{21} \text{ and } w_{11} = w_{22}$$

The frequencies for which the average yield, W, is maximum are $p_1^{\bullet}$ and $p_2^{\bullet}$, such that, at equilibrium, $dW/dp_1^{\bullet} = 0$ for $p = p_1^{\bullet}$. We can write

$$W = p_1 (p_1w_{11} + p_2w_{12}). + p_2 (p_1w_{21} + p_2 w_{22})$$
$$W = p_1^2 (w_{11}-w_{12}-w_{21}+w_{22}) + p_1 (w_{12} + w_{21} - 2w_{22}) + w_{22}$$
$$dW = 2p_1 (w_{11}-w_{12}-w_{21}+w_{22}) + (w_{12} + w_{21} - 2w_{22})$$
$$dW = 0 \text{ for } p_1 = p_1^{\bullet} \text{ if and only if}$$
$$p_1^{\bullet} = (w_{12} + w_{21} - 2 w_{22}) / 2(w_{12} + w_{21} - w_{22} -w_{11}$$

The two values, p_1^* and $p_2^{\bullet}$, are equal only when there is symetry, when $w_{12} = w_{21}$. when $w_{12} > w_{21}$, p_1^* is higher than $p_1^{\bullet}$, and the yield of the mixture is no more at the maximum ($dW/dp < 0$). Natural selection does create equilibrated "natural composites", not optimal ones.

3) A plant breeder is selecting at all levels, from the individual to the species

It finally appears that for most traits of the genetic systems, the evolutionnary stable state (when it exists) will not be an optimum (when there is one). In other words, the agronomist is looking for the good of the whole, while natural selection operates through individuals, and is therefore acting for the good of the individual, given the others (i.e., given the genetic environment). In most cases, this process will even act against the good of the whole.

II - Selection index and fitness in changing environments

Plant breeders and population geneticists share at least one common problem: defining the goal of the selection. In case of artificial selection, the problem will be to construct a selection index. In case of natural selection, one has also to use fitness in a such a way that it allows prediction. The preceeding examples have shown that there cannot exist one value of fitness, since its value depends on what the others are doing. However, even when selection is not apostatic, the environment may be variable for other reasons, such as spatial and temporal heterogeneity. Again arises the problem of defining fitness, or of selection index. In most cases, the classical "fitness" will not be the function to maximise for a genotype to invade. For instance, population geneticists (Haldane & Jayakar, 1963) have shown that a genetic polymorphism in a one locus, two alleles model, in a temporally variable environment, will be protected

- when there are three phenotypes (A_1A_1, A_1A_2, and A_2A_2): if there is overdominance on the geometric means of fitnesses in the different environments (with $w_{ij,t}$=fitness of genotype A_1A_2 at time t:

$$(w_{12,1}w_{12,2}\ldots w_{12,T})^{1/T} > (w_{11,1}w_{11,2}\ldots w_{11,T})^{1/T}$$

and $$(w_{12,1}w_{12,2}\ldots w_{12,T})^{1/T} > (w_{22,1}w_{22,2}\ldots w_{22,T})^{1/T}$$

- when there is complete dominance (two phenotypes: A and a): if the arithmetic mean of the recessive phenotype is higher, and its geometric mean is lower, than those of the dominant phenotype:

$$(1/T)\sum_{t=1}^{T} w_{a,t}. > (1/T)\sum_{t=1}^{T} w_{A,t}$$

and

$$(w_{a,1}w_{a,2}\ldots w_{a,T})^{1/T} < (w_{A,1}w_{A,2}\ldots w_{A,T})^{1/T}$$

A higher geometric mean for fitness, other things being equal (in particular the arithmetic means), means that the genotype is less variable.

This example shows that what population geneticists call "fitness" is a concept to be considered for one genotype (or phenotype), in one particular genotypic environment (population), and in one type of environment. In the preceeding examples, the "index of natural selection" was sometimes the geometric mean of fitness over time. In the case of spatial heterogeneity of the habitat, it is sometimes the harmonic mean (Levene 1953), although in this case the quantity which is maximized can be shown to be the total geometric mean.

Evolutionnary biologists have indeed adressed the problem of selection in a changing environments (Levins 1968): the knowledge of the fitnesses of a given genotype in different environments allows to predict the kind of "strategy" (set of rules defined **a priori**, as are the set of possible phenotypic expressions of a genotype) (Maynard Smith 1982) which will be selected for in a given heterogeneous environments. There may exist the possibility for a polymorphism to be maintained under certain circumstances, either as a genetic polymorphism with different pure strategies (i.e., different genotypes with only one phenotype each), or as a mixed strategy with only one genotype -(having different possible phenotypes, each more or less adapted to one environment), which can be called a "plastic" genotype.

The methods which are used can easily be applied to artificial selection, they should even be more useful, because the plant breeder usually knows exactly where he is going to grow his varieties. For instance, the knowledge of, say,

1) the optimal date of flowering in different environments, that is the one which provides the maximum yield of a given species in the different environments (this maximum is completely theoretical, and may be often approximately calculated), associated with the knowledge of

2) the cost of plasticity (difference between the maximum yield of a plastic phenotype and the overall theoretical maximum yield),

3) the frequencies of the different environments,

will tell us something about the kind of genotypes that we should be looking for: for instance, either one genotype for each environment ("**specialists**"), or one common genotype which will have an average phenotype, or which will respond to the different environments ("**generalist**", or "**plastic**", which may be either homeostatic or variable (Heyer et al, in prep.)).

Of course, the selection index will not only include the crop environment, but also the genetic correlations, between characters. Then the fitness itself can be considered as an index of natural selection. The same approach can be used by considering the maximum theoretical yield which would be brought about if an individal was investing all of its photosynthesis products towards one of the two characters considered: for instance, disease resistance versus insect resistance, or versus frost tolerance, etc. The knowledge of the genetic correlations between these two characters, of the probability of occurence of the desease, or the frost, or the insect in the different habitats, and of the variation of the correlations with habitats, will help to the decision making.

As it is in evolutionnary biology, game and optimization theory can be used with great profit in plant breeding. One only needs to have an "evolutionnary thinking" (Charnov, 1982) in mind. On the other hand evolutionnary biology may also profit from "artificial selection thinking". Indeed, quantitative genetics is now being used with success by evolutionnary biologists.

Not only this kind of thinking is useful for the definition of selection index, but also for the prospection of genetic variability within natural populations: population biology tells us a lot about the genetic structure of natural populations, as is shown in the next section.

III - Spatial population structure and the maintainance of genetic diversity: neighbourhood and metapopulation:

In this section we would like to show how the knowledge of pollen and seed dispersal, and of selfing rate influence

1) the sampling strategy of natural populations, in order to maximize the genetic diversity of the samples.
2) the designs for polycross
3) the maintainance of genetic diversity under artificial selection

Felsenstein (1976) has realized an excellent review on the population genetics of the consequences of the spatial structuration of the landscape on the effects of mutation, selection, migration and drift. Briefly, population geneticists have often distinguished between two kind of spatially structured populations (Olivieri et Prosperi 1986):

- populations which are a priori divided into panmictic sub-units, exchanging genes through a common pool of migrants ("island model") or though intermediate, closer units ("stepping-stone model")

- continuous populations, in which gene flow is restricted through distance, as in the stepping stone model, but where no sub-units may be a priori defined.

Results on the first type of populations indicate that, in the absence of selection, genetic differenciation will occur between the sub-units under certain circumstances (effective size times migration rate very low). (Wright 1940, Kimura and Maruyama 1971). The second type of populations involves the introduction by Wright (1965) (see also Levin and Kerster 1971, Crawford 1984, Cahalan and Gliddon 1985). of the concept of neighbourhood area: this is defined as the area within which the parents of the central individual have a high probability to be located, and from which these parents can be considered as drawn at random. Assuming a normal distribution of seed and pollen dispersal, the neighbourhood area is easily calculated from the parent-offspring dispersal variance of pollen (σ_p^2) and seeds (σ_s^2), and from the outcrossing rate (t) (Gliddon and Salem 1985):

$$N_A = 2 \Pi (\sigma_s^2 + t\sigma_p^2 / 2)$$

Wright has shown that, depending on the effective size within that area (the neighbourhood effective size), genetic differenciation within that continuous population will or will not occur. This will obviously influence the optimal methods of prospection of the genetic variability in natural populations.

It will also influence our designs for polycross or seed multiplication, since the neighbourhood area or effective size is also the larger panmictic unit (when the proportion of deviation to Hardy-Weinberg frequencies due to selfing is not considered), and should therefore be known when random mating is desired. It is actually sometimes implicitely taken into account (plant breeders do sometimes measure pollen dipersal distances).

One can inversely consider a subdivided population such that between populations migration rate is very low, and then arrive to the concept of metapopulation (Levins 1968, Slatkin and Wade 1979, Olivieri and Gouyon 1985, Couvet et al 1985). This model considers another factor, namely the probability of local extinctions. There is therefore two different process, one at the population level (classical population genetics), and one at the metapopulation level (regular extinctions and creations of new populations from the extant populations). If we then add selection within that metapopulation, it can be shown, by simulation methods (Olivieri, in prep) that, for instance, the selection pressure on some characters such as high reproduction, precocity, high migration rate, will be different depending on the time from foundation, i.e., the age of the population. Therefore, if one is looking for, say, characters of juvenile vigour, one should first look for young populations, instead of some ecological characteristics of the habitat, although it is often possible to relate both, unless seed dormancy is important. Indeed, in a survey of 20 natural populations of Medicago lupulina, and 8 of subclover (grown in an experimental nursery), we found almost no correlation between quantitative characters related to fitness (seed production, life-cycle) and ecological data from the original population (Olivieri, in prep.). We suspect that the age of the population might be the primary factor determining the type of life-cycle that we will observe in a particular site.

This has implication for the maintainance of genetic variability as well: it has been shown for instance (Couvet et al 1985) that cytoplasmic male-sterility in Thymus vulgaris was maintained in young populations through the combined effects of selection (much higher fertility of females compared to hermaphrodites) and restricted gene flow (no restauration genes in the neighbourhood of females). As populations get older, restauration genes finally arrive and are strongly selected for, given the high proportions of females (up to 90%). Therefore, if one wanted to maintain a reservoir of variability for cytoplasmic male-sterility, the last thing to do would be to create a large, undisturbed "natural" population, because the male-sterility would be then surely lost. Obviously, the same process arises with the maintainance of genetic variability for longevity in pasture plants: in the absence of disturbance, only the very prennial genotypes will be maintained, all of the annuals will disappear.

There is a great deal of discussion in the world around this aspects of conservation and others such as about the landscape fragmentation (is it good or bad ?). The only reasonnable answer we can think about is "it depends on the species, and even worse, on the genotypes you want to preserve !

In conclusion, it appears that natural selection may sometimes act in a favorable sense, sometimes in a unfavorable one, but it is never neutral...and therefore it should be worthwhile to know about it in the first place.

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The use of lucerne in a modern rotation is based on the improvement of forage feeding value through intensive management, both under dryland and irrigated conditions. Weed free, leafy green material is needed to produce a high quality lucerne crop harvested as baled hay, haylage, ventilated hay, ensilage, greenchopped, cubed or dehydrated forage. The increase in forage quality cannot be detrimental to yield levels in order to maintain lucerne as a competitive crop; at the same time, the duration of the stand under intensive management often determines lucerne profit potential. Cutting at early stages, which is required for modern lucerne exploitation, hastens stand decline (Feltner and Massengale, 1965; Robinson and Massengale, 1968) and decreases total dry matter yield (Veronesi _et al_., 1981).

On the basis of the above reported information, one of the main targets of lucerne breeding is the synthesis of varieties adapted to modern utilization technology, which requires long-lived stands, able to give qualitatively and quantitatively good results when the cutting interval is shortened.

The objective of this investigation was to evaluate the effects of 2 cycles of phenotypic recurrent selection for tolerance to frequent harvest on persistence and dry matter yield over a 3 year period.

MATERIALS AND METHODS

In 1980 sixteen plants, derived from 2 cycles of phenotypic recurrent selection (PRS) for high forage yield under frequent cutting regimes, carried out within the Italian ecotype 'Casalina' (Veronesi _et al_., 1986), were intercrossed by hand under a pollination cage. Equal amounts of cross seed were taken from each plant and mixed to produce a selected seed lot.

Cuttings of 120 plants from the selected seed lot and 60 plants from the original seed lot of 'Casalina' (control) were established in a glasshouse in the Spring of 1981. Seventy-two randomly chosen clones from the selected materials and 36 clones from the control were transplanted into a nursery in April of 1982; a split-plot design with 3 replications was used. Three different cutting frequencies were applied, under dryland conditions, on the main plots; selected and control materials were grown in the subplots where each genotype was represented by 2 plants (a total of 18 cuttings per genotype). The experimental design permitted each of the 108 clones (72 selected and 36 checks) to be subjected to the following harvest treatments during the 1983-85 growing seasons: i) very frequent (_VF_), cut when plants were 25 to 35 cm high (20 cuts); ii) frequent (_F_), cut when plants were 40 to 50 cm high (17 cuts) and iii) infrequent (_I_), cut at 1/10 bloom stage (13 cuts).

For each treatment, data were collected on: a) dry matter yield (g/plant) per cut, b) harvest tolerance, measured as number of live plants at the end of each growing season (a last evaluation of the number of live plants was performed in the Autumn of 1986) and c) regrowth rate, measured as plant height (cm) 14 days after the cutting in August 1983, 1984 and 1985. Arcsin transformation was performed on the percentage of surviving plants. Data are reported in actual units.

RESULTS AND DISCUSSION

Average percentages of surviving plants at the end of the growing seasons in the 1983-86 period are reported in Fig. 1; in 1983 survival of the different materials ranged from 60% (VF harvest treatment, control) to 78% (I harvest treatment, selected); from 58% (VF, control) to 76% (I, selected) in 1984; from 39% (VF, control) to 72% (F, selected) in 1985 and from 30% (F, control) to 56% (I, selected) in 1986. Fig. 1 shows that within each growing season there were no significant differences among survival percentages of selected materials. On the contrary, in control materials, survival percentages under infrequent harvest treatment appeared significantly higher than VF harvest treatment beginning in the Autumn of 1983 and were also higher than F harvest treatment beginning in the Autumn of 1984. Looking at the differences between selected and control materials submitted to the same harvest treatment, a significant difference was present at VF harvest treatment in the Autumn of 1983. In 1984 significant differences were present both for VF and F harvest treatments while significant differences between selected and control materials were present at all harvest treatments beginning in the Autumn of 1985. In agreement with our previous findings (Veronesi et al., 1986), selected materials appeared to have a higher average survival over time and to be less subjected to the negative effect of the increase of cutting frequency on persistence than control materials.

Regrowth rates after the 1983, 1984 and 1985 August cuts are reported in Tab. 1 in relation to the 3 different harvest treatments. Even if the average values of selected materials were always higher than those of controls, the differences were significant only in 1984 for VF ($\bar{x}$ = 19.5 vs $\bar{x}$ = 17,5 cm) and I ($\bar{x}$ =34.2 vs. $\bar{x}$ = 29.0 cm) harvest treatments. Therefore, our data are only in partial agreement with the results of Bocsa et al. (1983) who, looking at the regrowth of lucerne plants tolerant to frequent cutting, found height values remarkably higher than the average of control plants.

Concerning the differences among cutting frequencies, in 1983 the average regrowth of materials under I harvest treatment was similar to those of materials under F and VF harvest treatments while both in 1984 and 1985 I harvest treatment showed average regrowth significantly

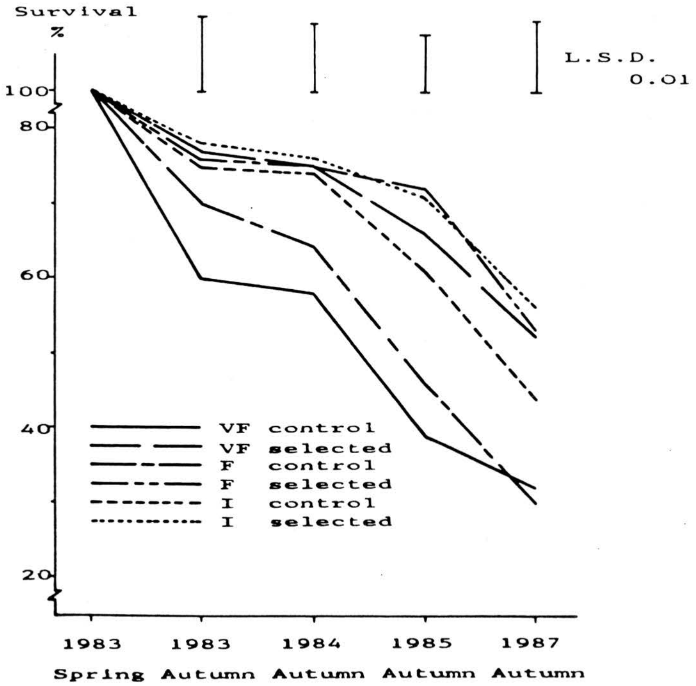

Figure 1. - Survival percentages (100 % = number of live plants at 1983 spring) of selected and control materials under 3 different harvest treatments.

Table *1*. - Regrowth rates measured as plant height (cm) 14 days after cuts made in August 1983, 1984 and 1985 in relation to the 3 different harvest treatments.

Cutting frequencies	Materials	Years		
		1983	1984	1985
Very frequent (VF)	Control	25.5	17.5	11.7
		n.s.	*	n.s.
	Selected	27.2	19.5	12.1
	$\bar{x}$	26.4	18.5	11.9
Frequent (F)	Control	26.1	21.1	12.9
		n.s.	n.s.	n.s.
	Selected	27.8	22.0	13.5
	$\bar{x}$	27.0	21.6	13.2
Infrequent (I)	Control	24.6	29.0	18.8
		n.s.	**	n.s.
	Selected	26.3	34.2	20.1
	$\bar{x}$	25.5	31.6	19.5
LSD 0.01 among harvest treatments means ($\bar{x}$)		-	5.3	4.7

n.s. = not significant; * = significant at 5% level; ** = significant at 1% level.

higher than F and VF both for selected and control materials.

Looking at the behaviour of materials over time, average regrowth under VF harvest treatment decreased from 26.4 cm in 1983 to 18.5 cm in 1984 and to 11.9 cm in 1985; it decreased from 27 cm in 1983 to 21.6 cm in 1984 and to 13.2 cm in 1985 (F harvest treatment) while, under I harvest treatment it increased from 1983 (25.5 cm) to 1984 (31.6 cm) and decreased in 1985 (19.5 cm). As regrowth after top removal is connected with accumulated carbon compounds (Brown et al., 1972), the more pronounced decrease of average regrowth under VF and F harvest treatments compared with I harvest treatment is probably a consequence of the higher number of cuts, which have been found to reduce the carbohydrate reserves (Demarly, 1957; Cooper and Watson, 1968; Talamucci, 1970). Generally speaking, the results show that the 2 cycles of PRS which produced a clear increase of persistence under frequent cutting regimes did not greatly affect the capacity for fast recovery under the same cutting conditions both within and among years.

Total dry matter yield of surviving plants per clone (sums of 1983 + 1984 + 1985 cuts) in relation to the 3 different harvest treatments are reported in Fig. 2. As expected, I harvest treatment resulted in the largest total DMY; yield reduction for selected materials from I to F and from F to VF harvest treatments were 39.5% (5459 vs. 3304 g/clone) and 39.3% (3304 vs. 2007 g/clone), respectively and for controls the yield reductions were 50.5% (4337 vs. 2148 g/clone) and 70.8% (2148 vs. 964 g/clone), respectively. Selected materials appeared significantly more productive than controls within each harvest treatment; in particular, selected materials yielded 25.9% more than controls under I harvest treatment, 53.4% more under F and 108.2% more under VF harvest treatment. As a consequence, selected materials under VF and F harvest treatments yielded 36.8% and 60.5% compared with the same materials under I harvest treatment while controls under VF and F harvest treatments yielded 22.2% and 49.5% of the controls under I harvest treatment. Therefore, selection produced a general increase in DMY under all harvest treatments and was successful in decreasing the negative influence of frequent cutting regimes on DMY. At the present time, it is our hypothesis (to be assessed through research) that the better performance of selected materials could be partially due to an unintentional selection for resistance to bacterial or fungal diseases. Nevertheless, even if this hypothesis needs to be corroborated by experimental results, it helps to explain the higher survival and total DMY of selected materials but does not explain the decrease of DMY differences among harvest treatments in selected, with respect to DMY differences among control materials. Consequently, a growth analysis is needed to understand when, in the growth cycle, the selected materials show a different behaviour.

On the basis of these results, PRS for tolerance to frequent

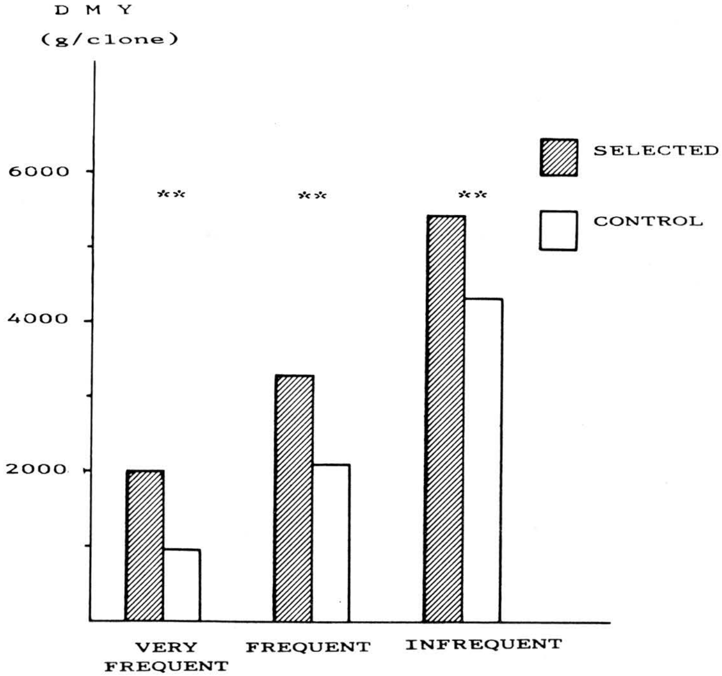

Figure 2.- Total dry matter yield (DMY) of selected and control materials (g/clone) in relation to the 3 different harvest treatments.
** Means within harvest treatment are significantly different at 1% level.

harvest of lucerne appears to be a breeding scheme able to allow both direct responses on persistence and correlated responses of agronomic interest in DMY. Besides, it is interesting to evaluate the possibility of a further decrease in the yield gap between F and I harvest treatments. In Autumn 1986, 10 clones of selected materials, which had maintained at least 12 out of 18 cuttings alive from transplants made in Spring 1982, were transferred in the glasshouse; we are presently in the process of intercrossing them to produce a new lot of seed. Average values of survival percentage and total DMY (g/clone) of these 10 clones are reported in Tab. 2, together with the relative selection differentials (S).

Table 2.- Survival percentages (Autumn 1986) and total dry matter yield (g/clone) of selected materials in relation to the 3 different harvest treatments; x and selection differentials (S = x - x) with a selection pressure of 14 p. 100 (10 clones selected out of 72 clones evaluated) are reported.

Harvest treatment	Survival percentage (Autumn 1986)			Total dry matter yield (g/clone) (1983 + 1984 + 1985)		
	$\bar{x}$	$\bar{x}_S$	S	$\bar{x}$	$\bar{x}_S$	S
Very frequent	52	71	19	2007	2536	529
Frequent	53	87	34	3304	4491	1187
Infrequent	56	83	27	5459	6112	653

Due to the noteworthy S levels for survival percentage (ranging from 19% to 34%) and to the good response to selection for this trait, we expect a further increase in persistence of the selected materials. At the same time, the 10 clones show total DMY selection differentials ranging from S = 1187 g/clone (F harvest treatment) to S = 529 g/clone (VF harvest treatment). Lastly these clones produced, under F harvest treatment, 73.5% of DMY produced under I harvest treatment (4491 vs. 6112 g/clone) while, as already shown, the 72 clones produced, under F harvest treatment, no more than 60.5% of DMY under I harvest treatment.

ACKNOWLEDGMENTS

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RÉSUMÉ

Pour la lucerne (*Medicago sativa* L.), une étude a été faite pour évaluer les effects de deux cycles de sélection phénotypique récurrente sur la tolérance à la coupe fréquente du fourrage, sur la persistance et sur la production de substance sèche. 108 clones (72 pour le matériel sélectionné et 36 pour les contrôles) ont été transplantés sur les champs au printemps 1982. Un schèma expérimental capable de soumettre les 108 clones (18 plants par classe), pendant la période d'essai 1983-85, aux rythmes de coupe suivants a été adopté: très fréquent (20 coupes), frèquent (17 coupes), peu frèquent (13 coupes). Les résultats obtenus ont démontré que deux cycles de sélection phénotypique récurrente augmentent la persistance et la production de substance séche de la lucerne et ceci pour chaque rythme de coupe. De plus, la diminution de la production de substance séche augmentait avec la fréquence de coupe, mais elle était moins remarquable, même si elle était consistante, pour le matériel sélectionné que pour le contrôles.

En général, la sélection phénotypique récurrente pour la résistance à la coupe fréquente semble être une méthode d'amélioration génétique qui permet d'avoir, pour la lucerne, des résponses directes et des résponses en corrélation avec un intérêt agronomique.

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Further results in breeding lucerne for frequent cutting

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Summary

In four fenotypical selection cycles spaced plants were cut in a 21 day cutting period.

According to our results, the withering within the vegetation period was less, and the winter survival was higher in the selected population, than those of the control population.

In every selection cycle the starting material was selected from the plants showing the best regrowth after the 4th, 5th and 6th cuts of the previous cycle. These plants were significantly higher, than the average height of the selected population. However, the relative advantage (expressed by the ratio of the average height of the selected population and the height of the plants with an outstanding regrowth) of the selected plants decreased from cycle to cycle as the average height of the population increased.

The winter-survival, by the end of the 4th selection cycle, of the selected population was significantly higher, than that of the control, which may indicate a positive effect of this type of selection on the persistence. The extent of destruction during the vegetation period was the same or higher (due to the effect of frequent cutting), than the winter destruction.

Introduction

Earlier attempts to producing a lucerne variety tolerant to frequent cutting were primarily aimed at increasing the protein yield of lucerne. However, these endeavours did not lead to spectacular results, therefore the researchers' attention turned to other aspects of tolerance to frequent cutting. It was so that the tolerance to diseases (Bócsa et al. 1976), further the competition and the physiological bases (Rotili 1979, Bócsa et al. 1983), and a possible increase in persistence (Chatterton et al. 1977, Veronesi et al. 1981, 1982, 1986) became the main subjects of investigation. Veronesi et al. (1982)

found significant differences in persistence after a single cycle of phenotypic selection, and after two cycles even the yield could be increased, though it did not compensate for the yield-reducing effect of frequent cutting compared to the control, a treatment with a normal number of cutting. In the present paper the increase in persistence and plant height as well as in the proportion of plants with extremely rapid growth under conditions of frequent cutting is discussed.

Materials and methods

The wide spaced plants were cut 6-7 times a year, every 21 days, always in the 2nd year of four cycles of phenotypic selection. In the end of the 2nd year from the number of plants in spring and of those left by the autumn the effect of frequent cutting, while in the spring of the 3rd year from the proportion of surviving plants persistence were determined. Only after the second cutting in the 3rd year the plants found best in regrowing ability were open pollinated, and their seed formed the initial material of the next cycle. After the 4th, 5th and 6th cutting of each cycle the plants were measured for height instead of weight, and the proportion of those quickest to regrowth compared to the whole selected population was recorded. Two kinds of control were used: for the effect of frequent cutting and for survival in winter the average of 3 known varieties, while for the height of plant and the proportion of rapidly regrowing plants our own selected population.

Results and discussion

The proportion of surviving plants after stressing, was the lowest, i.e. destruction the highest after cycle 1. As a result of a cycle of selection survival after cycle 2. in the selected material was significantly better than in the control. After cycles 3. and 4. the difference between the selected population and the control further increased, the control practically remained at the same level (Table 1.). It should be noted that under the influence of stressing the difference in spring- and autumn plant numbers in cycle 4. was very great between the selected population and the control in favour of the former. It was also found that the extent of destruction during the vegetation period generally was the same as or higher than the winter destruction.

Earlier (Bócsa et al. 1983) we reported a lower than 1 per cent proportion of plants showing extremely rapid regrowing in a population. This proportion increased to 3,48 per cent by the end of cycle 4. (Table 2.). The cytoquinine-synthetizing ability in these very rapidly regrowing and growing plants is essentially higher than in the control. The growth of number of these plants, though apparently little, is significant, which indicates an average increase in the vigour and persistence of the population.

After the 4th, 5th and 6th cutting the height of the rapidly regrowing and growing plants significantly exceeded the height of the control plants on the 21st day in each respective cycle. At the same time, the difference in the rate of growth between the selected population and the control (non-outstanding) plants decreased in the course of the successive cycles, which can be explained by the effect of selection, namely, in the regrowing ability and height of the whole population increased (Table 3.).

As for the increase in persistence we obtained practically the same results as Veronesi et al. (1982) after one and two cycles of selection, respectively, with the difference that we left the dry matter production out of consideration and only measured the plant height, which in spite of the known positive correlation of height (fresh weight does not allow drawing far-reaching and final conclusions). However, since the effect of selection for tolerance to frequent cutting can be seen after three or four cycles only, we thought of setting up the close-space performance experiments only after cycle 4., in the syn-3 generation.

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Table 1.

Destruction in response to frequent cutting and winter stress

Samples	Starting plant number in the 4th cycle	Destruction during the vegetation period (CF effect) % (1)	Destruction during winter % (2)	Total destruction % (1+2)
Breeding material for frequent cutting	6294	14,4	14,2	28,6
Control	378	46,4	16,1	62,5
LSD 5%				17,6

Table 2.

Plant number per cycle and percentage proportion of outstanding plants

Number of cyclus	Per cent of outstanding plants	Number of surviving plants
1 (1976)	o,82	5755
2 (1979)	1,24	2441
3 (1982)	2,26	5374
4 (1985)	3,48	43o4

Table 3.

Height rates of rapidly regrowing plants marked out in the 5th, 6th and 7th growths

Cyclus	5th	6th	7th
	growths before cutting %		
1 (1976)	134	137	147
2 (1979)	143	146	14o
3 (1982)	125	121	125
4 (1985)	126	122	124
$\bar{x}$	132	131	134
LSD 5%	8,8		

Genotype/management interactions for plot dry matter yield in Lolium perenne

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SUMMARY

The extent of genotype/management interactions for annual dry matter yield in Lolium perenne and their prediction from standard single-management plot trials is discussed with special reference to recent trials at Aberystwyth. It is concluded that such interactions can be important but that the performance of genotypes with similar ear emergence dates under a wide range of managements can be predicted to a large extent from data on yield at the reproductive and vegetative stages of growth, on persistency and on nitrogen content of herbage. However, more research on genotype/nitrogen rate interactions and on the possible importance of wear tolerance for performance under grazing is required.

INTRODUCTION

Breeding for improved productivity in perennial ryegrass (Lolium perenne L.) is complicated by the possibility of genotype/management interactions. The main management variables affecting annual dry matter yield in most of Western Europe are: the frequency, timing and height of defoliation, the level of applied nitrogen, the method of harvesting, and the frequency of resowing. Grassland may be cut only three or four times during the year to maximize dry matter yield, or, at the other extreme, grazed continuously. Levels of applied nitrogen vary from 0 to 500 kg/ha/year with only 6% of UK grassland farmers applying more than 400 kg/ha/year. Under grazing however, levels of available nitrogen are higher than the figures on applied nitrogen would suggest due to return both through the animals and through decay of uneaten herbage. At high stocking rates, the benefits of nitrogen recycling can be offset by the detrimental effects on yield of severe defoliation and treading, treading being generally the less important of the two factors (CURL & WILKINS, 1986). Pastures may be ploughed two years after sowing or left indefinitely, with the result that the influence of persistency on yield will vary greatly.

In the long term, improvement of the crop depends on genetic recombination and the accompanying necessity to evaluate large numbers of genotypes or families in a limited time: a difficult enough task using even a single management. It is therefore, important to assess the degree of genotype/management interactions and to understand their origins so that genotype performance under the full range of conditions can be predicted adequately from the results of standard single-treatment trials.

The influence of flowering date

Perennial ryegrasses vary widely in flowering date and this has a profound effect on the optimum timing of both the first conservation cut and any early spring harvest. BERENCLONE (1984) found that the ranking in total annual yield of cultivars with different flowering dates was reversed when they were cut at different dates. This makes data on genotype/management interactions very difficult to interpret in any terms apart from flowering date. One possible solution to this problem is to cut each genotype at a particular morphological stage of growth. During 1986 at the Welsh Plant Breeding Station (WPBS), simulated plots (300 plants/square meter) of a very early-flowering clone originating from the uplands of Northern Italy (Ba 8596), a late-flowering cv. Perma clone, and an F_1 hybrid clone (also early-flowering) were compared in an orthogonal randomized split-plot trial with two levels of applied nitrogen (200 and 600 kg/ha/year) and two cutting treatments (6-cut and 19-cut). In the 6-cut treatment, the first cut was in April and the next at 7 days after 50% ear emergence (23 May, 3 June and 23 June). Differences in mean total annual yield among clones were not significant, but there was a significant clone/cutting frequency interaction (Table 1).

TABLE 1. Mean annual dry matter yield (t/ha) of an extremely early-flowering clone (Ba 8596), a late-flowering clone (Perma) and an F_1 hybrid clone. Numbers in brackets=yield as a percentage of the Perma clone

	Cutting frequency		
Clone	6 cuts	19 cuts	Mean
Ba 8596	11.28 (88)	4.70 (101)	7.99 (91)
Perma	12.86 (100)	4.66 (100)	8.76 (100)
F_1	11.85 (92)	5.31 (114)	8.60 (98)
SED	0.506 (4)	0.127 (3)	NS

Under frequent cutting the F_1 hybrid yielded significatly more than the two parental clones, but under infrequent cutting the Perma clone gave the highest yields. This interaction could be attributed entirely to the very high yield of the Perma clone at the first conservation cut (cut 2 under the 6-cut treatment) which more than compensated for poor yields of vegetative growth in the other cuts and was much greater than the total annual yield of any clone in the 19-cut treatment (Fig. 1). It does not follow that late-flowering ryegrasses necessarily are better for making silage or hay since the higher temperatures prevailing later in the year result in increased fibre content and decreased digestibility at any particular morphological stage of growth.

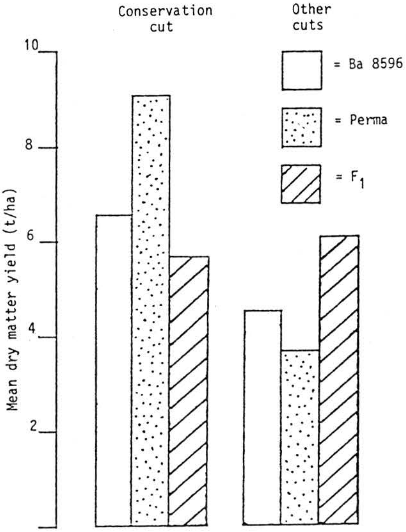

FIG. 1. Dry matter yield under the 6-cut treatment of three clones of perennial ryegrass at cut 2 and over the other 5 cuts. Ba 8596 and F_1 clones are very early-flowering and the Perma clone late-flowering

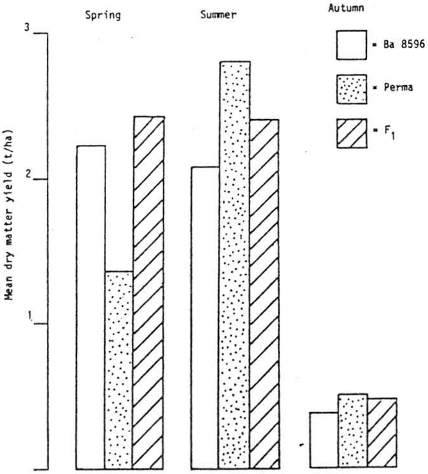

FIG. 2. Seasonal distribution of dry matter yields of three clones of perennial ryegrass under the 19-cut treatment. Ba 8596 and F_1 clones are very early-flowering and the Perma clone late-flowering

Time of flowering also has a marked effect on the seasonal distribution of yield under frequent cutting (Fig. 2). Total annual yields of such contrasting genotypes could be influenced by several factors including the timing of nitrogen applications (early application favouring the early-flowering types and late application the late-flowering types), and water shortage in late summer (favouring the early types). So it is clear that in order to achieve a better understanding of genotype/management interactions it is essential to compare material of similar ear emergence dates.

Genotype/harvesting frequency interactions

The existence of genotype/harvesting frequency interactions in material of similar flowering date is acknowledged implicitly in the use of two cutting frequencies in UK National List Trials. Such interactions have been attributed primarily to growth habit and its effects on light interception (e.g. RHODES 1971). However, the importance of light interception as a factor limiting yield under practical conditions is questionable. In the UK at least, plot yield often increases linearly with increasing nitrogen application up to 800 kg/ha/year (e.g. LAZENBY & ROGERS 1965) - a level far in excess of those used on most farms - showing that nitrogen rather than light is the primary factor limiting annual yield in most situations.

At WPBS, a trial is in progress comparing the performance of four perennial ryegrass varieties with similar ear emergence dates: Ba 10761, S.321, Talbot and the tetraploid variety Tove. Ba 10761 was derived from hybrids between North Italian upland ecotypes and selected clones of cvs Melle Pasture and S.23 (WILKINS 1986). S.321 was bred at WPBS and released in 1964 while Talbot and Tove both are recommended for general use in England and Wales. The trial is orthogonal with two cutting frequencies (infrequent and very frequent), three levels of applied nitrogen (200, 400 and 600 kg/ha/year) and two wear treatments (with and without wear applied with a wear machine following each cut of the 6-cut treatment). In the first harvest year (1986), cultivar/wear and cultivar/nitrogen interactions were not significant. There were highly significant differences between cultivars in mean yield and also a highly significant cultivar/cutting frequency interaction (Table 2).

TABLE 2. Variety/cutting frequency interaction for mean annual dry matter yield (t/ha)

	Cutting frequency 6 cuts	 19 cuts	Mean
S.321	11.61 (102)	4.18 (100)	7.90 (102)
Talbot	11.34 (100)	4.19 (100)	7.77 (100)
Tove	12.27 (108)	4.45 (106)	8.36 (108)
Ba 10761	11.66 (103)	4.91 (117)	8.29 (107)
SED			0.158 (2)

Ba 10761 outyielded Talbot by 17% when harvested frequently but by only 3% when harvested infrequently. So far this year, results have been similar except that S.321 and Tove have performed relatively poorly, which was expected because of their poorer persistency. The cultivar/cutting frequency interaction was attributable entirely to the relatively poor yield of Ba 10761 under both managements during May and June. Growth during this reproductive period accounted for more than half of the total annual yield under the 6-cut management but only about one quarter of the total under the 19-cut management. Ba 10761 was similar to Talbot in leaf:stem ratio at the first conservation cut (cut 2 under the 6-cut treatment). Such genetic independence of vegetative and reproductive growth has been reported in Festuca arundinacea but, unlike the present case, it was associated with differences in the ratio of fertile to vegetative tillers (NELSON, SLEPER & COUTTS 1986). A further generation of family selection for plot yield from within the same gene pool which produced Ba 10761 revealed families with good yields during both vegetative and reproductive phases of growth (Table 3). Thus it may prove possible to breed directly for high yield using a single 6-cut management in a way which will give good performance under both infrequent and frequent harvesting systems.

TABLE 3. Mean plot dry matter yield (t/ha) of two selected half-sib families and three control cultivars over two sites (WPBS and East Craigs, Edinburgh) and two harvest years (1985 and 1986)

Cultivar or family	Conservation cut	Other cuts	Mean
Talbot	6.11 (100)	8.72 (100)	7.42 (100)
Tove (tet)	6.67 (109)	8.33 (95)	7.50 (101)
Fantoom (tet)	6.57 (107)	9.07 (104)	7.82 (105)
Family 13	7.01 (115)	9.60 (110)	8.31 (112)
Family 79	6.86 (112)	9.77 (112)	8.31 (112)
SED			0.206 (3)

Other genotype/management interactions

In the trial with Ba 10761 cultivar/nitrogen level interactions for annual dry matter yield were not significant. Several other trials have produced the same result (e.g. COPEMAN & SWIFT 1966). However, significant interactions have been reported from trials employing N levels outside the 'normal' range. Over a five-year period, cv. Melle Pasture gave similar yields to six other perennial ryegrass varieties at 212 kg/ha of N but significantly higher yields of both dry matter and nitrogen than all the others at 425, 637 and 1062 kg/ha of N (LEE, DAVIES, ARMITAGE & HOOD 1977). ALCOCK & HARVEY (1965) compared the productivity of cvs S.23 and Melle Pasture at four levels of nitrogen (0, 100, 200 and 400 kg/ha/year of N). In this trial, S.23 yielded significantly more dry matter and more nitrogen than did Melle Pasture at 0 and 100 kg/ha/year of applied N but not at 200 or 400 kg/ha/year. The relevance of these low levels of nitrogen applied to cut plots to grazed pastures is a matter for debate but taking the two trials together it seems that genetic differences in response to nitrogen do exist in perennial ryegrass.

Differences in yield of nitrogen could be important under grazing because the higher the proportion of nitrogen taken up by the crop, the greater the amount available for recycling. This is a possible source of genotype/harvesting method interaction. But it is wrong to assume that increased dry matter yield automatically results in increased yield of nitrogen. At some cuts in the Ba 10761 trial, Ba 10761 and cv. Tove had significantly lower nitrogen contents than cvs Talbot and S.321 but since nitrogen analyses are incomplete it is not yet known if there were genetic differences in annual yield of nitrogen. Another possible source of genotype/harvesting method interaction is differences in tolerance of treading by grazing animals. Perennial ryegrass cultivars do differ in their tolerance of severe wear applied with a wear machine, as shown by amenity grass trials (ANON 1986). However, superior performance under grazing of wear-tolerant cultivars compared with less tolerant cultivars of similar yield potential has yet to be demonstrated.

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RÉSUMÉ

Cet article concerne le degré d'interactions entre le génotype et l'exploitation pour le rendement annuel en matière sèche pour Lolium perenne et les prévisions à partir d'essais en parcelles standard à exploitation unique, en se référant plus particulièrement aux récents essais menés à Aberystwyth. En conclusion, on note que de telles interactions peuvent être importantes, mais qu'il est possible dans une large mesure de prévoir la performance des génotypes soumis à différents types d'exploitation avec des dates semblables quant à l'apparition de l'inflorescence. Ces prévisions sont effectuées à partir de données sur le rendement aux stades reproductif et végétatif, sur la persistance et sur le contenu en azote de l'herbage. Il serait cependant nécessaire d'effectuer des recherches sur l'interaction entre le génotype et le taux d'azote et sur l'importance possible de la résistance à l'usure pour une meilleure performance en tant que pâturage.

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Breeding for improved winter hardiness in perennial ryegrass

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SUMMARY

The effects of winter on grasses influences sward yields during the following spring and even well into summer. Increasing winter hardiness through breeding can therefore make a very important contribution to efficient livestock farming.

Winter damage may result from a range of environmental factors including low (freezing) and fluctuating temperatures, low light intensities, dessication, wind, snow and ice cover, frost heaving, pests and disease. The relative importance of these factors depends on local climatic conditions and on sward management. Consequently when breeding grasses for good winter hardiness a broad range of adaptations must be incorporated into varieties. However, some understanding of the physiological and genetical relationships between relevant plant characteristics is essential when using such a multitrait approach.

Well designed breeding trials which incorporate suitable screening techniques provide good estimates of genetic parameters and correlations for relevant traits. These can be used in multivariate analyses to develop further research, identify useful sources of breeding material and aid in the construction of selection indices for improving winter hardiness.

INTRODUCTION

Winter conditions can have a severe and long lasting effect on the growth of grass. Sward damage sustained during the winter, ranging from complete plant death to chronic inhibition of growth as conditions improve in spring, results in reduced herbage yields in spring and even well into summer (MUNRO & DAVIES 1973). In terms of costly resowing or expensive extra feed requirements, the economic effects of winter are very important factors in determining the efficiency and hence the profitability of grassland farming.

Despite its obvious importance, breeding for improved winter hardiness in grasses is generally not well developed. In many breeding programmes, natural survival is used to provide the necessary selection pressures together with visual estimates of plant damage. Even with multi-site trials it is unlikely that local natural selection will, in the long term, produce sufficient adaptation to the range of winter conditions which grass varieties may experience in an expanding world market. However, in order to develop more sophisticated breeding strategies it is necessary to have some understanding of plant attributes which are relevant to improving winter hardiness and their physiological and genetical inter-relationships.

Winter hardiness depends on an ability to tolerate a wide range of environmental stresses including freezing, rapidly fluctuating temperatures, low light intensities, dessication, wind, snow and ice cover and disease. The relative importance of these factors depends on local climatic factors as well as management practices. In northern and eastern parts of Europe, where snow cover exceeds 2-3 months, diseases such as Fusarium nivale (pink snow mould), Typhula incarnata (grey snow mould) and Sclerotinia borealis (snow scald) can cause considerable damage. In parts of central and western Europe, where snow cover is more sporadic, the effects of freezing. wind and dessication become important. The occurrence of occasional frosts and rapidly fluctuating temperatures can be damaging to winter growing grasses in southern Europe while in northwestern maritime areas mild, wet, low light intensity conditions can cause problems. The ability to survive these various stresses often depends on the development of resistance through a process of hardening (TRONSMO 1984).

Hardening is induced by low temperatures (2°C to 5°C) (KACPERSKA-PALACS 1978) and is assisted by high light intensities, short days and a decrease in plant and soil water content. It results in complex changes in plant metabolism (LEVITT 1980) involving plant hormones, water relationships, enzymes, nucleic and amino acids, proteins, sugars, pH, protoplasmic viscocity, phospholipids and fatty acids, cell membranes, pigments and available energy. The physiological state of a plant, particularly in terms of growth activity and accumulation of energy reserves, will affect its hardening response. Therefore, winter hardiness must be considered in the context of overall grass growth during the winter which is also affected by the autumn growth and management of swards (HUNT et al. 1976).

HUMPHREYS AND EAGLES (1987) estimated the freezing tolerance, in terms of LT_{50}, of 86 Lolium perenne (perennial ryegrass) accessions using a glycol bath freezing test on tillers from naturally-hardened field plants. A range of other characters relevant to the winter growth and hardiness of these accessions were also measured and this paper briefly discusses relationships between them.

MATERIALS AND METHODS

Details concerning the origins of the 86 perennial ryegrass accessions studied are given in HUMPHREYS & EAGLES (1987). They represent a wide range of winter conditions from areas such as S.W.England, N.Italy, Belgium, Switzerland, Netherlands, France, Poland and Hungary. The accessions were grown during 1981 and 1982 as spaced plants at 0.6 m spacings in 20 plant plots in each of 2 blocks. Their management and the measurements made on them are listed in Table 1.

TABLE 1. The management of spaced plants of 86 perennial ryegrass accessions and the assessments made

Date	Action
1981	
23 Feb.	Plants sown in heated glasshouse.
14 Apr.	Seedlings cut.
14 May	Plants transferred to field.
22 May	Application of 377 kg/ha 20:10:10 NPK fertiliser.
7 Sept.	Plants cut to 10 cm and fresh weight yield g/plant recorded (SEPT81YLD). Analysis of % nitrogen on dried, ground samples (SEPT81%N).
10 Sept.	Application of 377 kg/ha 20:10:10 NPK fertiliser.
11 Nov.	Crown rust scored on 1 (low infection) - 5 (high infection) scale (NOVDIS).
1982	
19 Jan.	Green (undamaged) leaf scored on a 1 (little) - 5 (lot) scale (JANLF).
2 Feb.- 2 Mar.	Tillers removed for freezing tests (LT_{50}).
4 Mar.	Green (undamaged) leaf scored on a 1 (little) - 5 (lot) scale (MARLF).
18 Mar.	Plants cut to 10 cm and dry weight yield g/plant recorded (MAR82YLD). Analysis of % nitrogen (MAR82%N) and % water soluble carbohydrate (MAR82%WSC) on dried samples.
4 May	Spring growth scored on a 1 little - 5 lot scale (SG).
April-June	Third ear emergence recorded as number of days after 1 April (HDATE).
June-Aug.	Three monthly cuts each followed by an application of 377 kg/ha 20:10:10 NPK fertiliser - final cut 11 August.
12 Oct.	Plants cut to 10 cm and dry weight yield g/plant recorded (OCT82YLD). Analysis of % nitrogen (OCT82%N) and% water soluble carbohydrate (OCT82%WSC) on dried samples.

For 13 of the characters measured (excluding LT_{50} estimates), the presence of significant genetic variation between populations was tested by a two-way analysis of variance. LT_{50}s were estimated and tested using probit analysis. Analyses of covariance were carried out on all possible pairs of characters and genetic correlations were calculated using appropriate components of variance and covariance. Principal components analysis was carried out on the genetic correlations.

RESULTS

There was significant genetic variation between populations for all 14 characters measured. The genetic correlations between characters are shown in Table 2.

TABLE 2. Genetic correlations between 14 characters measured in 1981 and 1982 (character abbreviations as described in Table 1). Levels of significance (84 df); 0.2-5% P; 0.3-0.1% P.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	SEPT81YLD	1.0													
2	SEPT81%N	-0.4	1.0												
3	NOVDIS	-0.9	0.2	1.0											
4	JANLF	0.4	0.3	-0.8	1.0										
5	LT_{50}	-0.1	-0.4	0.2	-0.4	1.0									
6	MARLF	-0.1	0.2	0.0	0.7	-0.3	1.0								
7	MAR82YLD	0.9	0.0	-0.9	1.0	-0.4	0.6	1.0							
8	MAR82%N	-0.4	0.3	0.5	-0.4	0.3	0.0	-0.3	1.0						
9	MAR82%WSC	-0.9	0.5	0.7	-0.2	-0.1	0.3	-0.5	0.0	1.0					
10	SG	0.4	-0.4	-0.2	0.1	0.2	0.3	-0.4	0.5	0.0	1.0				
11	HDATE	0.5	0.4	-0.6	0.7	-0.5	0.2	0.6	-0.5	0.0	-0.4	1.0			
12	OCT82YLD	1.0	-0.3	-0.8	0.6	0.0	0.2	1.0	-0.1	-0.8	0.3	0.4	1.0		
13	OCT82%N	-0.5	0.0	0.8	0.6	0.5	-0.3	-0.5	-0.4	0.4	0.6	-0.7	-0.5	1.0	
14	OCT82%WSC	0.4	-0.1	-0.4	0.6	-0.3	0.3	0.6	0.6	0.0	-0.1	0.7	0.6	-0.7	1.0

Table 2 shows that:

a) JANLF was strongly negatively correlated with NOVDIS, and strongly positively correlated with MAR82YLD, OCT82YLD and HDATE. Thus, high January greenness was associated with active autumn and winter growth generally shown by later heading populations. The correlation of JANLF with LT_{50} was negative but relatively low (-0.4).

b) Corresponding correlations with MARLF were mainly lower. In particular the correlation with NOVDIS was reduced to 0. However, the correlation with SG was increased and that with MAR82%WSC changed from negative to positive. It appears that in March, greenness was dependent on spring growth factors as well as winter effects. Again the correlation of MARLF with LT_{50} was negative but low (-0.3).

c) Crown rust infection (NOVDIS) was highly negatively correlated with SEPT81YLD, OCT82YLD and HDATE, but highly positively correlated with MAR82%WSC. Thus those populations which showed most crown rust infection were early heading, they showed little autumn/winter growth but had high water soluble carbohydrate contents in March. It follows that MAR82%WSC showed strong negative correlations with SEPT81YLD, MAR82YLD and OCT82YLD i.e. populations with high water soluble contents at the end of winter showed little autumn/winter growth.

d) In general OCT82%N and OCT82%WSC were correlated in opposite directions with other characters, but the relationships with MAR82%N and MAR82%WSC were much more variable.

e) Relationships between freezing tolerance (LT_{50}) and other characters were complex. Nearly all had a significant effect in some way. In an attempt to elucidate these patterns of correlation further, a principal components analysis was carried out (LAWLEY & MAXWELL 1971). The results are given in Table 3.

TABLE 3. Loadings for the first three principal components derived from genetic correlations between 14 characters measured in 1981 and 1982

	1	2	3
SEPT81YLD	0.29	-0.40	-0.11
SEPT81%N	0.04	0.35	0.02
NOVDIS	-0.34	0.28	0.08
JANLF	0.34	0.07	0.36
LT_{50}	-0.30	-0.51	0.13
MARLF	0.12	0.17	0.64
MAR82YLD	0.29	-0.12	0.31
MAR82%N	-0.17	-0.02	0.30
MAR82%WSC	-0.12	0.42	0.13
SG	-0.01	-0.12	0.28
HDATE	0.48	0.22	-0.29
OCT82YLD	0.22	-0.29	0.19
OCT82%N	-0.28	-0.04	0.17
OCT82%WSC	0.31	0.09	0.07
% Total variance	49	21	10

Principal components represent uncorrelated variates derived from the original correlated variables. The relationship between them is indicated by the size and sign of the loadings of the original variables on the derived components. Interpretation of component loadings requires considerable caution although they can prove helpful in attempts to identify broad patterns of character relationships. For example, the first component shown in Table 3, which accounted for nearly half of the variance over all 14 characters measured, could be identified in terms of general winter growth factors. HDATE, JANLF AND MAR82YLD loaded heavily in a positive direction while NOVDIS loaded heavily in a negative direction. This suggests that good winter growth was associated with late heading and low autumn disease infection. The second component, which accounted for 21% of the total variance, appeared to describe general winterhardiness. Freezing tolerance (LT_{50}) loaded heavily on this component as did MAR82%WSC. SEPT81YLD and OCT82YLD also loaded quite heavily on it but in the opposite direction to MAR82%WSC. Thus good freezing tolerance appeared to be associated with low autumn growth and high water soluble carbohydrate content in March. Loadings for both JANLF and MARLF were low which suggests that leaf damage was not a good indicator of hardiness. The third component was related to aspects of spring growth such as early heading date and green leaf in March. Together these first 3 principal components accounted for 80% of the total variance in the original data. A scatter diagram of the 86 accessions in terms of their scores on the first principal component plotted against their scores on the second principal component is shown in Figure 1.

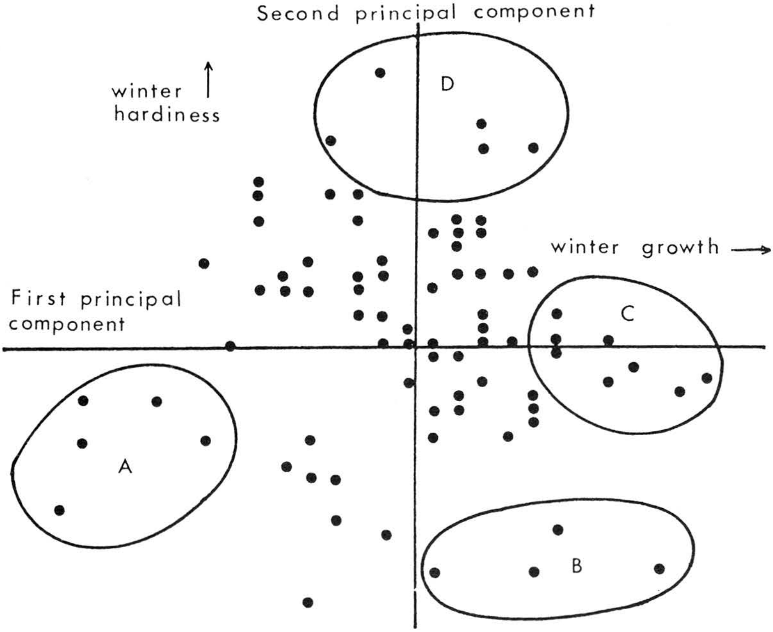

FIG. 1. A scatter diagram based on first and second principal component scores for 86 perennial ryegrass accessions

Four groups of accessions which showed contrasting responses to autumn and winter conditions are ringed in Figure 1. Group A consisted of accessions with little autumn/winter growth and poor winter hardiness. They were very early heading, susceptible to autumn crown rust infection and originated mainly from N.Italy. Group B had good autumn/winter growth but very poor winter hardiness. It comprised accessions from New Zealand and selections from the old winter growing cultivar Aberystwyth Teilo which originated from material collected in Normandy. Group C showed good winter growth and average winter hardiness. It comprised late heading cultivars from Belgium and the Netherlands such as Melle, Chieftain and Perma. Group D had average winter growth but excellent winter hardiness. As well as accessions from Hungary and the Pays de Herve in

Belgium, it included some amenity cultivars from the Netherlands (Majestic and Sprinter).

DISCUSSION

Unlike previous work on winter-hardiness related characters (THOMPSON 1974), in the present paper genetic correlations were used for multivariate analysis rather than phenotypic correlations. Because measurement errors as well as other environmental effects are largely removed in this approach, clearer relationships are obtained. When based on genetic correlations, the first three principle components accounted for 80% of the total variance; while they accounted for only 62% of the total variance when derived from phenotypic correlations. Genetic correlations also provide more relevant information concerning decisions on breeding strategy in order to maximise response to selection, for example, in a selection index approach.

The results described here agreed with those of THOMPSON (1974) in showing that visual estimates of winter burn were not good indicators of winter hardiness. In some populations leaf damage due to autumn disease infection certainly reduced winter hardiness. However, in other populations a natural decline in autumn growth, with a consequent build up of senescent leaves, resulted in good winter hardiness. On the other hand freezing tests indicated that actively growing winter green populations could be severely damaged by late winter/early spring frosts.

The identification of a winter hardiness component which was independent of a winter growth component is of interest. It suggests potential for combining both traits by breeding. Thus winter hardy cultivars, if the climate allowed, would produce useful growth at low temperatures in late autumn and in early spring. The amount of water soluble carbohydrate maintained through the winter appears critical. Factors which reduce carbohydrate reserves such as autumn disease or disease under snow cover, forced autumn growth through high nitrogen application, late cutting, or uncompensated respiratory loss through growth at low light intensities, can all seriously reduce winter hardiness. If carbohydrate levels can be maintained during periods of winter growth a decline in hardiness may not occur.

It is through a combination of field and laboratory measurements including assessment of freezing tolerance, analysis of carbohydrate content and determination of potential for growth at low temperatures that progress in breeding for improved winter hardiness is likely to occur. Principal component scores are a crude method of indexing populations. As more information becomes available on genetic and physiological relationships between characters and their role in winter survival, it should prove possible to define character weights more realistically in a biological sense and proceed further with an index approach to selection at the population, family and single plant level.

RÉSUMÉ

Les rigueurs de l'hiver sur les herbes ont des répercussions en ce qui concerne les rendements des prairies gazonnées au cours du printemps suivant et même jusque dans l'été. Une résistance accrue aux rigueurs de l'hiver par sélection, peut contribuer de façon importante à accroitre l'efficacité de l'élevage.

L'effet de l'hiver peut être dû à divers facteurs dans l'environnement: températures basses (gel) et variables, basses intensités de lumière, déssèchement, vent, couverture de neige ou de glace, effets du gel sur le sol, parasites et maladies. L'importance relative de ces facteurs dépend des conditions climatiques locales et de l'exploitation de la prairie gazonnée. En conséquence, il est nécessaire d'incorporer dans les variéties un vaste choix d'adaptations si l'on veut sélectionner des herbes résistant bien aux rigueurs de l'hiver. Cependant, il est essentiel, quand on utilise une approche à traits multiples, de comprendre les rapports physiologiques et génétiques entre les caractéristiques des plantes concernées.

Des essais de sélection bien concus, faisant appel à des techniques appropriées de dépistage, permettent d'obtenir de bonnes estimations quant aux paramètres génétiques et aux corrélations pour les traits intéressants. Ceux-ci peuvent être utilisés dans des analyses à variance multiple en vue de recherches plus poussées, en vue de l'identification de sources utiles de matériel de sélection, et en vue de la construction d'indices de sélection pour améliorer la résistance aux rigueurs de l'hiver.

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BREEDING HERBAGE SPECIES FOR NORTHERN SCANDINAVIA

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SUMMARY

In the northern regions of Scandinavia agriculture is to a large extent based on herbage production, both from long-term pastures and leys. Herbage species bred for more southerly regions have proved to be poorly adapted to the severe climatic conditions in northern Scandinavia, and the breeding of locally adapted varieties with high yield potential is an important factor in improving grassland productivity in these areas.

This paper outlines a joint Scandinavian breeding project set up in 1981 to develop herbage varieties with the ability to give a high and stable production throughout the region, and describes some preliminary results from the more than 80 field trials set up under the project, involving some 60 varieties or populations of Festuca pratensis, F. rubra, Phleum pratense, Poa pratensis and Trifolium pratense.

INTRODUCTION

Grassland plays a dominant role in the agriculture of northern Scandinavia, and livestock farming is the most profitable farming enterprise in this region. However, both climate and management place heavy demands on the plant material grown in this region. Winters are long, often with severe frost and prolonged snow cover. Summers are short, but with long days, and consequently plant growth during this period is very intensive.

The problems confronting herbage production in these northerly regions are to a large extent of the same nature. Apart from a short and variable growing season, winter damage is the most common cause of grass failure in these regions, even though different causal factors can be involved, such as ice encasement, snow mould fungi, etc. Herbage varieties bred for more southerly regions have proved to be poorly adapted to these harsh conditions and, hence, a breeding effort based on

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[*] Presented on behalf of the NORDGRASS project group: B. Dennis, K.J. Frandsen (Denmark); K. Egede (Greenland); S. Ravantti, O. Nissinen (Finland); Á. Helgadóttir, T. Tómasson (coordinator) (Iceland); A. Larsen, I. Schjelderup (Norway); E. Lindvall, A. Wiberg (Sweden).

locally adapted material of high yield potential is themost efficient means of improving grassland productivity in these areas.

Breeding locally adapted herbage varieties has for many years received high priority in the northern Scandinavian countries. However, the seed market in each country is small and the individual breeding projects are relatively expensive. As a consequence a joint breeding project was started in 1981, under the auspices of the Nordic Council of Ministers, with the aim of developing herbage varieties adapted to Northern Scandinavia (MANNER 1983). This paper gives an outline of the project, its present status and future work, and briefly describes some of the preliminary results.

CLIMATE AND SPECIES

Plant breeders from the following research and experimental stations are currently taking part in the project:

Denmark	The Royal Veterinary & Agricultural University, The Experimental Station Højbakkegård, Taastrup
Finland	The Agricultural Research Centre, The Experimental Station for Lappland, Apukka
Greenland	The Experimental Station in Upernaviarssuk
Iceland	The Agricultural Research Institute, Keldnaholt
Norway	The State Experimental Stations, Vågønes and Holt
Sweden	Svalöf AB, The Northern Branch, Röbäcksdalen

The geographic location of experimental stations and trial sites are shown in Figure 1. With the exception of Højbakkegård in Denmark, all locations lie north of the 63°N latitude, and the northernmost station, Holt in Norway is at 69° N.

The climatic conditions at the main centres are listed in Table 1. The photoperiod varies to some extent from station to station, particularly in the autumn and winter. HEIDE (1985) has reviewed a number of physiological aspects of adaptation to high-latitude environments, and has emphasized the importance of photoperiodic effects for plant productivity in the northern areas.

Precipitation is considerably greater in northern Norway than in Finland and Sweden, especially in winter, whereas the precipitation in Iceland lies somewhere in between these two extremes. Similarly, the temperature during winter is lower in Finland and Sweden than in Norway and especially in Iceland. In general, the summers are shorter but warmer in Norway, Sweden and Finland than in Iceland. The different conditions during winter at the research stations lead to different causes for winter kill. In Finland and Sweden the ground remains frozen throughout winter and snow cover is prolonged. Snow mould fungi, therefore, often cause damage to grasses. In Norway and Iceland, on the other hand, where winters are relatively mild and wet, repeated freeze-thaw cycles often lead to ice cover that can remain up to three months causing extensive winter kill. Where the ground remains without a snow cover for lengthy periods severe frost damage can occur.

The main breeding objectives in herbage species are dry matter productivity, yield stability, persistency and nutritive value, but different emphasis is placed on these criteria in the northern regions than would be the case in more southerly environments.

Dry matter productivity per se is not the major breeding objective in the northern areas, where high light intensities can give extremely high biomass production rates (SIMONSEN 1985). Under these conditions yield stability and persistency are of much greater significance, and the most important breeding objective in the Nordic countries is winter survival capacity. LARSEN & ÅRSVOLL (1984) have reviewed the biotic and abiotic factors responsible for winter damage in the Nordic region, and concluded that the shorter the growth period and the more severe the winter, the more important is the use of well-adapted, resistant varieties, coupled with management forms enabling the plants to store sufficient reserves for winter survival.

Grasses form the predominant part of animal feeds in the northern regions and nutritive value is obviously of importance. High light intensities and low temperatures, however, tend to improve digestibility (DEINUM 1985), and consequently somewhat less emphasis can be placed on this factor than in more southerly areas.

Of the herbage species cultivated in these areas, timothy (Phleum pratense) is by far the most important, although meadow grass (Poa pratensis) and red fescue (Festuca rubra) are also grown throughout the whole area. Red clover (Trifolium pratense) is commonly grown in mixtures with timothy and meadow fescue (Festuca pratense) in northern Sweden, Finland and Norway, but not in Iceland, Greenland and the Faeroes, where the non-adapted plant material previously grown has generally shown insufficient winter hardiness.

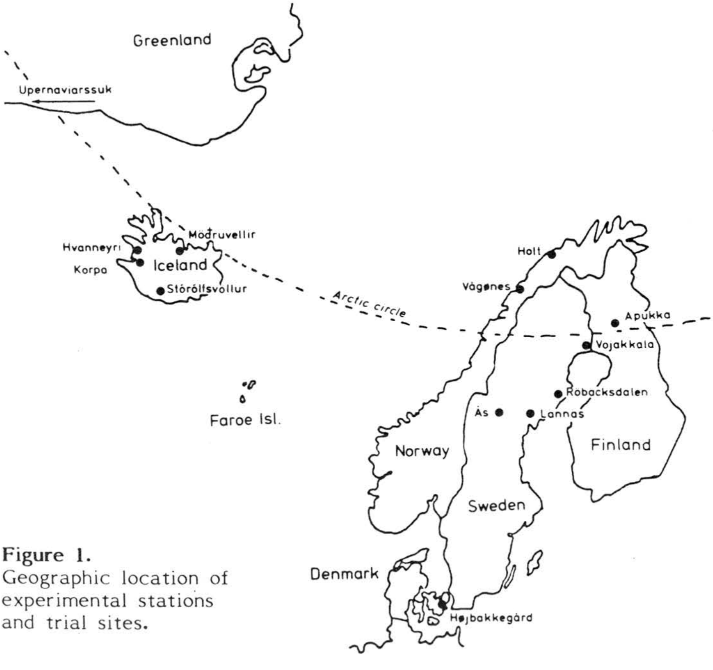

Figure 1.
Geographic location of experimental stations and trial sites.

Table 1. Climatic conditions at the research stations.

	FINLAND	ICELAND	NORWAY		SWEDEN	DENMARK
	Apukka	Korpa	Vågønes	Holt	Röbäcksdalen	Højbakkegård
Latitude	66°35'	64°09'	67°17'	69°39'	63°49'	55°44'
Photoperiod, h						
June	23.8	21.0	24.0	24.0	20.5	17.4
December	2.6	4.3	1.3	0.0	4.3	7.1
Precipitation, mm						
May-Sept	287	258	410	365	279	265
Oct-April	229	593	632	629	322	332
Total	512	851	1042	994	601	597
Temperature, °C						
May-Sept	10.6	9.0	10.4	8.7	11.8	14.0
Oct-April	-6.8	1.5	0.5	-1.3	-3.1	3.0

THE NORDGRASS BREEDING PROJECT

During the initial stages the project concentrated on cooperative Nordic trials in which both early and more advanced breeding material was tested at all the experimental stations. In this way it was possible to study the response of different species to the contrasting climatic conditions at the various locations. As the project developed emphasis was placed on evaluating material at an earlier stage in the breeding process in order to obtain varieties of greater stability for the northern regions. To meet this objective a cooperative breeding programme was initiated in timothy and genotypes from all the participant stations were pooled in a joint polycross.

In addition to these two main projects, seed production characteristics of northerly varieties have been studied at Højbakkegård in Denmark and genecological studies are being initiated in connection with the breeding programme in timothy.

Cooperative trials

The joint variety trials began in 1982 and have included local populations and advanced breeding materials as well as early breeding material for further selection, e.g. after topcross, polycross or pair crosses. The trials with the advanced materials have, in general, been sown at all the participating stations, often in more than one location and year, whereas the testing of the early material has been more restricted. Trials have been carried out with timothy (*Phleum pratense*), meadow grass (*Poa pratensis*), meadow fescue (*Festuca pratense*), red fescue (*Festuca rubra*), cocksfoot (*Dactylis glomerata*) and red clover (*Trifolium pratense*). In all 88 separate experiments with a total of 4480 plots have been sown (Table 2), but not all of these plots have been harvested as a result of poor winter survival. As a rule each experiment is harvested for three years. Dry matter yields in two cuts are determined and the plots are scored for various characters, such as type and degree of winter damage, botanical composition, disease attack, etc. In addition, quality factors, such as digestibility, protein and fibre content, are determined.

Table 2. Summary of the herbage variety trials sown in the Nordgrass project.

Species	No. of varieties	No. of experiments	No. of plots
Phleum pratense	10-21	23	1139
Poa pratensis	12-15	19	872
Festuca pratensis	9-21	12	487
Festuca rubra	12-14	15	850
Trifolium pratense	10-12	17	728
Dactylis glomerata	48	2	384
Total		88	4460

As a supplement to these trials the potentiality of other herbage species is also being assessed. Trials are currently in progress with Berings hairgrass (Deschampsia beringensis), a species which in terms of winter survival, yield and quality has shown promise under Icelandic conditions (TOMASSON 1984).

The statistical analyses of data collected from the main variety trials are as yet far from complete. For the present purpose we have selected a number of trials with timothy, meadow grass and meadow fescue over two harvest years, and standard analyses of variance have been carried out for total dry matter yields (Table 3).

The results for timothy show that the varieties differed significantly in yield, but there were no significant interactions between varieties and locations or varieties and years; varieties generally showed the same ranking order in all environments, despite varying growing conditions. For meadow grass and meadow fescue, on the other hand, there were significant interactions between varieties and locations and between varieties, locations and years. This differential response of varieties to conditions at the various locations during the two years is not totally unexpected in meadow grass, which is an apomict and therefore does not possess the same buffering capacity as an outbreeding species, such as timothy. Meadow fescue, however, although an outbreeder, did not show the same degree of plasticity as timothy. It is possible that meadow fescue is not as suited to these areas as timothy, as demonstrated by the fact that the former species is unable to survive the harsh conditions that prevail for example in Iceland, and is therefore more sensitive to extreme conditions at the test sites.

The Nordgrass timothy programme

The varieties that have been included in the cooperative yield trials have been bred in northern regions where climatic conditions are harsh but still vary from location to location. The statistical analyses from the trials with timothy demonstrate, however, that in contrast to the other two species studied, the relative performance of the varieties is not affected by the growing conditions at each location. In other words, the genotype x environment interaction is very small for this species. With this in mind the project group decided to set up a joint breeding programme in timothy based on pooled germplasm from all the participating research stations.

Table 3. Analysis of variance for total dry matter yields in variety trials with timothy, meadow grass and meadow fescue.

Phleum pratense	DF	MS
Locations (L)	5	413.4731***
Varieties (V)	8	1.3049***
L x V	40	0.3600
Blocks within locations (B)	15	1.5090***
Error	113	0.4472
L x Years	5	132.3747***
V x Years	8	0.5941
L x V x Years	40	0.4324
B x Years	15	1.3189***
Error	113	0.2430
Poa pratensis		
Locations (L)	4	153.2879***
Varieties (V)	11	2.7303
L x V	44	1.6863***
Blocks within locations (B)	12	1.7972***
Error	132	0.2513
L x Years	4	95.0000***
V x Years	11	1.0874
L x V x Years	44	0.5361***
B x Years	12	1.2717***
Error	132	0.1636
Festuca pratense		
Locations (L)	3	241.2433***
Varieties (V)	9	3.1439**
L x V	26	0.9638***
Blocks within locations (B)	11	1.9893***
Error	96	0.4160
L x Years	3	94.4150***
V x Years	9	0.8550
L x V x Years	26	0.6050***
B x Years	11	1.1231***
Error	96	0.2965

The aim of the breeding programme is, firstly, to develop varieties of timothy that possess a broad adaptation to a range of Nordic climates and managements, and thus can be grown throughout the northernmost part of Scandinavia. Secondly, the programme is expected to provide information on ecological and physiological factors that are important for adaptation to the growing conditions that prevail in the northern areas. Thirdly, the development of a joint breeding programme of this type with timothy as a model is expected to lead to similar Nordic breeding programmes in other species.

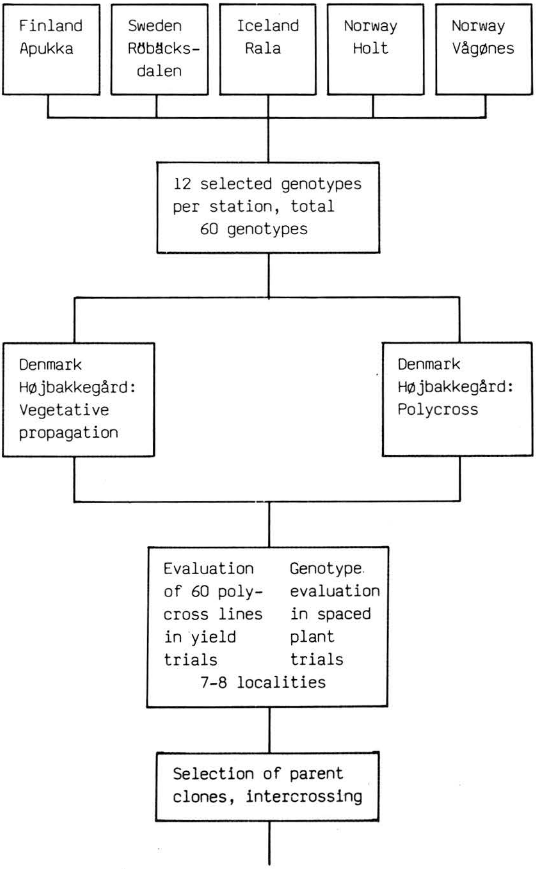

Figure 2. The joint breeding programme in timothy.

The breeding programme is outlined in Figure 2. Each participating research station originally contributed 12 genotypes of timothy giving a total of 60 genotypes. These genotypes were planted out for evaluation in a spaced plant trial at all the experimental stations in the spring of 1985. At the same time a polycross was set up at Højbakkegård in Denmark. Seed was obtained already in autumn 1986 and the half-sib families were sown out at the research stations the following spring. On the basis of results obtained from these field trials, parent clones will be selected and intercrossed to form synthetic populations. After comparable testing of the synthetic populations a new variety will hopefully emerge.

SEED PRODUCTION ASPECTS

The Danish climate is far more favorable for grass seed production than in the more northerly areas of Scandinavia. For this reason the joint timothy polycross and studies of seed production characteristics in timothy, cocksfoot, red fescue, meadow fescue and meadow grass have been located at Højbakkegård. Additionally, a number of Icelandic populations of red fescue, meadow grass and red clover are under multiplication, and several red fescue and meadow grass populations collected in south Greenland are currently being studied.

Despite the different origins of the populations studied, only limited differences have been found in earliness (date of ear emergence) within species. OEEC trials with perennial ryegrass, cocksfoot and meadow grass in 1955-57 showed that differences in earliness became more pronounced the more southerly the comparisons were made (EUROPEAN PRODUCTIVITY AGENCY 1960). A similar tendency has not been found amongst the selected timothy genotypes, a factor of importance in connection with the joint polycross described earlier.

In seed production trials at Højbakkegård, northern varieties of timothy have given high seed yields and have in several cases outyielded the adapted Danish control variety, particularly in the first harvest year, as shown below:

Seed yields, kg/ha:

		Northern varieties	Danish control
Timothy	1st year	848-1206	606
	2nd year	618- 899	768
Meadow grass	1st year	3- 258	199
	2nd year	401- 898	526

Acceptable seed yields have also been obtained from red fescue and meadow fescue. Northern varieties of meadow grass, however, do not generally give reasonable seed yields until the second harvest year under Danish conditions.

Seed production under climatic conditions differing from those to which the plant material is adapted can result in changes in earliness and productivity. Photoperiod and temperature are amongst the major factors determining reproductive growth, and differential responses of genotypes to changes in environmental conditions can result in changes in the genetic structure of the population. Experiments have recently been started to determine the extent of genetic shift in a composite timothy population as a result of seed production under Danish conditions.

CONCLUSION

To achieve a high degree of adaptation and hence a stable production under varying climates and managements it is essential that breeding material is tested under a wide range of environmental conditions. The Scandinavian experimental stations participating in the Nordgrass project cover a range of environments varying from maritime to continental and from 63°N to 69°N, which gives a valuable interaction between temperature and photoperiod. These different environments can only with difficulty be obtained within the individual countries, and the Nordgrass project has contributed significantly to a wider and more efficient testing of plant material from the participating countries.

In its later phases the project has formed the basis for a joint breeding programme with timothy as a model species. The programme aims at developing timothy varieties with the broadest possible stability within northern Scandinavia, and will subsequently be adapted for use with other herbage species.

The cooperation achieved through the Nordgrass project has made possible a joint evaluation of some of the many mutual problems facing herbage breeders in these northernmost regions in Scandinavia. An example of this is a recently started genecological study which aims at investigating the effects of different climates, managements and seed production conditions on the genetic structure, adaptability and stability of northern timothy genotypes.

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LUCERNE BREEDING FOR ADAPTATION TO COOL SUMMER ZONE

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SUMMARY

Selection for seed setting in lucerne grown for 3-4 weeks in unfovourable conditions (6-14°C)in the field and climatized chambers was carred out. Honey bees and hand pollination were employed for pollination. The trials were set in April and October when the temperature did not exceed 14°C otherwise phytothrone chambers were used. Pod and seed setting were evaluated. The clones which set pods in 90 per cent of the flowers produced were selected from "Varminska" (PL) x "Du Puits" derivates.

GCA of seed yield character was evaluated for 47 selected clones using five top-cross testers. In addition plant vigour was recorded.The field trial was performed for 3 years.

The best performing nine clones were used to produce a new synthetic population RAH 183. Syn 2-RAH 183 produced seed yield surplussing the standards by 15-25 per cent. The seed set was doubled compared to initial variety grown under 14°C temperature. The great physiological variability of lucerne enables selection of the strains with lower temperature requirements for seed production.

Lucerne produces 10-16 t of dry matter in three to four cuts per year in Poland. But this crop is not entirely adapted to the Polish climate because the periods with unfavourable conditions for seed setting often occur at the summer time. JABŁONSKI (1977) stated that the best conditions for seed setting were when air temperature was 25-35°C and relative humidity 50-60%. In the field trials seed yield decreased by 50% at the temperature 18°C as compared to that 20°C.In Poland temperature of 16°C is the limiting factor causing that seed production of grown varieties reaches only 10-20% of mean yield that is usually obtained in optimal growth conditions. Melitta leporina Panz., Eucera longicornis L., Megachile Latr., Rophitoides canus Ev., Taeniandrena Hed., and Bombus Latr. are the main pollinators of lucerne in Poland (DYLEWSKA et.al. 1970). Most of them are inactive at temperature below 18°C(JABŁONSKI 1973). Blondon et al. (1979) and STASZEWSKI et al. (1979) also found strong effect of temperature on lucerne fertility.

The aim of this work was the selection of the clones for obtaining a synthetic population which would be less susceptible to cool and rainy periods of Polish summer.

MATERIAL AND METHODS

The initial material consisted of 112 families of F4 progeny which were improved with respect to Verticillium wilt resistance and set setting. They were selected from the stock resulted from cross cultivars Varminska x Du Puits. Varminska was bred in Olsztyn region which is characterized by severe and cool winters.The study was completed in the following steps:

- Evaluation of the families in field trials and selection of 247 clones from the best performing ones.
- Selection of the clones with 90% seed setting grown in the temperature below 14°C. Crossing the clones with 5 testers to obtain F1 progeny.
- Evaluation of GCA of selected clones with respect to yields and observations of plant vigor in the field trials.
- Seed multiplication of the synthetics RAH 183 consisting of 9 clones which displayed highest GCA. Evaluation of RAH 183 yield in field trials and seed setting in the greenhouse and phytothrone under low temperature.

RESULTS

The weather conditions at Radzikow are unfavourable for lucerne production (Table 1).

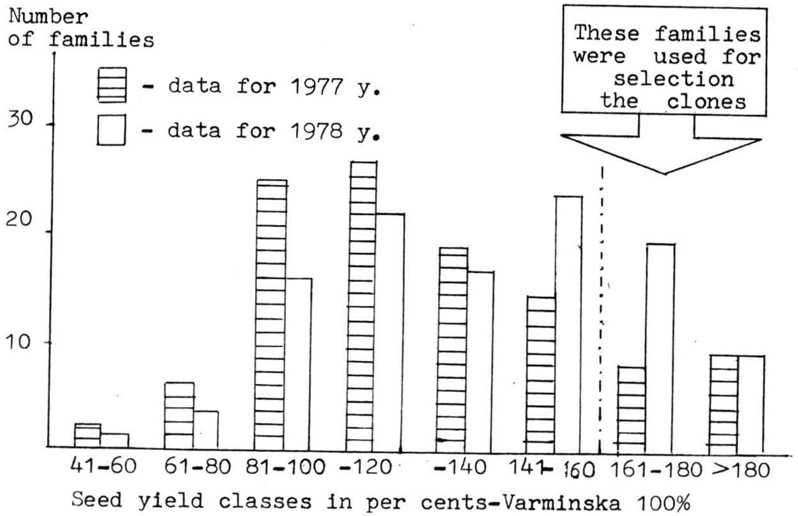
Table 1. Mean temperature and rainfalls at Radzikow

Month	Mean day-temperature in months							
	Years							
	1951 -1980	1977	1978	1981	1982	1983	1984	1985
April	7.5	5.3	6.3	5.6	5.4	9.6	8.8	8.1
June	17.0	11.0	15.5	16.9	15.6	17.2	14.4	14.9
July	18.1	16.0	16.0	17.4	18.7	19.5	15.6	17.5
August	17.4	16.2	15.9	16.4	18.7	18.1	18.1	17.9
October	8.4	12.7	8.6	8.8	8.8	8.7	10.5	8.1
	The sum of rainfalls (mm)							
April	34	41.9	40.4	11.2	22.4	44.8	13.2	36.3
June	64	39.4	48.1	105.4	91.4	23.0	54.9	64.7
July	76	94.4	75.0	133.8	12.8	28.9	81.4	74.5
August	58	203.1	114.0	34.2	45.6	42.9	5.5	78.9
October	39	40.5	34.4	57.0	20.5	22.3	11.0	13.7

Mean temperature in the summer is about 6-10°C lower then in France. Frequent rainfalls and low temperatures occuring in July and August have negative effect on insect pollination, seed setting and also cause flower falling. However these conditions are conducive for selection of plants superior with regard to seed setting.

In 1976 field trials for evaluation of seed yields of 116 families were set. Seed yields were evaluated in 1977 and 1978, which were rainy and cool. Twenty two families proved to have good seed yields (Fig.1). Single plants exibiting excellent seed setting and upright and strong stems were selected from those 22 families. Each selected plant was cloned and planted in three pots: two plants per 6 kg pot.

Fig. 1. The distribution of seed yields of families



The next step of selection was performed with respect to seed setting and was carried out concurrently in pot and field conditions in the following manner.The investigated clones were grown in the greenhouse under optimal conditions until October '78 (first growth) and April '79 (second growth) when they reached flowering stage. Then the plants were transferred for 4 weeks to the place near bee hives in the orchard. Honey bees collecting pollen pollinated plants intensively.Air temperature was as low as 8.6-6.5°C,then the plants were moved to the greenhouse again for six weeks to produce seeds. Seed setting on the same clones were scored two times (Table 2).

These trial enabled to select 47 clones that showed excellent seed setting. Subsequenty selected clones were used for crossing in order to obtain F1 progeny. The crossing were performed in the greenhouse. Each clone was hand pollinated by 5 testers to obtain 400 seeds for each top-cross combination.

Table 2. Pod setting on selected clones under low temperature.

Variant	Total number of clones	Number of clones in the groups					
		Per cent of flowers which set pods					
		<20	21-40	41-60	61-80	81-90	> 90
I test-October	'78(8.6°C):						
Clones	247	20	39	67	63	21	47
Varminska-cv.	40	25	14	1			
II test-April	'79(6.5°C):						
Clones	247	28	33	60	55	20	51
Varminska-cv.	40	29	6	2	3		

Selected for top-cross

The field experiment for GCA evaluation were set in spring 1980. Two months old seedlings (140) were planted in each plot (10 m sq.) in two replications. The best nine clones were intercrossed in the greenhouse in the winter '81 to obtain Syn-1. The seeds of Syn-2 were reproduced in the field in 1982 under the name RAH 183.

Table 3. GCA of selected clones

Number of clone	Mean seed yield g/10 m^2	GCA effects	Plant vigour in % of the standard
127/1	591.0	209.6	111.8
304	533.4	152.0	102.8
126/1	521.2	139.8	104.2
328	517.8	136.4	117.8
399	501.0	107.1	102.0
423	494.8	113.4	98.7
518	493.0	113.0	101.3
327/2	435.2	96.2	97.5
337	422.4	71.0	94.5
Vertibenda	173.8		100.0
Europe	212.0		106.6
Verminska	265.0		100.0
Diff. at alpha =0.05	67.52	67.52	

The greenhouse phytothrone and field experiments were carred out to compare new population with the standard varieties. The plants for greenhouse and phytothrone experiments were grown under optimal conditions in pots until they reached full flowering stage. The groups consisted of 20 plants represented: RAH 183 Syn-1, Syn-2 and Varminska cv. In each group, 50 flowers of each plant were pollinated with the mixture of pollen. Immediately after pollinations pots with plants were located in phytothrone (Variant 1) and greenhouse chamber (Variant 2). The plants during seed setting period were grown for 3 weeks in the following conditions (December '86):

	Phytothrone:	Greenhouse:
mean temp.	14°(constant)	$14^{\circ}(9.5^{\circ}$night, 18.5°day)
air humidity rel.	75 %	70 %
light intensity 16 klux	18 h	18 h

Table 4. Pod and seed setting of RAH 183 Syn-1 and Syn-2

Object	No of plants	Number of clones in the groups					Pod setting per 100 flowers	Seed number per pod
		Per cent of flowers which set pods						
		>20	21-40	41-60	61-80	91>		
Variant 1 Phytothrone								
Varminska cv.	20	7	13				30	0.7
RAH183-Syn.1	20	6	6	3	6		61	1.4
RAH183-Syn.2	20	2	8	4	4	2	53	1.2
Variant 2 Greenhouze								
Varminska	20	1	2	10	7		56.3	2.9
RAH183-Syn.1	20			5	6	9	77.0	3.2
RAH183-Syn.2	20		2	3	8	7	77.4	3.4

Subsequently plants were moved to the greenhouse where were maintained for 6 weeks in fovourable conditions for pod development. These results are given in table 4. Both Syn-1 and Syn-2 plants displayed better then initial variety Varminska pod and seed setting. The constant 14° temperature proved more unfovonrable then variable $14^{\circ}(9.5-18.5^{\circ}C)$.

Table 5. Seed and dry matter yields of RAH 183 and standards

Object	**Seed yields**					Dry matter yield 9 cuts (3 years)
	Years					
	1983	1984	1985	1986	Mean	
RAH 183	5.57	2.83	0.98	2.20	2.90	112.1
Kleszczewska	3.52	1.79	0.31	1.70	1.83	109.0
Varminska	3.61	2.00	0.55	1.74	1.72	108.0
Kometa	2.34	1.31	0.23	1.15	1.26	100.8
Europe	3.77	2.56	0.67	1.42	2.11	107.4
Vertibenda	2.49	1.34	0.48	1.06	1.34	
Diff. at alpha = 0.05	1.25	1.30	0.32	1.05		8.34

The field trials aimed at evaluation of seed production were carried out employing $20m^2$ plots in 5 replications according to complete block design. Seeds were sown in rows 0.5 m apart (150 seeds/m sq. and 0.2 m for forage (400 seeds/m sq.). In the several trials RAH 183 produced seed yields by 15 - 25% higer then standard variety. Dry matter yield of the new synthetics proved equal to that of the standard cultivars (Table 5).

The great physiological variability of lucerne enables selection of the strains with lower temperature requirements for seed production.

L´AMÈLIORATION DE LUZERNE ADAPTÉE POUR LA RÉGION DE LA TEMPERATURE BAISSÉE

SOMMAIRE

La sélection pour la formation des sémences a été ménée en mettant les plants qui étaient dans la période de la floraison et de la création des sémences dans des conditions infavorablé dans les 4 semaines. La pollination a été fait á main ou en utilisant les abeilles. La température de 14° a été assuré en déhors au mois d´avril et au mois d´octobre et dans le phytotrone. La formation des semences et des gousses a été bien analysé. Les clones qui avaient formé les sémences dans 90% de fleurs ont été selectionés de la $4^{\text{ème}}$ génération "Varminska" (PL) x "Du Puits".

On a fixé GCA pour 47 clones en utilisant 5 testeurs de la méthode top-cross. On a analysé le rendement de sémences et la matiere sechè dans la période de 3 ans.

On a crée une nouvelle population synthétique RAH 183 de 9 meilleurs clones. Syn 2 RAH 183 a donné le rendement de sémences qui étaient supérieures qui les variétés de controle de 15-25% ainsi qu´il a formé de sémences dans les conditions de 14° deux fois meilleurs que la varieté de base.

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Breeding for adaptation of perennial grasses to binary mixtures with lucerne (Medicago sativa L.).

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Seventy-eight cultivars and experimental populations of four genera [cocksfoot, Dactylis glomerata L.; smooth bromegrass, Bromus inermis Leyss.; ryegrass, Lolium perenne L. and L. hybridum; and Festulolium] were evaluated for persistence and competitive ability in binary mixtures with lucerne. Entries were seeded in 3-m single row plots, spaced 0.9 m apart, at Arlington (Wisconsin, U.S.A.), and overseeded with 'Saranac' lucerne (Medicago sativa L.) in April 1983. A second experiment consisted of 114 grass/legume mixtures: 19 grass cultivars in factorial combination with six legume cultivars [two each of lucerne, red clover (Trifolium pratense L.), and birdsfoot trefoil (Lotus corniculatus L.). All plots were managed for 3 years following the seeding year by taking three cuttings at late bud to early flower of lucerne before 1 September.

Maturity, vigor prior to cutting, recovery after cutting, and ground cover differed significantly for entries within each genus, except for maturity of smooth bromegrass. Vigor, recovery, and ground cover of cocksfoot entries tended to decrease with later maturity. Selection for increased persistence of smooth bromegrass was successful, and due mainly to increased vigor and faster recovery. Differences among ryegrass entries was related mainly to species and ploidy. The intermediate ryegrass and Festulolium entries had the greatest vigor and recovery; the Festulolium entry had the greatest ground cover.

Three-year mean forage mixture yields did not differ among grass entries for any of the three legume species. Ryegrass cultivars differed in percentage ground cover after 3 years of growth in mixture with legumes; this variation was not closely related to ground cover of the legume companion. Sufficient variation appears to exist in ryegrass to breed for greater persistence in binary mixture with legumes.

Grass/legume forage mixtures are important to animal agriculture. They offer several potential advantages over pure grasses or pure legumes, such as erosion and weed control and prolonged stand longevity (DROLSOM and SMITH, 1976). On the negative side, differences in growth habit, regrowth potential, and physiological growth requirements among components of an interspecific mixture often preclude the effective use of management practices to maintain components in the mixture. Even if both components can be maintained in a binary mixture, it may be difficult to maintain each component at a specified level (SMITH et al., 1986). This becomes an even more difficult proposition when management of the mixture is based on factors related to only one component of the mixture (SMITH, 1968).

Cocksfoot (Dactylis glomerata L.) is more competitive in binary mixtures with lucerne than smooth bromegrass, Bromus inermis Leyss., (SMITH et al., 1973) and perennial ryegrass, Lolium perenne L., (JUNG et al., 1982; SOLLENBERGER et al., 1984a and 1984b). Excessive competitiveness with lucerne is partially responsible for a decreased popularity of cocksfoot in some regions of the U.S.A. Poor persistence of smooth bromegrass (DROLSOM and SMITH, 1976) and perennial ryegrass (CASLER, 1987, unpublished data) in mixture with lucerne have made this trait an objective of breeding programs on these two species. Genetic variation for persistence with lucerne is known to exist in smooth bromegrass (DROLSOM and SMITH, 1976), while cultivars were known to vary in persistence under the old two-cutting system (CHURCHILL, 1947; WILSIE, 1949). Cultivar variation exists for perennial ryegrass competitiveness with white clover, Trifolium repens L., (EVANS et al., 1985), but is not documented for lucerne.

The objective of this research was to evaluate a broad range of cultivars and experimental populations from four perennial grass genera for performance differences in binary mixtures with lucerne. Cocksfoot was chosen to determine if entries with less competitive effects than those used in published studies can be identified and targeted for mixtures with lucerne. Smooth bromegrass was chosen to evaluate the recent populations developed from genetic selection for increased persistence with lucerne. Ryegrass (L. perenne L. and L. hybridum) and Festulolium were chosen because of the recent interest in their use as forage crops in temperate North America and because of greater potential nutritional value than cocksfoot (JUNG et al., 1982).

MATERIALS AND METHODS

Seventy-eight cultivars and experimental populations (40 cocksfoot, 20 smooth bromegrass, and 18 ryegrass) were seeded in April 1983 at Arlington, WI. Cocksfoot and ryegrass entries varied in adaptation to Wisconsin environments. Cocksfoot entries were separated into early, medium, and late maturity classes. Smooth bromegrass entries were grouped based on their selection history for improved persistence with lucerne (selection vs. no selection). Ryegrass entries were grouped according to a) maturity: medium vs. early, and b) ploidy and species.

Plots consisted of a single 3-m row, with rows spaced 0.9 m apart in six randomized complete blocks. Species were whole plots and entries within species were sub-plots in a split-plot restriction on randomization. Seeding rates for each 3.0-m row approximated the amount of pure live seeds expected in a 0.3 x 3.0-m broadcast area, with seeding rates of 13.4 kg ha^{-1} for smooth bromegrass and ryegrass and 8.1 kg ha^{-1} for cocksfoot. Rows were spaced 0.9 m apart for convenience and to maintain genetic integrity of each row. Immediately after seeding, the entire experimental area was broadcast with 16.8 kg ha^{-1} of 'Saranac' lucerne.

Plots were clipped twice in the establishment year and three times (late May, mid-July, and late August) before 1 September, usually when lucerne was in the late bud to early flower stage, in each of the 3 following years. Fall fertilization with P and K in each year was according to soil test recommendations for lucerne.

Vigor prior to each cutting was determined as the height of grass tillers minus the height of the lucerne canopy, recovery after each cutting was determined as plant height 2 weeks after cutting, and ground cover was visually rated in fall and expressed as a percentage of ground cover after seedling emergence.

A second experiment including 15 of the above ryegrass entries, 'Baylor' smooth bromegrass, 'Hallmark' cocksfoot, 'Palaton' reed canarygrass (Phalaris arundinacea L.), and 'KY31' tall fescue (Festuca arundinacea Schreb.) was seeded adjacent to the first study. Grass entries were drilled in five rows, 15 cm apart, in 0.9 x 4.6-m plots. Plots were overseeded with six legume cultivars [two each of lucerne, red clover (Trifolium pratense L.), and birdsfoot trefoil (Lotus corniculatus L.)]. Binary mixtures were created with the six legume cultivars arranged in factorial combination with the 19 grass cultivars. The 19 grass cultivars were also seeded in two randomized complete blocks without a legume companion and fertilized with 56 kg N ha^{-1}.

Forage yield was measured on three cuttings in each of the 3 years following establishment. Percentage ground covered by the grass and legume components was rated in fall 1986, the end of the third production year.

RESULTS AND DISCUSSION

Entries within species differed significantly for all traits except maturity for smooth bromegrass (Table 1).

Previous studies have also reported a lack of significant differences in maturity among smooth bromegrass entries (eg. REICH and CASLER, 1985). Entry x year interactions were significant for vigor, recovery, and ground cover of all species and for maturity of cocksfoot and ryegrass. Entry x year interactions were mainly due to changes in ranking within the categories listed in Table 1. The relative performance of most traits in most categories was similar for each year.

Cocksfoot

This species was the most diverse of the three included in the study (Table 1). Maturity of individual entries at first cutting ranged from early boot to anthesis, averaged over 3 years. Some entries were so poor in vigor that as little as 1-2 cm of growth appeared above the lucerne canopy

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Table 1. Three-year mean performance of perennial grass cultivars and experimental populations, evaluated in mixture with lucerne. Entries are grouped into meaningful categories for purposes of data reduction and generalization.

Species and category	#	Vigor†	Recovery†	Maturity†	Ground cover
		------ cm	-------		%
Cocksfoot					
Early	18	37a	17a	6.0a	127a
Medium	16	23b	15b	3.9b	140a
Late	6	16b	11c	2.4c	94b
Sm. brome					
Selected	8	24i	9i	4.0i	97i
Non sel.	12	17j	6j	4.0i	69j
Ryegrass					
Early	7	11m	13m	3.0m	55m
Medium	11	6n	12m	1.6n	50m
2xLp‡	7	4t	10t	1.9s	41u
4xLp‡	9	7t	13s	2.3r	55tu
Lh‡	1	30r	18r	2.6r	57st
FL‡	1	16s	16r	2.2s	88r

† Vigor = sum over three cuttings, recovery = mean over three cuttings, maturity: 1 = vegetative, 3 = late boot, 5 = full elongation, 7 = anthesis.

‡ 2xLp = diploid *Lolium perenne* L., 4xLp = tetraploid *L. perenne* L., Lh = *L. hybridum*, and FL = Festulolium.

at each cutting; other individual entries grew up to 40 cm above the lucerne canopy at first cutting and 25 cm at later cuttings. Vigor and recovery were highly correlated with maturity differences, early maturity leading to greater vigor at cutting and faster recovery after cutting, the opposite for late maturity.

Early and late maturing entries averaged 9 and 33% lower ground cover than medium maturing entries, respectively. Late entries were poor mainly due to their poor regrowth potential and subsequent shading by lucerne. Early entries may have been poorer than medium entries because of a greater susceptibility to stand loss due to cutting at a more advanced reproductive growth stage (SMITH et al., 1986). Data to explain this observation in a more definitive manner were not collected during this experiment.

Ten cocksfoot entries had less ground cover at the end of the experiment than at the beginning. Eight of these entries typically have forage yields less than 80% of the highest yielding cultivar at Arlington (CASLER, 1981-87, unpublished data). Each of these eight entries were bred outside the North Central U.S.A. These observations may indicate agronomic potential for low-yielding or relatively unadapted cultivars in binary mixtures with lucerne.

Smooth bromegrass

The eight populations previously selected for increased persistence with lucerne averaged 7 cm greater vigor over all cuttings, 3 cm greater recovery after cutting, and 28 percentage units greater ground cover than the 12 unselected populations. Apparently selection for increased persistence with lucerne was successful, and was at least partially due to improved vigor and regrowth potential of the selected populations.

Ryegrass

Some ryegrass entries were not visible prior to any cutting in any year. The cultivars with the greatest vigor were similar to the most vigorous cocksfoot entries in second and third cuttings. The most vigorous cultivar at second cutting was 'Bison', which attempted to reproduce during the second growth period each year. High and low cultivars for recovery after each cutting had values similar to those for cocksfoot. Most cultivars were still vegetative at the time of first cutting, with the earliest maturing cultivar just emerging from the boot stage.

Medium maturing cultivars were slightly less vigorous prior to cutting than early cultivars (Table 1). There were no other significant differences between early and medium cultivars in this study. The cultivars representing the

Festuloliums ('Tandem') and the intermediate ryegrasses (Bison) tended to have the greatest vigor prior to cutting and the greatest recovery after cutting. Diploid perennial ryegrasses tended to have slightly poorer recovery than tetraploid ryegrasses. In general, these differences were closely related to ground cover ratings. Tandem Festulolium had the best ground cover (88%). Bison intermediate ryegrass was similar to the average tetraploid perennial ryegrass, and ranked higher in ground cover (57%) than the seven diploid perennial ryegrasses.

Three-year mean forage yields of grass/legume mixtures did not differ significantly (Table 2). Ryegrass/legume mixtures were similar in forage yield to those of the other perennial grasses. Differences in ploidy, species, or inherent yielding ability in pure stands did not influence ryegrass/legume mixture yields. This was similar to results of CASLER and DROLSOM (1984) for smooth bromegrass, tall fescue, and cocksfoot. Pure legume means were near the median value for grass/legume mixtures of each legume species.

Large differences were observed for percentage grass ground cover of the grass/legume mixtures (Table 2). Cocksfoot and tall fescue competed well against each legume, while smooth bromegrass was a poor competitor. Ryegrass

Table 2. Forage yields (3-year mean) and percentage grass ground cover after 3 years for binary mixtures of grasses with three legume species (lucerne = Ms, red clover = Tp, and birdsfoot trefoil = Lc).

	Mixture yield			Grass ground cover		
Grass entry	Ms	Tp	Lc	Ms	Tp	Lc
	Mg dry matter ha^{-1}			----- % ------		
<u>Ryegrass</u>						
High cultivar	16.8	10.8	11.0	15	49	45
Low cultivar	15.8	9.6	9.5	5	24	14
Sm. bromegrass	15.6	9.7	10.3	1	11	5
Cocksfoot	16.0	9.8	9.4	64	81	81
Reed canarygrass	16.6	9.6	10.7	13	24	64
Tall fescue	16.8	10.8	11.2	43	70	91
No grass	16.1	10.2	10.2	2	0	5
LSD (0.05)	ns	ns	ns	8	17	21

cultivars did not compete well against lucerne, but several competed well against red clover and birdsfoot trefoil. There was only a slight negative relationship between ryegrass and legume ground cover in binary mixtures (r = -0.36 for lucerne, r = -0.49 for red clover, and r = -0.25 for birdsfoot trefoil, all nonsignificant). Sufficient variability appears to exist among cultivars to provide a basis for selection for improved persistence in binary mixtures with legumes.

RÉSUMÉ

Soixante dix-huit variétés et populations expérimentales de quatre genres de graminées (dactyle: *Dactylis glomerata* L.; brome: *Bromus inermis* Leyss; ray-grass: *Lolium perenne* L. et *L. hybridum*; et *Festulolium*) ont été étudiés pour leur pérennité et leur aptitude à la compétition en association avec la luzerne (*Medicago sativa* L.). Chacune des variétés et populations a été semée en une ligne de 3 m (avec 0,9 m entre chaque ligne), à Arlington (Wisconsin, Etats-Unis) en avril 1983. La luzerne variété Saranac, a été semée à la volée par-dessus les semis de graminées. Une deuxième expérience a consisté à réaliser 114 associations binaires: 19 variétés de graminées en combinaison factorielle avec 6 variétés de légumineuses (deux de chacune des espèces suivantes: luzerne, *Trifolium pratense* L.; et *Lotus corniculatus* L.). Toutes les parcelles ont été cultivées pendant trois ans aprés l'année du semis, avec trois coupes annuelles réalisées entre le stade fin bourgeonnement et le stade début floraison de la luzerne.

Le stade phénologique, la vigueur avant les coupes, le redémarrage aprés coupe, et la couverture du sol diffèrent significativement pour les différents génotypes au sein d'un même genre, excepté pour le stade phénologique du brome. La vigueur, le redémarrage, et la couverture du sol des différents génotypes de dactyle ont tendance à décroître avec la tardivité de la culture. La sélection pour une pérennité accrue du brome a été efficace; elle est due essentiellement à une augmentation de la vigueur et de la vitesse de redémarrage. Les différences entre les génotypes de ray-grass sont dues principalement à l'espèce et au niveau de ploïdie. Les génotypes de ray-grass hybride et de *Festulolium* présentent les meilleures vigeur et reprise, et le *Festulolium* possède la couverture du sol la plus importante.

La moyenne sur trois ans du rendement fourrager des associations ne varie pas selon les graminées pour chacune des trois espèces de légumineuses. Les variétés de ray-grass diffèrent par le pourcentage de couverture du sol après trois années de culture en association avec des légumineuses. Cette diffèrence n'est pas liée avec la couverture du sol de la légumineuse associée. Une

variabilité suffisante semble exister dans le ray-grass pour permettre une sélection pour une meilleure pérennité en association avec une légumineuse.

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A selection strategy for persistency-testing of grasses on hydroponics.

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ABSTRACT

The present study discusses a nutrient-flow system (hydroponics) as a utility for plant breeding, specially related to breeding for persistency.

The regrowth of shoots and roots after frequent cutting was studied.The cutting of the roots hardly influenced the growth of shoots.The heritabilities of the cumulative harvested dry matter were 0.77 (shoots) and 0.40 (roots). Comparison with field experiments showed a significant correlation between the root regrowth on hydroponics and the ratings for persistency in the field.

Nitrogen limitation caused a decrease of the nitrogen content of the leaves but the variation coefficient was only 3.2%. The correlation between yields under optimal and limiting nitrogen supplies was 0.86.

Drought stress can be simulated by addition of Poly-Ethylene-Glycol to the nutrient solution. The advantage of these treatment above drought experiments in soil is the better defined control of water potential in the rooting zone. This is illustrated by an experiment with 6 grass species.

INTRODUCTION

Persistency of grasses is the result of various morphological and physiological processes. Selection for persistency in field trials is often impeded by the complex interference of many individual processes with environmental factors. In addition it is very difficult to study the functioning of the roots in field trials or pot experiments. The use of a nutrient flow system, widely used in physiological research, provides the possibility to quantify both root and shoot responses to environmental variations. Numerous applications of nutrient solutions have been reported in literature both as hydroponic systems (STEINER, 1983) and with solid substrates (SMITH et al.,1983). For plant breeding, however, this technique is hardly used as a practical selection environment.Nevertheless the flexibility of the hydroponic system provides a challenge for a combination of breeding methods and physiological research.

MATERIAL AND METHODS

The growing system.

A diagram of part of the experimental growing system is shown in Fig. 1. The nutrient solution is supplied from four reservoirs with a capacity of 0.2 m^3 each. Four series of 15 containers with a capacity of 0.035 m^3 are connected in parallel with each reservoir. The solution flow through each container is 2 dm^2 min^{-1}. The inlet is connected with a perforated frame mounted at the bottom of the container to establish a homogeneous flow past the roots.The flow through each individual container is maintained at a constant and equal rate by the introduction of glass capillaries with a resistance 25 times higher than the resistance in the remaining circuit. Seedlings are growing on tubes (d =2.8 cm; l=5 cm) filled with rockwool, pierced through the covers of the containers.The lower tube edges are in contact with a flowing nutrient solution. The solution is drained from a hole at the top of the backside of the container and recirculated to the main reservoirs. To compensate for water losses due to transpiration and evaporation, the level in the reservoirs is maintained automatically within a narrow range, governed by a simple electrode system that switches a valve to let tap water in, below a minimum level. The pH in the main reservoirs is measured each minute in a bypass cuvette and deviations from the setpoint are automatically readjusted by small peristaltic pumps injecting acid into the reservoirs. The added amount is registered by a data-acquisition system connected with a personal computer. In a similar way anorganic nitrate is added. The addition rate is controlled on line by a computer system and as such it can be made dependent on the measured light intensity or on other desired control-algorithms.

Irradiance is measured at the west and east side of the greenhouse just below the greenhouse cover and photosynthetic active radiation is measured at plant height by quantum flux meters integrating over a length of 1.2 m. To improve the light distribution in the greenhouse reflecting aluminium foil is attached to the side walls. In addition each container is surrounded by a reflecting aluminium screen adjustable to the height of the simulated swards. This appears a satisfying solution to improve the light distribution within individual subplots.

The temperature distribution in the greenhouse and in the nutrient solutions are recorded by Pt-100 sensors.

Plant material

Seeds of 22 populations of timothy (Phleum pratense), selected from field experiments and two varieties were sown in small pots. After germination they were transplanted to the hydroponic system. The planting scheme was designed as a split plot, with 60 subplots each containing all 24 entries, randomized within each subplot. The 24 plants in each subplot occupied an area of 0.145 m^2. The main plot was divided in five blocks. Leaves were harvested at three weeks time intervals. Besides the leaves, roots were harvested from half of the 60 subplots, randomized in each block. Dry matter of leaves and shoot was determined after oven drying at 80 °C. The total amount of nitrogen was determined according to method 2.058 and NO_3 according to method 7.041. The latter was slightly modified adopting a continuous flow technique. The numbers refer to the Official Methods of Analysis of the Association of Official Chemists, 13th edition, 1980, Washington D.C.

RESULTS AND DISCUSSION

Growth analyses

The pattern of shoot regrowth after different harvests is summarized in Fig 2. The growth rates of leaves on hydroponics appears to be rather indifferent for dissecting the roots, indicating that for plants growing on nutrient solution, the major part of the roots in fact is superfluous. Similar results were obtained for ryegrass by KLEINENDORST AND BROUWER, 1969. This however, does not implicate that the observed rate of root regrowth would be irrelevant to the achievements of genotypes in the field. In general, plants showed different growth strategies for soil culture and hydroponics (Table 1).

Table 1 Relative growth rate of shoots

Harvest nr	Soil	Hydro + roots	Hydro - roots
1	0.050	0.130	0.132
2	0.097	0.141	0.129
3	0.132	0.172	0.176
4	0.224	0.221	0.215
5	0.231	0.172	0.163
6	0.251	0.215	0.202

Plants on hydroponics settled very fast in comparison with plants on solid substrate.According to the classical growth analyses, the RGR in the exponential growth stage is the resultant of the net assimilation rate and the leaf area ratio (LAR). The LAR is mainly dependent on the unit leaf area that can be made from a unit structural dry matter, reflected by the SLA. The SLA was consistantly higher for plants grown on hydroponics (44 m2/kg) than for plants grown in soil (37 m2/kg). This resulted in a proportional effect on the RGR. The advantage of a high SLA is mainly due to the acceleration of the rate of canopy closure. A similar tendency was found for the dry matter percentage. Leaves grown on hydroponics contained more water than those grown in soil.

The RGR preceding the fifth harvest was considerably higher for the soil than for hydroponics, despite the high SLA in the latter. The period preceeding the fifth harvest was five weeks instead of three weeks and the average height of the swards was more than 60 cm. The big harvest apparently caused a delayed regrowth on the hydroponics but did not so in soil. It has been well established that the interaction between environmental conditions and the storage of non-structural carbohydrates is a dominant factor for regrowth of grasses after cutting. To overcome the temporary shortage of assimilates, enough storage should be available for a fast expansion of new leaves to restore photosynthesis. Under sub-optimal growth conditions (soil), carbohydrates are accumulating and thus, teleologically, enlarging the opportunity to survive. The optimal conditions in a nutrient solution tend to an optimistic investment of assimilates in growth, which consequently leads to a relatively strong depression after cutting.

Nitrogen limitation

The hydroponic system provides an excellent opportunity to study genetical variation of nitrogen utilisation. The nitrogen supply can be directed by "on-line" calculations of the momentaneous demands. Accordingly, the supply can be limited to realize a controlled deficit. This is illustrated by Fig.3, which shows the shoot production of the genotypes relative to the population mean for the fifth cutting (optimal nitrogen supply) and the sixth cutting (limited nitrogen supply). In the latter case nitrogen was supplied every 30 minutes based on calculations to reach a nitrogen content of 1.5% in the leaves. The average nitrogen concentration realized under these conditions was 1.93% (control) and 1.76% (plants with dissected roots). The measured concentration under optimal supply was 5.75% and 5.5% respectively. These preliminary results tend to the conclusion that nitrogen limitation provokes essentially different responses for different genotypes. Whereas some genotypes tend to maintain a constant value, others are more or less sensitive to nitrogen deficiency. Nevertheless, the correlation between both harvests was high: r=0.86 (treated) and 0.9 (control), which indicated that plants which perform well under limiting nitrogen supply also tend to produce well under optimal nitrogen supply. There was no significant correlation between the organic nitrogen content and shoot production. The coefficient of variation for the organic nitrogen content actually was low: 3.2% (limited supply) and 1.9% (optimal supply). This shows that several feedback mechanisms are operative. In pot plants the coefficient of variation was 7%, which can be attributed to heterogeneous conditions in the soil.

In conclusion it seems possible to select genotypes that are efficient nitrogen consumers in a sense that they produce relatively well under limited nitrogen supply, though caution should be taken to base selection procedures on the organic nitrogen contents.

Drought tolerance

Drought tolerance is an essential component of persistency. In a growing season (representative for dutch conditions), 30% of the production might be lost due to drought, illustrated by Fig. 4a, which shows the results of a simulation model (Schapendonk and Spitters in prep.) based on the reduction of CO_2 assimilation due to a reduced soil-water potential.

Selection procedures for drought resistence are difficult because numerous physiological processes are involved and because drought conditions in the soil are very hard to control in terms of quantified water potentials. Furthermore differences in transpiration rates cause unwanted variations of the water potential in the root zone. Fig.4b depicts the transpiration of different grass species, measured on nutrient solutions. From the data,it is evident that Phleum pratense would exhaust an imaginary soil much faster than Lolium perenne or Festuca ovina. The consequent differences of the water potential in the rooting zone will lead to different stress conditions. To avoid variations of the water potentials in the rooting zone, Poly-ethylene-glycol can act as a substance that simulates drought in a nutrient solution by lowering the water potential. This assures a constant, reproducible stress condition. Fig. 4c shows the relative water content of the leaves after 8 days on nutrient solution with two concentrations of PEG. The stress condition was essentially equal for all species,which greatly facilitates the interpretation of the results. For further details see SCHAPENDONK (1987).

Correlation with field experiments

The ultimate goal of the present study is a selection strategy, based on individual processes. Simulation modeling should support the synthesis of different components into a production model, that is capable of predicting the effect of individual genetical improvements. Nevertheless it seems worthwhile to consider the dry matter production as a whole and to look for correlations between the production in the field and on the hydroponic system. Fig.5a shows the production of individual plants of Phleum pratense , planted in a sward of Lolium perenne. There were 54 families and two commercial varieties in four replicates. Each replicate consisted of 20 plants. There appears a considerable variation for the observed production and the difference even exceeded a factor 2 between the best producing family and two commercial varieties (solid black squares). In is therefore possible to obtain much better progenies by appropriate selection. Table 2 summarizes the coefficients of variation, the heritability (h2) and the standard error (se) in the field experiment and the hydroponic system.

Fig.5b shows the correlation between the ratings for persistency in the field (autumn 1986) and the regrowth of roots on hydroponics. There was a significant correlation coefficient of 0.44. Similar calculations did not yield significant correlations for shoot productions.The results of the field experiments at the end of this year (second year of production), should be awaited before we can draw any firm conclusions.

Table 2 Coefficients of variation (mean :g DM/plant)

	field	shoot hydro	root
se	0.694	0.265	0.047
CVe'	0.228	0.473	0.712
CVe(mean)	0.114	0.086	0.130
CVg	0.222	0.158	0.107
CVp	0.249	0.180	0.168
h2	0.790	0.770	0.402
mean	6.082	3.062	0.363

se;standard deviation of means CVe';coefficient of variation of individual plants (hydroponics) or individual plots with 20 plants (field). CVe(mean);coefficient of variation between families. CVg;coefficient of genetic variation between families. CVp; coefficient of phenotypical variation h2;heritability.

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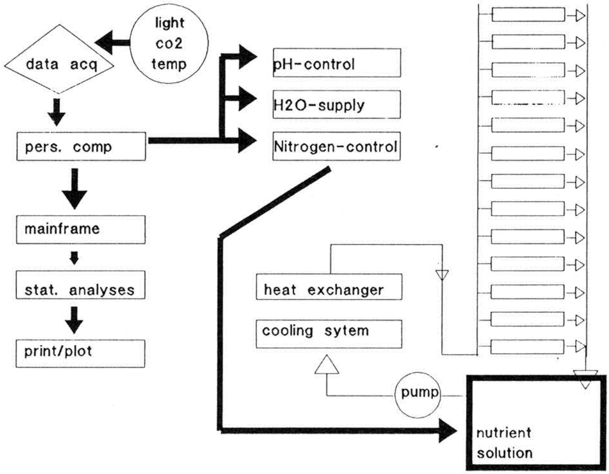

Fig.1. A diagram of the experimental setup.

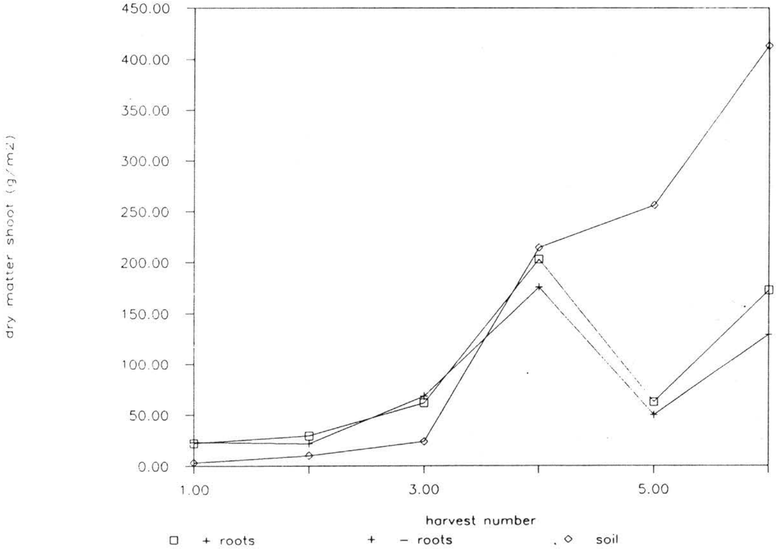

Fig.2 Dry matter of shoots at the successive harvests of plants grown in soil (diamonds), in nutrient solution with intact roots (open squares) and in nutrient solution when roots were removed at each harvest (crosses).

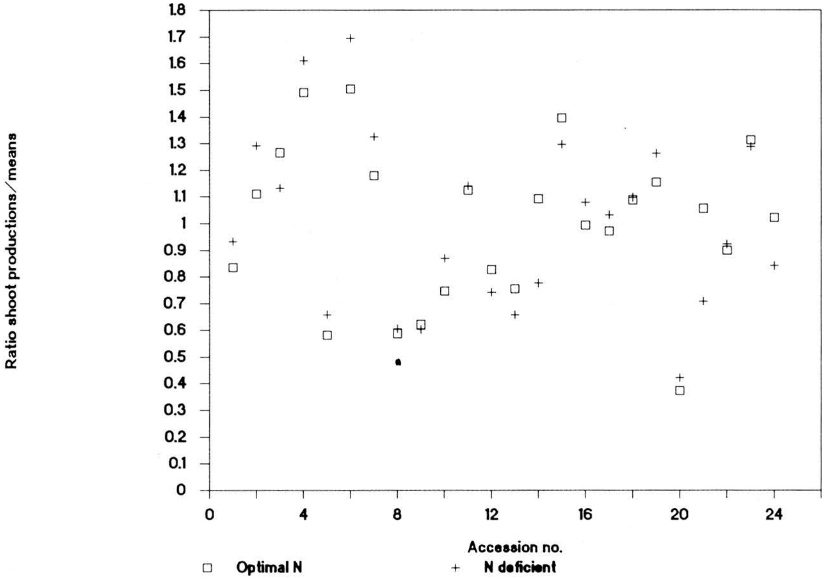

Fig.3 Shoot dry matter production of selected populations of Timothy , relative to the mean shoot dry weight, under optimal and suboptimal nitrogen supply.

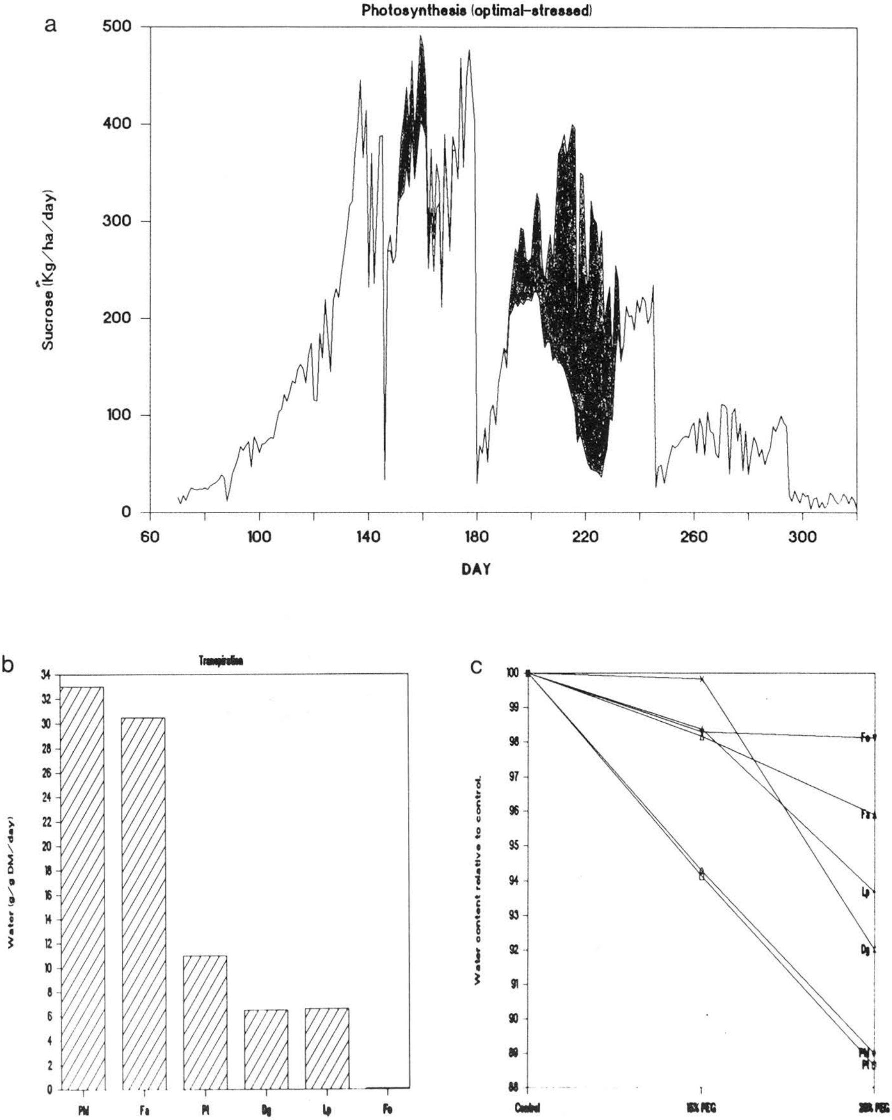

Fig.4. a) Simulated assimilation of a sward of ryegrass in 1982. The black area corresponds with the simulated losses in assimilation due to drought. b) Transpiration rates for 5 grass species: Phl, Phleum pratense; Fa, Festuca arundinacea; Pt, Poa trivialis; Dg, Dactylis glomerata; Lp, Lolium perenne; Fo, Festuca ovina. c) Relative leaf water content of the species in b., after a drought treatment of 8 days on PEG (15% and 20%)

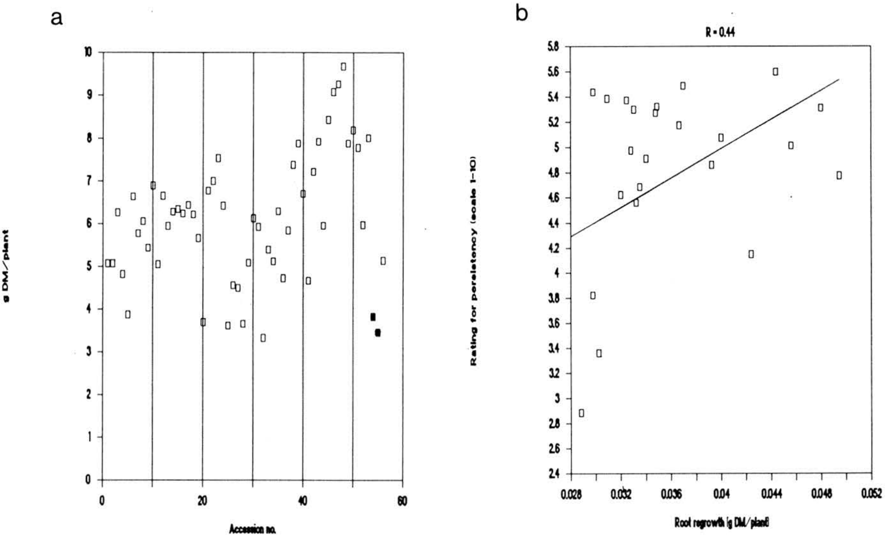

Fig.5 a) Dry matter of 54 selected families (open squares) and two commercial varieties (black stained squares) in the spring of 1987. b) Relation between ratings for persistency in the field in autumn 1986 and root regrowth (sum of three successive harvests in the hydroponic system (spring 1987).

POSTERS

ABSTRACTS

Electrophoretic variation in Festuca ovina

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Sheep's fescue, Festuca ovina L., is a diploid (2n = 14), allogamous grass, which is widely distributed in Scandinavia and occurs in a variety of habitats.

Four populations from South Sweden were sampled, two from Öland and two from Scania. 4 panicles were taken from each population, producing 10 offspring each.

Starch gel electrophoresis was carried out on leaf samples.

The following enzyme systems were studied: ACO, ACP, ADA, ADH, ALD, DIA, ES, GAPDH; GOT, G-6-PD, GPI, GPT, IDH, MDH, PEP, 6-PGD, PGM, SDH, SOD, TPI and UV-ES. Various buffer systems were investigated to obtain optimum separation.

Four enzyme systems, GPI, 6-PGD, PGM and TPI were found to be suitable for routine analysis because they showed distinct bands, were polymorphic and had a banding pattern which was possible to interpret. These systems will be used for future studies in Festuca ovina.

Variability of some physiological characteristics in lucerne plants regenerated by somatic embryogenesis

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The variability of chlorophyl content, water holding capacity, total and cuticular transpiration as well as the number of ovules and pollen stainability was evaluated in lucerne plants which regenerated from callus cultures by means of somatic embryogenesis. The evaluation was made on the field conditions in the phase of flowering.

Callus cultures were derived from one plant of lucerne. Seven plants regenerated from callus cultures were cloned and some physiological characteristics were evaluated with the whole experimental material /40 plants/.

We didn´t find differences in pollen stainability and in number of ovules by means of confidence interval estimation.

The values of some chosen physiological characteristics were significantly higher or lower in comparison with the average of the set.

It appears from that the selection of drought resistant and high-yielding plants is possible and the system of somatic embryogenesis is promising for selection of such improved lucerne genotypes.

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The ECP/GR Forages Working Group

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The Forage Working Group of the European Cooperative Programme for the Conservation and Exchange of Crop Genetic Resources (ECP/GR) is dealing with major forage temperate crops. 11 Institutes have accepted to act as European forage data base and have collated for *Bromus*, *Dactylis*, *Festuca*, *Lolium*, *Medicago*, *Poa*, *Phalaris*, *Trifolium* and *Vicia* most essential passport data on accessions held in European collections. More than 20,000 accessions are actually registered and the work is now aimed towards completion of passport information for each accession, so that breeders may have comprehensive lists (under print-out or computerized form) of available forage genetic resources in Europe. At long-term each European forage data base should play a leading role for its species of concern by identifying genetic diversity not yet collected and enhancing collaborative action.

To facilitate comparison of evaluation data between countries, lists of reference varieties are under implementation.

A programme or urgent collecting missions to rescue disappearing material was issued and many Institutes have undertaken collecting in accordance with these recommendations.

Training courses were organized in Welsh Plant Breeding Station, Aberystwyth, UK, and Servicio de Investigación Agraria, INIA, Badajoz, Spain.

Genetic variation in reed canarygrass (*Phalaris arundinacea* L.) populations of different origin.

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100 pure gramine genotypes from 10 populations were planted in a two-replicated trial in 1983, two ramets per plot. The populations originated from Western Norway (6 local pop.), USSR (2 pop.), USA (MN-76) and Canada (Castor).

Gramine genotypes (gramine is the less harmful indole-alkaloid in reed canarygrass), existed as a greater proportion of all populations studied. 5 pop. were pure graminetypes. Low alkaloid genotypes were accessible in all pop., and sufficiently high heritabilities were found to expect progress from selection. The observations of alkaloid concentration corresponded with lognormal distributions.

The local populations were high yielding, but they were inferior to all the other populations as to quality, estimated as lab-IVDMD and NIRS-IVDMD, CP and CF. Moderate heritabilities were obtained for yield and quality characters. Genotypic correlation between lab- and NIRS-IVDMD was 0.88. High negative genotypic correlations were obtained between NIRS-IVDMD and grassyield and between NIRS-IVDMD and CF.

A very late heading date characterized the Norwegian populations. As to their seed yielding capacity they were inferior only to Castor, which was superior in all seed characters. The WIR-populations showed genetic instability. High heritabilities were found for earliness, moderate to low for seed yield, No. of panicles and seed yield per panicle.

Concerning the total investigation wide genotypic variations were obtained in all populations for most of the characters studied. Clear differences were revealed between the local adapted pop., still with wild type characters, and the selected pop.. No significant relationship were found between the alkaloid concentration and the other characters studied, but the populations were different in this aspect.

Genetic resources of berseem (*Trifolium alexandrinum* L.)

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Berseem or Egyptian clover is a major annual forage crop cultivated in several Mediterranean and Near Eastern countries, and in some W. European countries for green manure and/or forage. Variation is found between varieties with respect to regrowth after harvesting, including growth form, response to temperature, susceptibility to disease and growth rate. Variation is also found in seed weight and flowering time.

Germplasm of berseem is considered under the 4 eco-genetical groups:

1. Cultivars - Single and multi-cut varieties are commercially available from Egypt, Greece, Israel, Italy, Morocco and USA.

2. Land races - Genetically diverse farm-grown populations. The genetic diversity between and within these populations is poorly assessed nor are there attempts to collect and preserve this important germplasm. This category is currently highly vulnerable to displacement.

3. Wild relatives - Four wild Eastern Mediterranean taxa of *Trifolium* have been identified, all of which intercross with berseem. *T. berytheum* and *T. salmoneum* are considered to be closely related to the wild progenitor of berseem (PUTIYEVSKY, KATZNELSON and ZOHARY, 1975. Theor. Appl. Genetics 45 : 355-62). Additional taxa may also be valuable for inter-specific hybridization. The extent to which these germplasm resources are endangered needs to be ascertained.

4. Wild-growing *T. alexandrinum* - Considered spontaneous forms originating from cultivation and now prone to extinction.

The collection, documentation and preservation of the germplasm was initially supported by IBPGR. The material stored in the genebanks has not as yet been evaluated. IBPGR also initiated the establishment of a Berseem data base.

GENETIC RESOURCES OF THE GENUS MEDICAGO

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An important part of the work of the forage plant breeding laboratory is concentrated on collection and evaluation of genetic resources. Priority is actually given to the genus Medicago (annual and perennial species).

The research programs on the genetic resources are based on:

- studies of the caracteristics of natural populations through a better knowledge of the relationships between the genetic variability of plants (estimated in experimental plots) and the environmental factors (ecological, physical or human disturbance, ...) of the sites.

- studies of gene flow between wild plants (such as Medicago glomerata or Medicago falcata) and cultivated plants (Medicago sativa).

- studies on the influence of the neighbourhood (estimated from the parent-offspring dispersal variance of seeds, pollen and from the selfing rate) on the genetic structure of natural populations.

This work is based on collection missions that will be realized in mediterranean area. Actually several missions have already been done in different countries (Spain, Algeria and France).

For instance, for the biogeographic survey of the genus Medicago in Spain, with the collaboration of I. DELGADO (INIA of Zaragoza), different points are already quite clear:

- the distribution area of perennial Medicago and annual Medicago are different: perennials in high plateaux in east and center of Spain, annuals rather in south-west of Spain especially for very frost sensitive species such as Medicago scutellata, Medicago ciliaris or Medicago intertexta.
- the distribution of annual medics is quite correlated with the level of frost of the site. Medicago rigidula has a larger geographic distribution than other species, for instance Medicago aculeata.

Data analysis is in progress, so that no final conclusions can be drawn so far.

Genetic Resources of *Lathyrus* (*Fabaceae*)

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Genetic resources of *Lathyrus* species of economic value are being studied in Pau. These species include :

- *L.sylvestris* (flat pea, pois sylvestre), which is used as a forage, for stabilization of disturbed and bare areas and constitutes an effective cover on roadbanks, ski slopes, mine spoils,etc... This species is adapted to droughted soils, low fertility and very cold climate.
628 accessions are registered.

- *L.latifolius* (perennial pea, pois vivace), which is used in seed mixture to establish cover in eroding areas, along roadsides for beautification and also constitutes a food for wildlife. It grows well in silt loam to fine sandy soils with fair to good drainage.
286 accessions are registered.

- *L.sativus* (grass pea, pois carré) and the wild form *L.cicera*, which are used as forage crops. Pulses are eaten from southern France to India, where it is an article of diet for the poor in time of famine. But their overconsumption rises the problem of toxicity (lathyrism).
202 accessions are registered

- *L.tuberosus* (earth nut, macusson). Its tubers have been consumed by man until XIXth century in Europe and Asia. It is consumed by wild beasts, particularly wild boars.
231 accessions are registered.

A database for these species is being realized with the passport data. This is an agreement with I.B.P.G.R. and in cooperation with F.BISBY (I.L.D.I.S., University of Southampton) who is in charge of the other species of the genus (about 150).

Influence of environnemental factors on the comportement of different heading date perennial ryegrass

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For many years, we have determined the bad starting growth of late heading perennial ryegrass implanted on wet and cold soils.

It seemed to be interesting to specify this observation in connection with our perennial ryegrass breeding program.

In our trial, 29 varieties have been studied on 2 types of soil : a sound soil on which grass early grow, and a sodden and hydromorphic soil in which maximum spring's temperatures are 1.3°C lower.

Leaf starting growth is not correlated with heading date on sound soils, while late heading date varieties have a later starting growth on cold and sodden soils. Further, in such lands, blades have a reduced mean length.

For any type of soil, the number of living leaves per tiller is maximum for intermediate heading varieties.

Tillering, which is already considerable for late heading varieties, is reduced on wet and cold soils.

Variability of enzymatic systems in natural populations of Festuca arundinacea Schreb. from Morocco

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Isoenzyme patterns studies provide a rapid and easy tool for natural populations variability analysis as well as for inferring on phylogenetic relationships among related taxa. Description of tall fescue natural populations with different ploidy levels and geographical origin using the isoenzyme studies is the main aim of our project.

In the first stage twenty two natural populations of Festuca arundinacea Schreb. from Morocco (7 octoploid populations - F. arundinacea var. atlantigena f. pseudomairei $2n=8x=56$ and 15 decaploid populations - F. arundinacea var. letourneuxiana and cirtensis $2n=10x=70$) were investigated. The seeds were collected by J. JADAS-HECART in 1984. Isoenzyme patterns in three enzyme systems : esterase (EST), peroxidase (PX) and acid phosphatase (ACPH) were analysed in leaves of 7 week-old seedlings. Two different electrophoretic methods were used : thin layer isoelectrofocusing (EST and PX) and horizontal polyacrylamide gel electrophoresis (ACPH). Statistical analysis of electrophoretic data was carried out.

Great variability of all analysed enzyme systems was observed within and between populations. The highest variability between populations was observed in the esterase isoenzyme patterns (all the studied populations differed significantly at 0.05 level and 19 of them - at 0.01 level). In the case of acid phophatase the variability was the lowest (only 4 populations differed at 0.05 level and 2 - at 0.01). No significant relationships were found between the studied isoenzymatic systems variability and populations ploidy levels or their geographical locations.

The use of Hordeum spontaneum in breeding for self-regenerating pasture barley

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In dry Mediterranean climates there is a shortage of roughages, caused by the low precipitation and the long dry summers. Low fertility marginal lands and hilly or rocky uncultivated areas can become more productive by growing a pasture crop, which is able to regenerate itself. The traditional crops used to improve such pastures, namely medics and loliums, have been tested in some areas but with little or no success, especially in the most dry Mediterranean areas.

The wild progenitor of barley, Hordeum spontaneum, is a native crop in most Mediterranean islands and countries and it is adapted in the very dry areas better than other crops. It grows very fast after the first autumn rains, it competes with weeds more efficiently than medics and it produces large quantities of forage for grazing. The dry matter at tillering to stem elongation stages contains 20-30% crude protein, comparable to that of legume pasture crops. In addition it regenerates itself because of an efficient seed dispersal mechanism, mainly its brittle rachis and shrunken kernels. There is significant genetic variability between and within H. spontaneum populations for many traits. In Cyprus and other countries, progenies of natural outcrosses between H. spontaneum and H. vulgare were found, all having a brittle rachis and the seed type of H. spontaneum. It is very easy to cross the two species and their progenies are fertile.

A breeding programme was initiated at the Institute in 1983, which aims at developing self regenerating pasture barley by crossing H. spontaneum with H. vulgare. The available data are very encouraging. The best F_3 families were bulked and will be evaluated in hilly and rocky areas in order to increase their animal carrying capacity.

Bio-agronomic study on white clover cvs of different origins grown for seed and forage

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The results are given of two experiments carried out at Lodi (1982-1987) on White Clover cvs from different W.Europe countries and U.S.A.,in comparison with the local ecotype "Gigante Lodigiano", grown for seed and forage .

The experiments were a split-plot layout with four replicates having cultivars as main plots and two defoliation treatments (A : no defoliation or B : 1 defoliation before cutting for seed) as sub-plots .

Data on establishment, dry matter yield and percentage of ground cover were recorded in the first year (1982,Trial 1 and 1985, Trial 2). Percentage of ground cover, seed yield components, actual seed yield and dry matter yield were recorded in the subsequent two years of culture .

Particular emphasis is given to the following characters : flower number per m^2 at harvest , florets per flowering head and seed set/floret .

The analysis of the presented data shows significant or even highly significant differences among the 14 tested cultivars . With regard to seed yield components, actual seed yield, dry matter yield and ground cover best performing cvs prove to be Espanso, Lune de Mai, Gigante Lodigiano, Regal (Ladino types) and Olwen and Merwi (Hollandicum types).

Seed and dry matter yield is influenced by the origin of cvs and for a great extent by management techniques (in particular by defoliation or not before the seed harvest).

The choice of Italian Ladino cultivars for seed as main crop and forage as by-product is advisable for our modern dairy farmers . On the contrary, foreign cvs perform better without defoliation and are therefore not useful in our country .

Since actual seed yield is moderate or low, even in the plot, modern techniques of management must be investigated. In particular the optimum doses of irrigation water, the optimum rates of mineral fertilisation and the optimum density of hives should be defined with priority .

The breeding of a new Italian Ladino cultivar with a higher seed yield potential, vigourous and persistent might be very useful too .

Selfing effects in S_1, S_2 and S_3 generations of Dactylis glomerata measured in competitive conditions

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Selfing combined with selection in competitive conditions was utilized to create Dactylis glomerata cultivars suitable for intensive management systems like irriguous plains of Northern and Central Italy. Such a method was proved efficient in lucerne (Rotili 1970, Rotili et al. 1980-1982) in allowing a sufficient homogeneization of the physiological traits, in conserving such homogeneity in the subsequent generations of the new cultivars and in increasing selection efficiency for the additive traits by unmasking genes, gene combinations and "linkats" (Demarly 1977) favourable to vigour.

Two parental populations were utilized: Dora, derived by mass selection in the Lodi area, and Montpellier, selected in South France. 4000 plants of each population were studied in greenhouse at a density of 250 plants per square metre. Greenhouse allows the evaluation of yield capacity during the summer, at temperatures over 35° C.

After 8 cuts the plants selected on dry matter yield and earliness basis were cloned and submitted to selfing as well as to crossing. S_0 and S_1 families were studied in the same conditions and the same criteria were adopted for plants selected in S_1 and S_2 families.

The existance of either tolerant or sensitive plants to selfing was confirmed, this sensitivity reaching its maximum level in the summertime.

Two ways of variety creation should therefore be tried: 1) synthetic varieties and 2) "semi-hybrid" varieties, by exploiting pollen competition. By the second way it should be possible to comulate vigour linked to the maximum heterozigosity with vigour linked to the quality of genes, gene combinations and "linkats".

Resistance to winter stress-factors in Norwegian timothy populations.

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In Norway a co-ordinated breeding program for forage crops was started in 1980 (MARUM 1984). Good winter survival is a crucial trait in our perennial crops, and the basic breeding material is therefore tested for resistance to winter stress-factors.

A major part of the breeding material in timothy for southern Norway is local ecotypes which in this presentation is grouped according to their origin in 3 'regions'. The plant material was tested for resistance to freezing and to the snow mould fungi *Typhula ishikariensis* and *Fusarium nivale*, in controlled environment. The resistance mean to each stress-factor did not vary significantly between regions, but the frequency distribution curves for resistance to *T. ishikariensis* and to *F. nivale* clearly showed different patterns in the 3 regions.

For the whole plant material (122 populations) the resistance to *T. ishikariensis* was significantly correlated with resistance to *F. nivale* (r = 0.42). This indicates that selection for resistance to one of the fungi also should improve resistance to the other.

The resistance to freezing was correlated with resistance to *F. nivale* in 'East' (r = 0.35) and with resistance to *T. ishkiariensis* in 'Trøndelag' (r = 0.32). This reflects a coadaptation to predominant winter stress-factors in the regions.

No correlation between resistance to winter stress-factors and latitude of origin was found for these timothy-populations.

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GENETIC VARIATION FOR SEED YIELD COMPONENTS IN WESTERWOLD RYEGRASS (LOLIUM MULTIFLORUM VAR. WESTERWOLDICUM).

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Genetic variation for seed yield components was studied in Westerwold ryegras. The possibility was considered of using Westerwold ryegrass as a model crop for genetic studies of seed yield components in perennial ryegrass.

Four diploid varieties were used: Wewo, Weldra, Merwester and Baroldi. Per variety 19 genotypes were grown in the field in two clonal replications. The following characters were determined: inflorescences per plant (infl), spikelets per inflorescence (spkl) and florets per spikelet (fl), floret site utilization (fsu), 1000-grain weight (tgw) and seed yield per plant (y). Floret site utilization was calculated as fsu = (seeds / florets)*100%, and averaged over three spikes per plant. Path models were calculated according to the formula y = infl * spkl * fl * fsu * tgw, after logtransformation and standardization of the characters.

Within varieties, we found large variation in seed yield, but not significant differences for yield were present between varieties. For most seed yield components large variation was found within and between varieties. Path model analysis revealed, that relationships between seed yield components differ to a great extent between varieties. Fsu was a major yield component in three of the four varieties. There was ample opportunity for genetic improvement of seed yield in all varieties.

Large heterogeneity in inflorescence maturity and seed shattering tendency precludes the use of Westerwold ryegrass as a model crop for genetic studies of seed yield components in perennial ryegrass.

Multilocal recurrent selection of perennial ryegrass in France

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Every year, France imports about 10.000 tonnes of perennial ryegrass seeds, for pasture and amenity utilisations. There are practically no french perennial ryegrass varieties, and often, foreign varieties are not well adapted to our environnemental conditions.

To reduce the importation of seeds, one of the objectives of the plant breeding programme is to develop varieties better adapted to our own conditions.

The plant breeding programme is conducted at Le Pin au Haras and Clermont-Ferrand. Le Pin au Haras, in Normandy, has an oceanic climate. The Station of Clermont-Ferrand, in Massif Central, has two locations : the first one, near the town, on Limagne plain, (semi-continental climate), and the second one in the mountains (cold and weit climate), near Bourg-Lastic (840 m asl).

The selection method employed is a multilocal recurrent selection. For each cycle of selection (4 years), the choice of the best mother-plants is made independantly in each of these three locations. This method consideres the results of individual plants and their family.

The best mother plants are crossed inside big polycrosses according to their heading date. The new families descended from these polycrosses form genetic material for a new cycle of recurrent selection.

Selection objectives are to improve the value of broad populations and to create French varieties adapted to our environnemental conditions.

Diseases resistance is evaluated in plants nurseries at the 3 locations. Dry matter production is calculated on microplots in Le Pin au Haras, and macroplots in Bourg-Lastic. Drougth resistance and autumn regrowth are estimated on row trials in Clermont-Ferrand, while cold tolerance and persistency in mountain conditions are studied at Bourg-Lastic.

The Station of Le Pin au Haras allows the observation of perennial ryegrass under good conditions.

Adaptation for grazing (plants able to withstand poaching and soil compaction) is also one of our objectives. Finally compatibility with white clover will be considered at the three locations.

AN EFFICIENT PLANT TEST TO SCREEN FOR RESISTANCE TO DRECHSLERA POAE IN KENTUCKY BLUEGRASS (Poa pratensis L.)

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Persistency under turf conditions is a major selection criterium in breeding of Kentucky bluegrass (Poa pratensis L.). Persistency under a close cutting regime in a wet and cool climate depends heavily on resistance to the melting out disease, caused by Drechslera poae (Baudys) Schoem, formerly known as Helminthosporium vagans (Drechsl.) Resistance to this disease varies between cultivars and is usually assessed as part of the total persistency score during field trials over several years. Seedling inoculation tests have been described but they have not proved reliable enough to introduce into practical breeding programs. An efficient and reliable screening test was developed which screens individual plants in seedling boxes within a six months period.

Pre-germinated seedlings are transplanted in the boxes, established and regularly trimmed to promote turf development. Seedlings are inoculated two months after transplanting by spreading shredded dried diseased leaf litter uniformly across the boxes. Four months later plants are individually rated according to a disease severity scale of 0 - 9 (9 = resistant).

Seedlings of 37 breeding lines and 11 cultivars were assessed in the plant test in boxes, with four replicates (rows) of six plants each. The breeding lines were derived from interspecific crosses with Poa longifolia and Poa chaixii, both of which have excellent resistance to Drechslera.

The same 48 objects with different degrees of resistance were sown in a field trial in 4 replicates. Each replicate consisted of 3 sown rows, covering 0.5 x 1 m., with border rows of red fesque The trial lasted two years.

There is a close correlation between the ratings of the plants in the boxes test and the field trial (r = 0.82 **),as well as with the long year average rating in the field. Therefore the plant test can be effectively used in a breeding program.

CYTOLOGY OF SEED ABORTION AND SEED SHATTERING IN PERENNIAL RYEGRASS (LOLIUM PERENNE L.).

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In grasses, realized seed yield is much lower than potential yield. A major cause of low seed yield is low floret site utilization (FSU). Degeneration of ovules and abortion of seeds cause low biological FSU; losses during harvesting and cleaning cause low economical FSU (Elgersma, 1985). Cytological research was carried out to study seed abortion (Sniezko and Elgersma, 1988) and the machanism of seed shattering (Elgersma et al., 1988).

Seven genotypes of Lolium perenne with contrasting seed yield and seed retention were grown in the field. Spikes were fixed at weekly intervals and microtome sectioned.

About 25 % of the ovules degenerated very shortly after anthesis, and a few percent aborted either before anthesis, or during later developmental stages. Genetic differences for seed abortion frequency correspond with differences for seed yield. Although abortion occurred especially in apical florets, aborted ovules were found at all floret positions within the inflorescence.

Seed shattering is caused by abscission. Abscission layers are located just below each individual floret and are already present at heading. 3-4 Weeks after anthesis abscission layers break mechanically, opening initially from the epidermis. Although the genotypes had contrasting seed retention, no genetic differences were found for the mechanism of seed shattering.

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