NEW WAYS IN
FODDER CROP BREEDING

Proceedings of a meeting of the
Fodder Crops Section of Eucarpia

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held at

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Some experiences with hybridization of *Lolium* and *Festuca* species

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ABSTRACT

When conventional methods for plant improvement seem to near exhaustion plant breeders have to look out for other methods. One of these may be interspecific or intergeneric hybridization. The breeder may resort to such wide crosses when certain desirable characters are lacking in the species which he wants to improve, whereas they are highly developed in a related species or genus. The direct cross may sometimes provide hybrids that are sufficiently fertile to use them as a basis for a breeding programme. More frequently, however, complications occur which ask for special techniques to make the desired new combinations possible and accessible.

Interspecific and intergeneric hybridization often involve the combining of sex cells differing in chromosome numbers or in affinity of their chromosome sets. In such cases viable hybrids may fail to appear or they are too infertile to be of much use for breeding work. Chromosome doubling of the parents before hybridization or of the hybrids after the cross has succeeded, may produce allopolyploids with restored fertility. Such plants may be usefull and of agricultural value in themselves or they can be used for further crossing to produce genotypes which are difficult to obtain by direct crosses. Finally interspecific and intergeneric hybridization followed by repeated backcrossing may result in the creation of cytoplasmic male sterility which may be valuable in breeding.

At the Foundation for Plant Breeding attempts have been made to utilize intergeneric hybridization between *Lolium* and *Festuca* species in the various ways mentioned above.

1. *Lolium multiflorum* and *L. perenne* x *Festuca pratensis*

Hybrids between *L. perenne* (2n = 14) and *F. pratensis* (2n = 14) are not uncommon in old Dutch grassfields. In addition to diploid hybrids two triploid types can be distinguished: a festucoid triploid intermediate between the diploid hybrid and meadow fescue and a loloid triploid intermediate between the diploid and perennial ryegrass.

The festucoids are very similar to experimental triploids containing one *Lolium* and two *Festuca* genomes; they are considered LF_2 plants. The loloids closely resemble experimental triploids containing two L and one F genome; they are designated as L_2F plants. There are differences of opinion with regard to the origin and the mutual relation of the three hybrid types.

Diploid hybrids and festucoid triploids have non-dehiscent anthers and are nearly completely female sterile. Part of the loloid triploids are to some degree both male and female fertile.

Several of the natural hybrids compared favourably with plants of the parent species. None of them can be propagated by seed. The induction of artificial allopolyploids (L_2F_2, L_2F_4 and L_4F_2 respectively) might be the most rapid way of obtaining fertile and sufficiently constant types of agricultural value. This could be done by colchicining tillers from natural or experimental hybrids or by treating seeds from intergeneric crosses. All possible crosses between the parent species on the diploid and the autotetraploid level were made. Only the cross *Lolium* 2n x *Festuca* 4n always produced sufficient quantities of seeds to allow for a seed treatment. Emasculation and embryoculture were not requested. This cross became the cornerstone of a breeding programme for which the following working scheme was designed.

$L_2 \times F_4 \rightarrow LF_2 \rightarrow$ colch. $\rightarrow L_2F_4 \times L_2F_4 \rightarrow$ selection
$L_2F_4 \times L_2 \rightarrow L_2F_2$ $\quad L_2F_2 \times L_2F_2 \rightarrow$ selection
$L_2F_2 \times L_2 \rightarrow L_2F \rightarrow$ colch. $\rightarrow L_4F_2 \times L_4F_2 \rightarrow$ selection
$L_2F \times L_2 \rightarrow L\frac{+}{2}$ $\quad L\frac{+}{2} \times L_2 \rightarrow$ introgression

By intercrossing diploid ryegrass plants with autotetraploid meadow fescue several hundreds of festucoid triploid hybrids have been obtained. Colchicining seeds or tillers resulted in about 15 L_2F_4 hexaploids brought to light by the dehiscence of the anthers. Backcross progenies of these hexaploids to diploid *Lolium* consisted nearly completely of tetraploids or near-tetraploids which were not distinguishable from L_2F_2 amphididiploids.

By a second backcross to diploid *Lolium* the amphidiploids produced large numbers of triploids, apparently of the constitution L_2F. Successful seed treatments gave rise to L_4F_2 hexaploids.

From this survey one may conclude that the scheme designed several years ago really works. All three hybrid types found in nature have been converted into the allopolyploid form. The initial hybrid material has been used very efficiently to make genome combination which with other methods would come into being on a much more limited scale. Yet this part of the story is not a success story.

Some of the original L_2F_4 hexaploids looked very promising but several of them have been lost by virus attack and other mishaps without having produced hexaploid progeny. Seed production has been disappointing. Although initially more than 90% of the legitimate seedlings appeared to be hexaploids, later results do fear that most L_2F_4 plants are cytologically unstable. As functional female gametes nearly always have 21 chromosomes male sporogenesis must be the weak point. Perhaps the same applies to the L_4F_2 hexaploids. Very interesting plants have been produced but sexual reproduction is still unsatisfactory.

Only in the L_2F_2 amphidiploids stability and fertility do not offer great problems. They form an interesting new type of herbage plant. Families obtained by doubling outstanding natural diploid hybrids are vigorous and highly homogeneous. The progenies from the cross L_2F_4 x L_2, however, are often rather heterogeneous. As unbalanced chromosome sets might be responsible for this behaviour, intercrossing of original and derived amphidiploids should be performed with caution.

Earlier work by Jenkin in Aberystwyth indicated the possibility of transfer of separete genes from *Lolium* into *Festuca* and vice versa, but conclusive evidence was still lacking. We try to complete Jenkin's studies using his marker genes for red base-colour. Non-red ryegrass has been intercrossed with red meadow fescue followed by repeated backcrossing to non-red diploid ryegrass. The appearance of red diploid ryegrass plants in the last backcross progenies (L_2F x L_2 a.s.o.) would prove that introgression has taken place. Red trisomics will be useful for studying the effect of external conditions on the frequency of gene transfer. The material is waiting for a cytological check.

Anticipating the theoretical foundation of an introgression breeding programme thousands of backcross plants from natural and experimental hybrids have been screened for fertility, summergrowth, cold resistance and disease resistance during successive generations. Some populations are now being tested. In similar populations cytoplasmic-genic male sterility has been discovered. This character seems to be inherited in a simple way. It can be combined with good seed setting. Maintainer types appear to occur frequently in *Lolium perenne*. If the attempts would fail to breed stable and fertile hexaploids a technique might be developed to produce F_2L and Fl_2 hybrid populations by using tetraploid meadow fescue and amphidiploids as pollinators. Preliminary results are rather encouraging.

2. *Lolium multiflorum* and *L.perenne* x *Festuca arundinacea* (2n = 42)

Only a few words should be said here on the hybrids between ryegrass and tall fescue. As early as 1952 we found in old pastures off-type plants of tall fescue presumably resulting from intercrossing with ryegrass. Seed production was a great problem. A few small field experiments were laid out but lack of time and lack of interest from the upper circles caused us to stop this line of work.

We started again about 1960, stimulated by the work done in foreign breeding stations. In general we obtained similar results. The production of fertile amphidiploids appeared to be rather simple. Amphidiploids between *L. multiflorum* and *F. arundinacea* had about the same yield, the same distribution of yield and the same protein content as tall fescue whereas cattle clearly and constantly preferred the former. However, cold resistance and persistency were weaker. These shortcomings and initial developments might be improved by choice of parents e.g. *L. perenne* and selection.

Contrary to disappointing results on some other stations our initial data on seed yield were rather encouraging: in the Co up to 300 seeds per culm. Average seed yield per plant in the Co generation was 15,3 g and in the C_1 14.0 g. A small experiment produced 900 kg/ha. We see no reason to discontinue the breeding of amphidiploids. Might a loss of fertility in advanced generations be related to loss of chromosomes?

Seedling growth of allopolyploids from the cross *Lolium multiflorum* x *Festuca arundinacea*

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ABSTRACT

Allopolyploids from the cross *Lolium multiflorum (Lm)* x *Festuca arundinacea (Fa)* develop rather slowly at the seedling stage. This can be a great disadvantage when sowing species mixtures in which plants occur that have a rapid seedling growth. In fact, such is frequently the case with establishing grasslands in the Netherlands.

Of allopolyploid material obtained through the above cross at the Institute de Haaff (S.V.P.) 5 C_3 families, 2 *Lm*- and 2 *Fa* varieties were tested for some characters that play an important role when grassland is created.

Dense rows of these varieties and hybrids were grown in a heated and an unheated greenhouse and in the field. In the greenhouse sowing took place on 6 February and in the field on 12 May, 1970. Emergence, length, number of plants and of shoots and leaf width were scored at different times. At the end of the experiment yield in green weight and dry matter were determined, i.e. for the heated and unheated greenhouse and the field at respectively c. 6½, 11 and 6 weeks after sowing.
For many characters, as was to be expected, the hybrids showed values that were intermediate between those of *Lm* and *Fa*. Often there were significant differences with regard to the mostly lower values of *Fa*, while the hybrids mutually differed significantly too. In some cases the hybrids either were at a level with *Lm* or exceeded the values of both *Lm* and *Fa*. As far the field was concerned, the figures for length remained between the value of *Lm* and that of *Fa*.

The found variation is indicative of good prospects of selection. Some hybrids showed a clear interaction from the treatments. Which test method to use in preference to an other is hard to say, especially so since it concerns the field experience of only one year.

With one hybrid no or hardly any treatment interaction could be noticed for the characters length and yield. In practically all instances this population also appeared to be one of the two top-performers. It is therefore possible to choose between different testmethods when working with such a stable hybrid. The unheated room or the field is obviously the most simple solution. The field test requires more seed than the glasshouse because of the less uniform conditions.
To test small quantities of seed, e.g. of families, a pre-selection in the unheated glasshouse seems to be the recommendable method.

The development of *Lolium-Festuca* hybrids and their derivatives as breeding material

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ABSTRACT

Grass breeders have long recognized the agronomic potential of certain *Lolium-Festuca* hybrids and have made considerable effort to realize this potential in the form of acceptable cultivars. Work on such hybrids is currently in progress in no fewer than eight countries, indicating its wide acceptance as a worthwhile approach to breeding in this important group. Attention has largely been confined to the four intergeneric hybrids obtainable from *Lolium multiflorum*, *L. perenne*, *Festuca pratensis* and *F. arundinacea*, since it has been shown that they successfully combine many of the desirable characteristics of these species. The method of production and development of such material depends on several factors, including the ease with which the hybrids can be produced, their fertility and the number of characters to be combined.

Since, in general, it is desirable to combine several characters from each of the four species, work at the Welsh Plant Breeding Station is concentrated primarily on the production of amphiploids incorporating whole sets of chromosomes, and their development has been attempted along orthodox methods of synthetic cultivar production. Our experience has shown that by crossing highly compatible, chromosome-doubled genotypes, with the *Lolium* species as female parent, sufficiently large numbers of relatively fertile material can be produced for the orthodox method to be adopted.

The three combinations, *L. multiflorum* x *F. arundinacea*, *L. multiflorum* x *F. pratensis* and *L. perenne* x *F. pratensis*, have shown sufficient promise for development, and by using a range of material including some of the most recently developed cultivars a widely based group of amphiploids has been made available for screening.

Though possessing many agronomically desirable attributes, the *L. multiflorum* x *F. arundinacea* amphiploid predominantly resembles the *Festuca* parent, particularly in establishment characteristics, and accordingly it has been considered necessary to increase the proportion of *L. multiflorum* genomes in its complement. This has been achieved through back-crossing on to the tetraploid form, and the material has proved to be sufficiently amenable to this kind of manipulation to allow further back-crossing without any marked loss in fertility. However, the manipulation of genomes in this way cannot be achieved with precision, since there is an appreciable degree of homoeologous chromosome pairing in this material. Use may be made of this latter phenomenon, however, where single or relatively few characters need to be transferred from one species to another. Thus, repeated backcrossing of this material on to tetraploid *L. multiflorum*, together with careful screening, should result in plants resembling the latter but with enhanced winter hardiness and persistency. Three cycles of back-crossing have given encouraging results. Improved cytological techniques could give greater precision to these operations and may even open up the field of chromosome addition and substitution in this group.

In an attempt to identify at an early stage the most promising lines and the best genotypes within them, a two-stage screening procedure based on seedling and mature plant performance, has been adopted in our work at the Welsh Plant Breeding Station. Particular attention is paid to the seedling establishment phase, and heavy selection pressure is applied at the end of this phase, on the basis of emergence rate, coleoptile tiller production, tiller number, and seedling dry weight.

Evidence has been found indicating the importance of the coleoptile tiller in seedling performance; seedlings possessing a coleoptile tiller have a significantly higher mean dry weight than those without. The distribution of coleoptile tillers in the different amphiploids in relation to their parentage and genomic constitution is also interesting and appears to have a bearing on their relative performance.

For the second or adult phase screening, selected material is transplanted into drills of closely spaced plants, giving some degree of competition while maintaining individual plant identity. Two managements are applied, a frequent cutting and a conservation/aftermath regime, and genotypes giving the best performance throughout the programme are selected within each management. These are subsequently polycrossed to provide sufficient seed for plot evaluation.

The stability of the different amphiploids through consecutive seed generations is currently under investigation and their development as cultivars depends on the outcome of this and their performance under large scale agronomic assessment.

Influence of the parental genotypes on the performance of the *Lolium multiflorum* x *Festuca arundinacea* hybrid.

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Nyon

ABSTRACT

Before starting with an interspecific hybridization programme, the breeder has to decide if he will restrict himself to cross a rather small number of highly bred parental genotypes or if he will increase the genetic variation by taking material from various origins.

The following elements will influence his choice:

- the cross compatibility of the parental plants
- the influence of the parents on the characters of the hybrid
- the fertility of the hybrids at the final level

In 1968, five clones of Italian rye-grass, three of them being diploid, one tetraploid and one hybrid (*L. multiflorum* x *L. perenne*) were pollinated by 5 populations of tall fescue. The seeds have been grown on an artificial medium and transplanted in the field for observation. The results have been as follows:

Seed setting: With the exception of the hybrid rye-grass, which produced fewer seeds, no significant differences have been found which could be attributed to the rye-grass or tall fescue parent.

Number of plantlets likely to live: Noticeable differences have been observed concerning the germination and the number of living plantlets. The seeds issued from tetraploid rye-grass did not germinate at all.

Morphological characteristics of the F_1 hybrid: The observations of several authors concerning the branching of inflorescences, the presence of hairs on the leaf auricles etc. have been confirmed. Furthermore, the epidermic features and the transverse-section of the leaves were all of the tall fescue type.

Dates of heading: The figures presented show that the dates of heading of the hybrids were lying between those of the parents. They were distributed over one month.

Growth: During the first two years after planting and as far as the estimations are made at the same growth stage, no systematic difference in growth could be attributed to the choice of the parent. After two winterings, the progeny of non-hardy tall fescue was significantly less vigorous.

Fodder quality: Differences in the flexibility of the leaves have been found according to the tall fescue parent. The crude protein and crude fibre contents of the hybrid were intermediate between those of rye-grass and tall fescue.

Resistance to rust: The hybrids were more resistant than both parents to *Puccinia coronata f. lolii* and *f. festuceae*. The resistance seems to be heritable.

There is some evidence of the validity of these observations for the amphi-diploids and other euploid hybrids.

In conclusion, it seems that both cross-compatibility and physiological characters should be taken into account for the choice of the parents. In our material, emphasis will be given mostly on cross-compatability for diploid rye-grass, on agronomic characters for tall fescue.

Cytological studies of intra- and interspecific hybrids within the *Lolium/Festuca* group in relation to breeding.

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ABSTRACT

Interspecific hybrids in the *Lolium/Festuca* group have been made in an attempt to combine the attributes of different species in new synthetic species. Hybrids involving diverged species are often sterile and therefore cannot be used further in a breeding programme. On doubling the chromosome number of these hybrids fertility is restored and the amphiploids can be used in the improvement of the crop. In terms of plant breeding, the utilization of interspecific hybrids in crop improvement can be conveniently divided into procedures involving:- 1) complete genomes, 2) introduction of individual chromosomes from one species to another and 3) the transfer of chromosome segments or genes.

Amphiploids have the advantage of restoring fertility but still breed fairly true for a highly heterozygous state if the degree of chromosome pairing approaches the stability of natural polyploids. A number of amphiploids in the *Lolium/Festuca* group, that have been cytologically studied, have irregular meiosis compared with the natural polyploid *Festuca arundinacea*. Irregularities in meiotic behaviour affect the stability of the amphiploids in successive generations and limit their capacity to breed true to type. Procedures combining complete, distinct genomes to produce novel synthetic species have limitations in the *Lolium/Festuca* group. However, the recent discovery that amphiploids with B chromosomes have a more regular meiotic behaviour could have an impact on amphiploid breeding in the *Lolium Festuca* group.

The control of chromosome pairing in polyploids of the *Lolium/Festuca* group is not sufficiently rigid to enable methods involving the introduction of single chromosomes from one species to another to be used.

Chromosome pairing involving chromosomes of different species takes place in interspecific hybrids and amphiploids resulting in the recombination of specific characters. The reassortment of the chromosomes and genes of the species involved presents the breeder with new variation on which to base the selection of superior forms. However, the breeder is confronted with the task of harnessing this variation in genotypes which are sufficiently stable to breed true to type. Backcrossing the amphiploid to the parental species is one method of introducing new variation into the crop species. The cytological behaviour of backcross hybrids gives some indications of the feasability of following this approach in producing new forms within the *Lolium/ Festuca* group.

Interspecific crosses as a tool in breeding *Poa pratensis*

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ABSTRACT

In this paper apomixis is taken as reproduction by seed in which the embryo is produced without fertilization. It is rather widespread in nature. *Poa pratensis* is one of the apomactically reproducing grasses.

Different ways of apomictic reproduction are known. In *P. pratensis* the legitimate embryosac mother cell is replaced by a somatic one that develops into an eight-nucleate embryosac (apospory). One of these eight nuclei behaves as an egg and grows out into an embryo without fertilization (parthenogenesis). Finally fertilization of the central nucleus is needed for endosperm formation (pseudogamy). The embryo has the same genotype as the motherplant.

In *P. pratensis* plants also some sexual reproduction occurs: facultative apomixis. The degree of apomictic reproduction is mainly a character of the plant. A high degree is preferred.

The apomictic reproduction has some advantages. Varieties can be based on one single plant and are very uniform.

In the Netherlands *P. pratensis* is used in seed mixtures for pastures but its highest importance is the use in sports turf, lawns etc. Varieties must have resistance to close and frequent cutting, to *Drechslera Poae* (or *Helminthosporium vagans)*, rust (*Puccinia poarum*) and mildew (*Erysiphe graminis)*. A nice colour is desirable. A high seed yield is wanted; non-hairiness of the lemma could facilitate the cleaning of the seed crop.

In breeding *P. pratensis* collecting and testing of ecotypes is a well known method. Thanks to some sexual reproduction in most plants crossing is not impossible. At the foundation for Agricultural Plant Breeding the use of interspecific hybrids as a sexual phase in breeding *P. pratensis* is investigated. Hybrids have been obtained between *P. pratensis* and *P. nemoralis*, *P. arachnifera*, *P. chaixii* and *P. longifolia*.

The last mentioned *Poa* is mostly used, only results of this cross are discussed.

P. longifolia is a cross fertilizing species, growing in the Black Sea region (USSR). Seeds were received from Dr. G. Almgård, Sweden.

As *P. pratensis* parents Dutch ecotypes, varieties and Hungarian introductions were used. The crossings were made following the mutual bagging technique. The first hybrids were harvested on *P. longifolia*, only experiences with this type of hybrids are discussed.

Some contrasting characters of the parental species:

P. longifolia 2n=42, sexual, no rhizomes, height 100-150 cm, non hairy lemma.
P. pratensis 2n=35-90, apomictic, rhizomes, height 65-75 cm, hairy lemma.

F_1-hybrids were about intermediate in phenotype, they were sexual and nearly all sufficient fertile. The chromosome number could not immediately be derived from the parents.

In F_2-populations a vast range of types was observed. New combinations were found, which is shown by the characters plant height and hairiness of the lemma. Backcrossing of F_1-hybrids with *P. pratensis* gave more *P. pratensis* like plants in the offspring.

In F_2, F_3 and back-cross progenies plants with an apomictic reproduction were found.

It is concluded that interspecific crosses can be used as a sexual phase in *P. pratensis* breeding. Non-hairiness of the lemma can be introduced in *P. pratensis*.

DISCUSSION

Abbreviations used in records of discussions:

Lm = *Lolium multiflorum*

Lp = " *perenne*

Fp = *Festuca pratensis*

Fa = " *arundinacea*

Fr = " *rubra*

Chairman: Dr. Lackamp

Nitzsche: Has anybody any experience with hybrids of *Fp* x *Fa*? We have good results with these crosses.

Julén: What is your own result?

Nitzsche: We have fertile forms of amphidiploids of this hybrid. They grow well especially in the second and third year. These types between *Fp* and *Fa* are not so tough as *Fa*. They will give good forage crops for special conditions.

Dijkstra: Is the fertility of the amphidiploids as good as from the parents?

Nitzsche: Yes.

Wit: Do you know anything about the cytological stability and number of chromosomes in later generations of the amphidiploids of *Fp* and *Fa*?

Nitzsche: The polyploid *Fa* is not stable in chrom. number. We have produced *Fa* with 84 chrom. and the chrom. number goes back to 70 or lower. We have seen this also in our hybrids. I don't know exactly the chrom. number of our ("first") hybrids but I think it will be about 28.

Emecz: In what direction are you doing the crosses?

Nitzsche: In both directions.

Elci: Did anybody work with natural hybrids of *Fa* x *Lp*?

Lewis: The natural form of this hybrid has been reported in our country, but not recently.

Elci: We have found rhizomic types which are very suitable for erosion control of the edges of the channels. This material is not suitable from the viewpoint of seed setting and I treated it with colchicine and got material with 56 chrom.

Lewis: We have given this particular hybrid the lowest priority in our breeding programme because it is the least attractive agronomically.

In our collection of tall fescue we have found a form which is rhizomotous from N. Spain. The Festuca parent of the hybrid you found may have been rhizomatous.

Emecz: Is it possible to combine the excellent characteristics of *Lm* and *Fa*? For example the early vigour of *Lm* and the persistency of *Fa*. So far it appeared that the hybrids of *Lm* and *Fa* were not entirely successful.

Wit: I have only some experience with hybrids of *Lolium* and *Fa*. We found that especially in the first generation these amphiploids were rather fertile and gave a sufficient amount of seeds. We tested them for yield and they were as productive as *Fa*. Then we tested them under grazing conditions and they were much better eaten by cattle than *Fa*. The only drawback in our material was, that after two years the sward was becoming rather thin, so the persistency was not as good as that of *Fa* and we suppose that this has been caused by the *Lm* parent. So one of the things Mr. Dijkstra is trying to do is to improve persistency by using *Lp* instead of *Lm*.

We had, especially in the beginning, a very good seed setting. After some generations of multiplication however it seems that seed setting is becoming poorer. Perhaps that has something to do with loss of chromosomes.

Badoux: I think it is quite difficult to combine all the positive characters we want to have, but what we try to do is to go as far as possible. But it is possible that we can have a "competition". If you breed for quality, you may loose vigour or something else. This we have noted in some cases.

Lackamp: Do you expect that from this kind of material good varieties will arise for the Western parts of Europe where already such good species exist? Or do you expect that this kind of material will be suitable for much different climates not found in W. Europe, because the conditions here are too favourable for this kind of hybrids?

Lewis: I would expect ryegrass-fescue hybrids to be valuable for N. Western Europe provided they incorporate some of the most important characteristics of both parents. More tall fescue would be grown in the U.K. if its rate of establishment could be improved; introducing this character alone from Italian ryegrass would be worthwhile.

Thomas: The possibilities of combining characters of different species depends on the dominance relationships of the genes of the two species. Introducing a gene into a different background can often alter the expression of the character it controls. This is a factor to be considered in the synthesis of amphyploids aimed at combining the attributes of the parental species.

Julén: Mr. Emecz is asking an ideal plant, where he can combine all the various positive characters in one single plant or variety or population and I think that is to ask for too much.

There are some weak points in *Fp*. If we for instance could have a *Fp* variety, which was easier and quicker to establish, but still kept the other characteristics of *Fp*, then I think it would be an achievement even if it does not give the same production as *Lm*. But if we are looking for such more limited achievements my question would be: Are there greater possibilities to achieve this by crossing of the different species or can we achieve the same thing by looking for the widest possible distribution of genes inside the species and look and see if we can find this variation inside, for instance in this case in *Fp?* Is it easier to increase the variation by intergeneric crosses than to try to increase the variation inside the species?

Dijkstra: Now perhaps we are in the time that species crosser are popular, but it is an omission to neglect the possibilities within the species and not to select within it. I try to obtain hybrids between *Lp* and *Fr* in order to produce a lawn grass that is less course than *Lp*, but more treading resistant than *Fr*. But when you select within *Lp* it is possible to get much finer-leaved *Lp* grasses and I think you must use both approaches. We have the solution that selection within species is done by Dutch private breeders and crossing of genera and selection within hybrids at least the first steps of it, is done by Institute "de Haaff".

Julén: Another question. My impression is so far that the hybrid material used is on a rather limited and narrow genetic basis. In each case each cross is involved with a very limited number of plants. I think Mr. Badoux's material is very interesting in that respect that he has tried to get parents from various sources of different types and to combine them. This reminds me of the result from the tetraploid red clover, where we found that if we were working on the material based on a narrow genetic base our achievement was rather slight. The yield increase was very limited indeed. But we widened the genetic base by taking material from different sources and bringing them together to intercross. We got a very striking increase in dry matter production. And now I would like to ask Mr. Badoux: "Have you tried to intercross the hybrids from various sources, also the Finnish with the Portugese or different material, just in order to increase the entire genetic base and when you have done that, what is the result? Do you increase the vitality in that material compared with that of the more limited one?"

Badoux: We have tried to do that and the amphiploids from this material have been used in the polycross. The fertility of the progeny of this polycross is not the same for all the progenies. I don't know why. The idea is to have this material intercrossed.

Ahloowalia: I have few comments. In interspecific or intergeneric hybrids in general it appears that meiotic instability results when the polyploid level goes beyond the tetraploid level. And this meiotic instability is an antithesis of the uniformity, which the breeder wants at the end. Yet, it is extremely important for having the initial variability for selection. An answer to the second part of the question, which Dr. Julén asked. I think we still have yet a third approach towards incorporation of desired characters from one species into another: Producing trisomics in the beginning and replacing one chromosome at times rather then putting the whole genome in.

Thomas: In the first place I don't quite agree with the first remark that there is less regularity after the tetraploid stage, because there are natural chromosome lines of Fescue's with 56 and 70 chromosomes and they have regular bivalent pairing.

Ahloowalia: I was thinking of hybrid material. Did you ever obtain hybrids beyond the tetraploid level that remained stable after the second generation? According to my experiences in the third generation seedsetting problems arise, which have their origin in chromosomal instability. This has been the main problem in producing a variety in the end.

Thomas: This is also true of a tetraploid since you get a considerable aneuploidy at that level.

Ahloowalia: I agree, but tetraploids can be made stable. Nobody has succeeded yet in doing so in the hexaploid or dodecaploid hybrids.

Thomas: I don't think that it is impossible. I think that putting a B-chromosome in might give you such a system. I don't think that we know enough about it at present.

Ahloowalia: After my opinion it would be an easier way to put in one chromosome at a time.

Thomas: I don't think you can do that in the Lolium-Fescue group for the simple reason that the control of chromosome pairing is not rigid enough. You can do it in wheat and oats quite easily because you have genetic control of chromosome pairing and isolated monosomic lines. But the amount of chromosome differentiation or control of chromosome pairing that exists in the Fescue-Lolium does not allow this.

Ahloowalia: But I think you are having this question from an entirely different angle. If you want to introduce a character like increased tolerance to winter from fescue. If we had trisomics of meadow fescue we would easily shift one chromosome into the Lolium. Just that particular chromosome that carries the factor. Then you will not be coneerned with the rest of the material all together. Similarly increased persistency from Lp could be transferred very easily into the fescue group, provided you have the trisomics.

Thomas: Can I go back to the question which Dr. Julén asked concerning crossing diverse types within a species. We have tried this in *Lolium perenne* and found considerable meiotic instability in some hybrids involving climatic races.

Julén: It was not that problem I have taken. It was the problem of the limitation in the genetic source. It was not the question of bringing *Lolium* together from different areas but of all of the ordinary types, and to have crosses of various varieties just in order to widen your genetic base, still dealing with well balanced material.

Lewis: I would just like to add that we have used a wide base in the sense that we have used all the best cultivars in Italian ryegrass, as in tall fescue and we have even in some cases introduced some natural hybrid material We obtained a range of F1's which were polycrossed, so that we are incorporating a range of varied material into the hybrids.

Julén: What was the result?

Lewis: Our selected lines are really synthetics of several sources.

Lange: We have heard quite a lot of meiotic instability introducing losses of chromosomes and my question is: "Is there in this material any evidence for meiotic instability resulting in losses of chromosomes just during the somatic divisions?"

Thomas: Most chromosomes are lost during sexual reproduction and seed production. As far as I am awere there is no evidence for mitotic instability.

Nitzsche: I think we will have lost the chromosomes also in the mitosis in dodecaploid *Fa*. It is not only a meiotic reduction.

Badoux: I would just ask a question to the cytologists who are here. Do you think there is an optimum for normal chromosome set we should have in a hybrid? You know the work of Dr. Buckner done in America, who came to the conclusion that hybrids with a lower chromosome number than 8x may be more fertile. In this connection I would like to mention that we have found hybrids in which full sets of chromosomes have been lost. My question was: "Is there an optimum of chromosomes for the hybrids between tall fescue and *Lolium*?

Speckmann: There is an optimal chromosome number for different species as you know, but whether we could say there is an optimal chromosome number in the hybrid material, I can't think of in this moment. I know that Dr. Ahloowalia said that it seems that the complication arise at higher chromosome numbers and that might be an indication.

Ahloowalia: The optimal number can be justified only against the particular cytoplasmic background and the rest of the genome coming into it. You can for example take a diploid and add one extra chromosome. I have done that and

I can add two-three and four chromosomes at a time and the plant becomes increasingly sterile. It might start to form a very poor root system, and give plants as Dr. Wit pointed out. He showed the slide where leaves become extremely narrowed. We can associate in fact that with chrom. 6 or 7, which is one of the smallest in *Lp*. Now when you are going to high levels, when you are bringing in not only cytoplasm, but a huge mass of genome after genome into the hybrid, you are not actually concerned with the optimum number, but with the genetic background.

Elci: In the natural situation we have an Agropyron cristatum with 14 chrom. and we collected in our vicinity in the central part of Turkey a natural tetraploid. We checked this material from the view point of agricultural characteristics and in this material we have found that the 28 chrom. is better than the 14 chrom. But this does not always mean that with a higher chrom. number you will get better results, so in other words we could say not only the chrom. number is important but also the genetic background.

Jones: I think one has to remember in the breeding program for *Lolium* and *fescue*, what it is you are trying to do. The main aim of the exercise is to incorporate the desirable genes from one species into the other. And polyploidy here is a means of achieving this aim and I think it is probably correct to say that it is not polyploidy alone that is important but the genetics. There is no doubt that species do have optimum chromosome levels, but for different purposes you might say. In Sweden is shown that *Poa* species had optimal chrom. numbers for various sizes. Once you got beyond tetraploid to hexaploid and octoploid the size decreased.

Now in this case (*Festulolium*) we got complications in pairing between genomes. It would seem logical that with the fewer chromosome sets with a balance of an even number, the more regularity you get. And in point of fact the most successful hybrid, which is now appearing in Aberystwyth is reduced from the hexaploid to the tetraploid level and I think therefore that the optimum level is that which gives you the incorporation of the genes where you will have stability.

Nitzsche: I also have got in the last years hybrids on the diploid level of *Lm* x *Fp*. And I have got very good results from these hybrids. They are fully fertile.

Emecz: We have seen that it was possible to introduce more variation by manipulating chrom. number or by introducing interspecific hybrids. But many of the agronomic objectives are not reached due to various linkages I suggest. It seems that seedling vigour and early establishment is associated with a shorter live span. I would like to ask the panel: "Is there any strong evidence that by manipulating the chrom. either through polyploidy level or hybridization, we are breaking these undesirable linkages?"

Lewis: We have no information on this.

Wit: I suppose by chrom. doubling you have more possibilities of breaking undisirable linkages than on a diploid level. We have no special experience about persistancy and so, which you asked, but I think from the theoretical point and from the point we have seen from our selection for disease resistance, you should get more possibilities on a polyploid level than on a diploid level. That is the only answer I can give on the question. I think we know too little about it.

Emecz: If you go beyond the tetraploid level the possibilities of breaking linkages is even greater, even if you are loosing a little bit of stability.

Wit: I have crossed tetraploid *Lolium* with octoploid *Fp* and obtained very nice hexaploids. It appeared very difficult to maintain the octoploid parent plants. I had one octoploid *Lolium* plant and a few octoploid *Fp*, but they lived only for one year. So I think, what has been mentioned before, there is really an optimum. I suppose there must be an optimum of chrom. number in certain species, which optimum of course may be quite different in different species.

Julén: Is there in any of the places where one has been working on this, any variety of these hybrids ready for release and has any of these varieties been tested under practical conditions? And in that case with that with what result compared with material we have now?

Lackamp: (Chairman): Well, I think it will be the best thing to give an answer by nation.

For Great Britain. Lewis: We shall be registering two varieties with IVRO in the near future: *Lm* x *Fp* and *Lp* x *Fp*, both have performed well in trials. We are also developing backcrosses of *Lm-Fa* on to *Lm* but have no varieties as yet from this cross.

For Switzerland. Badoux: We hope to get something in the future. I would just like to mention that as far as I know a variety Kenhy has been released in Lexington in Kentucky, but I don't know exactly the composition of this hybrid.

Thomas: I think it is derived from *Lm-Fa*, it was backcrossed to tall fescue and than they got material of around 42 chrom. So it looks as it is *Fa* with a few chrom. of *L*.

For France. Gillet: Nobody in France is searching in this direction for the moment. Two Dutch varieties are tested for the French list of cultivars.

For Germany. Nizsche: In Germany there is also no strain in practice, because the persistence is not good in the material from Dr. Hertsch. But on the other hand nobody can have the breeders right on such hybrids, because it is not in the law.

For the Netherlands. Duyvendak: There are a few Dutch varieties in trial at the IVRO in the Netherlands. They fall into two groups. The originally octoploid varieties turned out to be instable in chromosome numbers. Most of the investigated plants had chromosome numbers which came down to around 49, which meant chromosome loss. These applications have already been withdrawn by the breeder, because of the difficulties in seed production. The other group is the group with the 28 level, the amphidiploid Lp_2Fp_2. These varieties are fertile and are being studied now.

For Scandinavia. Julén: No information.

For Turkey. Elci: We found a new hybrid in our plant introduction field. We have observed a procumbent leafy type with many rhizomes. The plant is suitable for water and wind erosion control. But the plant is not fertile.

Cytogenetic implications in the breeding of tetraploid hybrids between Italian and perennial ryegrass.

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ABSTRACT

The simultaneous exploitation of interspecific hybrids and polyploids in ryegrass improvement would depend mostly on their vigour, persistency, fertility and stability. The species of Italian (*Lolium multiflorum Lam.*) and perennial (*L. perenne L.*) ryegrass are diploid, 2n = 14 and very closely related. Hybrid ryegrasses based on the segregating generations of the two diploid species have been in commercial use for sometime now (Corkill, 1964). Recently, varieties from the segregating interspecific tetraploid hybrids have been considered as a commercial venture (Schumann, 1968; Stephens and Breese, 1971).

Tetraploid ryegrass varieties which have all the characters of the Italian ryegrass but carry persistency of the perennial parent would be highly desirable. Since both the species are self-incompatible at the diploid and tetraploid level (although the latter produce seed under forced selfing), they can be hybridized without emasculation by paired crossings. Such crosses were made during 1969 and 1970, by mutual inter-pollination of the unemasculated spikes under controlled growth conditions. The Italian ryegrass characters of fluorescent-roots and awned florets were used as genetic markers to distinguish the hybrids from selfs in the perennial (♀) x Italian (♂) crosses. Both the characters were dominant. On average, 53% of the tetraploid and 75% of the aneuploid progeny of the perennial parent was hybrid as shown by root-fluorescence. Awning as an additional genetic marker showed that nearly 83% of the tetraploid and 89% of the aneuploid progeny was of hybrid origin. In the reciprocal hybrids, F_2 segregation for the fluorescent-root phenotype showed that about 93% of the F_1 plants were of hybrid origin.

Cytogenetic studies revealed that the hybrids has a high multivalent frequency. The mean chromosome association at the first meiotic metaphase was 0.6 I + 6.5 II + 0.2 III + 3.5 IV in the perennial x Italian hybrids and 0.6 I + 5.8 II + 0.2 III + 3.8 IV in their reciprocals; thus about 50% of the genome was present as multivalents. The high multivalent frequency in the F_1 and segregation ratios of 20.8:1, 11.0:1,7.7:1, 5:1 for the root-fluorescence in the F_2 showed that homeologous rather than homologous chromosome association occurred and chromatid rather than chromosome segregation prevailed, which permitted a free recombination between the parental genomes.

Aneuploids (2n = 26, 27 and 29) were present in the progeny of tetraploid crosses; their frequency was about 9% among hybrids with the perennial and 23% with the Italian as the seed parent.

The hybrids were highly fertile with 90% pollen stainability and about 15 g seed per plant. These allopolyploids, therefore, behaved like the heterozygous autopolyploids. From breeding view point, a high degree of variation, an extremely valuable asset in selection, would be anticipated in their subsequent generations.

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Species crosses in the family *Brassiceae* aiming at the creation of new fodder crops

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ABSTRACT

Thanks to the pioneer work of Karpechenko, Morinaga and U and numerous investigations carried out since then, particularly in Japan and Europe, there is a wealth of information available concerning species relationships in the family *Brassiceae*. This information illustrates, among other things, in an excellent way one major instrument in the process of species formation, that of hybridization in combination with polyploidy. Of particular interest to plant breeders is the fact that the three polyploids in the genus *Brassica*, two of which are important crop plants, can be resynthesized comparatively easy. But many species hybrids, other then those, which have been successful in nature, are possible to create. This has been repeatedly established in various investigations. This fact should also be tempting to plant breeders since many of the diploid species in the family are important crop plants. In the present paper some preliminary results are given from recent investigations at the Cytogenetic division. These investigations are part of a project aiming at a more intense use of cytogenetic knowledge and technique in the breeding of *Brassica* crops.

Marrow stem kale is more productive than fodder rape under Swedish conditions. However, the growth rhythm of marrow stem kale is too slow to allow this crop to be grown under the short summers in northern Sweden, where most of the fodder rape is grown. One possible way of speeding up the growth rhythm and still retain the high productivity of marrow stem kale could be to produce a hybrid between marrow stem kale and fodder radish. This hybrid has already been successfully produced, i.a. in recent years by doctor I.H. McNaughton at the Scottish Plant Breeding Station, Pentlandfield. Four tetraploid fodder radish populations were used in the crosses. The marrow stem kale material

used consisted of 39 F_1-combinations of 15 different tetraploid populations. The majority of crosses were made with fodder radish as mother parent. A total of 1.123 hybrid seeds were obtained from 5.288 flowers pollinated in this combination. The reciprocal cross yielded 13 seeds from 227 flowers crosses, however, none of these were hybrids. Pollen fertility was rather low in the hybrid population, on an average 39 per cent. The seed setting was also extremely low, on an average 0.06 seeds per flower. Pollen fertility has improved to around 60 per cent after a rather weak selection in the two following generations. At the same time seed setting improved to 0.24 seeds per flower in F_2 and to 0.53 in F_3. Sufficient seed for a small hand-sown field trial was obtained already from the crosses on the F_1-material. Three *Raphanobrassica*-populations were compared with the best marrow stem kale variety available, the tetraploid Sv. Tema. Plants were harvested at three different occasions. The three hybrid populations out-yielded the marrow stem kale variety at all harvest occasions with the exception of one hybrid at the last harvest. The superiority in dry matter yield ranged from 4 to 75 per cent. Digestibility appears to be as good as or even better than that of marrow stem kale and the same holds true for protein content, this in spite of the fact that most of the hybrid plants were in full flower at the time of the harvests. Of particular interest is that some of the hybrid material produced a yield at the first harvest (15/7), which was almost the same at that of the marrow stem kale at the last harvest two months later. The leaf to stem ratio of this hybrid material was almost 1 - 1 at the first harvest occasion.

The fodder radish material has also been crossed with various *Brassica campestris* types, such as *chinensis*, *narinosa*, *pekinensis*, *perviridis* and *nipposinica*. The yield of hybrids was much smaller in these crosses (0.002 to 0.01 seeds per flower crossed) than in that between radish and marrow stem kale (0.21 seeds per flower). These hybrids appear to be even more sterile than the perviously mentioned ones.

Crosses have also been performed between marrow stem kale and the *Brassica campestris* types earlier mentioned. The yield of hybrid seeds in these crosses is also much lower than that from the crosses between fodder radish and marrow stem kale, the yield ranging from 0.001 to 0.006 seeds per flower crossed. *Brassica narinosa* is an exception to this both with regard to the crosses with marrow stem kale and with *Raphanos sativus*. The *narinosa*-material in both these crosses happened by accident to be diploid. The cross between this diploid parent and the tetraploid marrow stem kale yielded 0.36 hybrid seeds per flower crossed, that with tetraploid fodder radish 0.16 hybrid seeds. Naturally, all the hybrid seeds were triploid. However, these triploid plants produced gametes with full sets of parent chromosomes in a very small quantity. Allopolyploid plants were obtained from crosses between these triploids.

A preliminary study of interspecific hybrids between *Brassica napus* L. and *Brassica campestris* L. as alternatives to forage rape

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ABSTRACT

B. napus is an allotetraploid (2n = 38, aacc) derived from the diploid species *B. campestris* (2n = 20, aa) and *B. oleracea* (2n = 18, cc) (U, 1935). The latter two species possess well defined self-incompatibility systems, particularly thoroughly investigated in *B. oleracea* (Thompson, 1957), in which species it has been exploited in the production of F_1 and double cross (DX) hybrids both of agricultural and horticultural varieties. F_1 hybrids of forage rape and intervarietal hybrids of swede (*B. napus*) have been shown to exhibit significant heterosis for yield (Johnston, 1971, McNaughton and Munro, 1972), but since *B. napus* is generally self-compatible the production of F_1 and/or DX hybrids, and exploitation of this heterotic gain is at present precluded in these crops.

During the synthesis of Brassica napocampestris, an autoallohexaploid (2n=58, aaaacc) of *B. napus* and *B. campestris* at the Scottish Plant Breeding Station (McNaughton, in press) the initial sesquidiploid hybrids (2n = 29, aac) were observed to be very vigorous. Since *B. napus* and *B. campestris* hybridize extremely easily (Palmer, 1962) this raised the interesting possibility of utilising the self-incompatibility of the latter to enforce natural hybridization with the former, the subsequent sesquidiploids being grown as a higher yielding alternative to true forage rape.

As a first step, in examining the feasibility of this, single plants of *B. campestris* ssp. *rapifera* (turnip cultivars) were isolated each with a single plant of *B. napus* (rape cultivars). The seed from the turnips was then sown in a randomised replicated trial with the parental cultivars as controls. The frequencies of sesquidiploid hybrids amongst the turnip progenies ranged from 30-95%. The hybrids (turnip progenies) outyielded their respective controls, having an overall yield advantage of

20% fresh weight, and 13% digestible dry organic matter.

If this increase in yield can be translated into a field scale crop the production of these sesquidiploids would be an attractive proposition. There would be many problems, and an increase in the cost of seed production is inevitable. However, further work is in progress at the S.P.B.S., aimed at the isolation of self incompatible lines of *B. napus*. Using this material seed production problems would be greatly alleviated. Apart from the exploitation of the interspecific heterosis for yield, this particular combination provides a means for the production of a 'forage rape' with a broad spectrum of resistance to clubroot (*Plasmodiophora brassicae*) by introduction of resistance from both species.

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DISCUSSION

Chairman: Dr. Wit

Lange: Just one little question. I have not understood one figure. In table 1 you pointed out that in the cross *Lm* x *Lp* you got 88% fluorescence in your *F1*. What about the rest 12% non fluorescence? How do you explain that? You should expect 100%.

Ahloowalia: We thought the plants are heterozygous. What I am telling you is the mean of 7 different crosses. Now there are progenies were you find 94%, 100%, 78%. So if *Lm* was recessive for one locus certain non-fluorescence plants would appear. Each mean of a cross was based on 200 plants each.

Emecz: Would it not be worthwhile to use emasculated parent plants in the case of such a study, because you observed about 10% selfing and it would make your study easier and pure.

Ahloowalia: I think it is quite a laborious job if you want to handle 30.000 seedlings. We started the material for breeding not for genetic studies.

van Bogaert: How did Mr. Ahloowalia obtain the *F2* generation?

Ahloowalia: By keeping something like ± 200 F_1 plants in open pollination with each other. The group was isolated.

Nitzsche: Dr. Ahloowalia found about 60% of the crom. associated in quadrivalents and found poor chrom. segregation. Was it possible to estimate whether the same chrom. were always involved in meiotic pairing?

Is Dr. Ahloowalia of the opinion that the chromosome carrying the florescent character is always included in the quadrivalents?

Ahloowalia: It is not possible to distinguish the chromosomes in the quadrivalents. What I showed you is the mean quadrivalent frequency. There are *Lp-Lm* hybrids with all the genomes associated into quadrivalents, whereas in the recipricals anything between 45-71% of the genomes were associated in quadrivalents, which is nearly anything up to 4-5 quadrivalents. In other words the multivalent frequency was very high. It would seem to me that with this kind of multivalent frequency the particular chromosome which carries the fluorescent character must either be included in the quadrivalent or in the homoeologous pairing.

Duyvendak: What does the word sesquidiploid mean?

I can translate it, but why or how do you use the word?

Mackay: Rape (*Brassica napus*) is an allotetraploid of B. *campestris (AA)* and B. *oleracea (CC)* and has the genomic formula *AACC*. If you cross this with B. *campestris* the subsequent hybrid contains two *campestris* genomes and one *oleracea* genome. A plant of this genomic composition *(AAC)* is called a sesquidiploid, it has 1½ genomes.

Jones: I would ask why one expects heterosis to be correlated with frequency of hybrids?

Mackay: Hybrid vigour is heterosis.

Jones: That is hybridity, but not the frequency of crossing. These are two different things I think.

Mackay: I would have thought that heterosis, if it must due to hybridity, should have been positively correlated with a number of hybrids within a plot. The more hybrids the greater heterosis over the control. But in fact we did not establish this, which rather surprised us.

Jones: Are you saying that the degree of crossibility reflects the genetic difference between the parents?

Mackay: I don't think so. Can I put it in another way perhaps? If we obtained nothing except selfs of the turnip seed parents itself, then we had no hybrids at all in a particular plot. Then we would not anticipate any heterosis. If we had 100% hybrids we would expect maximum heterosis. When I took the anomalous families out the correlation was there. Unfortunately the comparison is not a very good one, because the yield of the hybrids was in fact the yield of the single paircrosses and the yield of the rape controls was the yield of the varieties, or samples of varieties. So I think the only figures that mean very much is the overall 20% rather then taking an individual paircross and looking at its control.

Toxopeus: The heterosis has been compared only with the rape parent for reasons of the fact that the production of these sesquidiploids are above-ground and they have not got a root like the turnip,(*B. campestris*). Now we are generally of the opinion in Holland that turnips will outyield rape mainly because they establish more easily, more quickly, they get into the crop stage earlier than rape does. Is this also the opinion in Scotland?

Mackay: I agree with you. Perhaps to make it clear the turnips we used were two varieties. They are not the more typical Dutch fodder turnips, what they call stubble turnip, which are extremely fast growing, and high yielding. These are Aberdeenshire Scottish type turnips, usually grown as an alternative to swedes. They are usually sown quite a lot earlier. Rape will be sown in Scotland any time from the beginning of June to the end of July, whereas the Aberdeenshire type turnips will be sown in May. And in this particular trial these turnips are included as well as controls. They were treated as a rape. They were sown late, they were not thinned to the same extent and they performed very badly. They formed very poor bulbs and their leaf yield did not compare at all with the rape or the hybrids. We will use Dutch material in later crosses and we are interested to see how they are in comparisons.

Toxopeus: About self incompatibility I did a little work on this myself and of course the Japanese did quite a lot. There is a percentage of plants that are selfcompatible. Of course you may say that their S-alleles action is very poorly defined. But this probably explains part of the variation that you get. You would first have to work more on the selection of S-allele inbreds. We found it difficult and too laborious to bud pollinate. The flowers are so much smaller than those of cabbage that you can't really compare the labour intensity of the two. How do you visualize the production of a fair amount of seed of your inbred lines before you make the hybrid-vigour cross?

Mackay: We have in fact successfully introduced selfincompatibility into the rape side as well. And if we can use the seed from both parents, I agree rape is easier to bud pollinate than turnip, than it may become a commercial proposition.

Ahloowalia: I want to ask Dr. Sjödin, what you would expect from normal euploids in Brassica crosses in the first generation. Do you run into trouble with aneuploids in subsequent generations?

Sjödin: I am quite convinced we have aneuploids too, but we have not checked it up. But you can expect a rather high frequency of aneuploids probably.

Ahloowalia: Do you find any morphologically deviating phenotypes from the *F1* hybrids or parents?

Sjödin: We have not found any distinct variation in the *F1*. You mean some plants should be monosomic or trisomic and so on?

Ahloowalia: Any kind of aneuploidy might lead to fertility problems as you have shown. At the first generation your fertility was low, at the second it was high. I was wondering if these aneuploids have also become part of the whole multiplication and they just stay, and your fertility will not go up any more, till you rooted them out.

Sjödin: Unfortunately I have no information about that. We have tried to run this as practically as possible and we have not made any cytogenetical investigations.

Mackay: I have just something to add to Dr. Ahloowalia about this question. In fact it is my colleague's experience that the initial *F1* hybrids do vary. You do obtain some of these very diminutive plants, but on checking them cytologically they are quite well balanced. And it is rather odd that the most fertile *F1* hybrid raphano-brassica has turned out later to be an aneuploid.

Ahloowalia: I was expecting that because aneuploids in ryegrasses survived for three years in very hard winters. And fertility can be on a quite good rate. They are not limited on their own.

Mackay: The Brassica's seem very tolerant with chromosomes. The sesquidiploids mentioned are more or less sterile, but one expects them to be sterile because they are allotriploids. But in fact if you leave them in a field situation you get quite a lot of seed.

Sjödin: Is there anybody who has information of the present situation of the work with *Raphanobrassicc* -t Aberystwyth?

Thomas: At Aberystwyth they have the same problems in getting fertile amphidiploids. We have looked at the cytology of the amphingploids and find that they are very regular, but the embryo's abort in the pods.

Sjödin: That is our opinion too. We have looked at the meiosis of some of the plants with very bad fertility and they too were surprisingly regular in the meiosis. So the bad fertility is probably caused by something that happens later on.

Toxopeus: What does the panel think of the work of people like Karpechenko and Howard before the war. They were apparently able to get amphidiploids after crossing on a diploid level. I do not think they used colchicine. And it is apparently very difficult in this stage at various places to cross *Raphanus* and *Brassica* on a diploid level and then despite the use of colchicine double up the chrom. number. I would like some comments.

Sjödin: It is out of question that it is much more difficult to cross at a diploid level. But I do not know what is the frequency of seeds these people obtained, probably not very much. It is much easier to double the parents first and then make the cross.

Toxopeus: Of course I do not think they quote figures about fertile plants. I remember that some of these people were working with a F_5, so I assume there would be a reasonable fertility and it was not particularly difficult to get seed and push them through generations. I do not know why they did not make use of it in agronomical terms. What I read and hear from McNaughton problems are more serious then one would gather from the old literature.

Sjödin: Well there is a wide variation within the *Raphanosativa's* and I think in these investigations they did not use the proper *Rapl..nus* type which is used today both in Scotland and in Aberystwyth and with us there are more agricultural crops than the earlier types used. That is also the reason why nothing has happened from the agricultural point of view.

Toxopeus: You are using fodder radish, whereas they were using consumption radish.

Sjödin: Probably yes.

Mackay: About the colchicine treatment. Mc.Naughton has attempted a cross on a diploid and a tetraploid level and as far as the frequency of hybrids concerned it is about the same. But he found that on doubling his haploid *Raphanobrassicae* although meiosis was stable, they were absolutely sterile. And he gave it up in favour of crossing at a tetraploid level with a little more success. About Karpechenko. He was very lucky. I think Howard did in fact have sterility problems. McNaughton has now got to F4, F5 with some of his material and seed set is improving.

Ahloowalia: I want to ask Dr. Sjödin about the use of the *Raphanus* parent, *Raphanus sativa* the edible type with a big root. I have seen some varieties, which can produce roots up to may be a foot long and about 4 inch diameter thick, which are often fed to cattle like turnips. If one tried to incorporate that character also in *Raphanus*, you would have root yield, as well as the shoot yield. I believe that all the *Raphanus-brassica's* have been selected only for the top growth rather than for the underground growth.

Sjödin: Karpechenko probably used such a *Raphanus* type but I really doubt that the plant would be capable to give a good production above and below the soil surface. That looks too good to me to believe it.

Mackay: Dr. McNaughton has some evidence on this. He has not tried the large ones, but has attempted the edible ones. He obtained no hybrids. I do not really think it is a practical proposition because in our experience, Dr. Sjödin mentioned that, his *Raphanobrassica's* are inclined to bolt. This is a new problem in fodder radish in the U.K. and this is probably the reason why there is not much gain in the ground. Dr. McNaughton has made selections within fodderradish of bolting types and he has been very successful, but as the bolting tendency decreases they tend to produce a bulb as root perennating organ. In our experience sheep don't like this very much. I do not know whether it is the taste or something else. In trials where they have been grazed they are left behind.

Toxopeus: From the *Brassica* and *Raphanus* species that have been used for interspecific or intergeneric crosses *Brassica oleracea* is the only one that has what might be called a vegetative stem, which sometimes may even be bulbous. *B. oleracea* will never form a bulb under ground level. I think from the experience of crossing cabbage *acephala* with the turnip, it has been found that apparently physiologically the combination of stem and bulbous root is impossible. It takes only two generations before you find either type and not a combination. In the first generation you can find the combination of root and stem but subsequently they segregate out and you do not get the intermediate type any more. Perhaps a similar situation would occur with *Raphanus* × *Brassica's*.

Sjödin: It sounds reasonable. I do not know anything about it. We do not use bulb types.

Kööts: I should like to ask Dr. Sjödin, what is the fodder value of the hybrids?

Sjödin: Well we have tested the digestibility. Of *Raphanus sativa* it is better than of marrow stem kale. And the digestibility of the *Raphanus-brassica* types which I was showing in the figures from the yield trial, at least at the first harvest was better than in marrow stem kale, but not as good as in *Raphanus sativa's*. That is all the information I have about this. We evaluated the digestibility by the normal in vitro method.

Registration of heterogenic populations

by

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ABSTRACT

Before entering into the homogeneity problem in variety registration which is the actual subject of my paper I am asked to spend a few words on a taxonomic problem. The question has been raised how hybrids between species or genera are being registered in the Netherlands.

In general we have tried to avoid the use of hybrid taxa as much as possible. The nomenclatural system is based on the type method. One individual is described in detail and the cluster of related individuals is given the same Latin name. The borderlines between the clusters are not defined, and neither are the gaps.

The simplest conception of this taxonomic system consists of a restricted number of type-specimen defining large genera and species.
In some cases we see introgression of genes from one taxon into another through wide crossings. *Solanum andigenum* genes, for example, have been introduced into the common potato, *S. tuberosum*. The genetic variation of *S. tuberosum* has been widened this way. But in our country this did not lead to the use of a different Latin name.

In another example crosses have been made between *Festuca arundinaceae* and *Lolium multiflorum*. The resulting fertile varieties were classified in our trials either as *F. arundinaceae* or as *L. multiflorum* according to the majority of their characters. We did not use the name *Festulolium*. But unfortunately no variety has as yet been inserted in the official Netherlands Register for Plant Varieties.

A case which is well known is *Brassica napus* (2n=38) which has been resynthesized from *B. campestris* (2n=20) x *B. oleracea* (2n=18). One should, however, take notice of the fact that *B. napus* is considered as a true species. The designation x *Brassica napus* is not being used.

Concluding I would like to say that in our country as far as the agricultural crops are concerned the taxonomic systems used as an aid for the classification of varieties. It is not felt to be restrictive for any plant breeding or hybridization work.

If a species is eligible for plant breeders rights the varieties which are enriched with genes from elsewhere are not being excluded.

In the Netherlands an official Plant Variety Register serves as a basis for the granting of plant breeders rights, for the admittance of varieties into the trade and for the seed certification.

A variety is entered into the register by the establishment of an authorized variety description. This description is drafted by a variety research institute and approved by the national registration authority. It expresses the conviction of the responsable experts that the new variety is different from those already known.

A plant variety is conceived as a distinct genetic unit that is capable of true reproduction and that might have an agronomic interest (taken in its widest sense).

The new variety in order to be registered not only must be distinct and stable, it must also be sufficiently homogeneous. The required level of homogeneity clearly is a compromis conditioned by biological, technical, economical and other aspects.

This introduction is meant to explain how the requirement of an efficient homogeneity for registration is understood in our country, especially with regard to the agricultural crops.

Dependent on the mode of reproduction of the crop there are different kinds of varieties. In article 2 of the Paris Convention for Plant Breeders Rights of 1961 are mentioned: clone, line, stock and hybrid, which we can define as follows:

- clone, offspring produced through vegetative propagation.
- seed clone, offspring produced by seed without reduction and fertilization,
- line, offspring through self fertilization,
- stock, offspring produced through cross fertilization,
- hybrid, offspring produced by crossing two or more clones, lines or stocks.

Clone varieties. Generally speaking in vegetatively reproduced crops the varieties consist of a single clone and are, therefore, isogenic. Impurities, mutations, etc. do not belong to the variety and are not described.

In dependance of the environment the genotype can produce different phenotypes. The variety description is the description of the average phenotype produced in a restricted and preferably well defined set of circumstances. It is expressed in words that may suggest to have an absolute meaning but the description only gives the position of the variety in relation to other described varieties.

Seed clone varieties. These occur for instance in crops with apomictic seed formation. From the viewpoint of variety registration the seed clone does not differ from the vegetative clone. Only varieties consisting of a single seed clone are capable of protection and unaltered reproduction. A limited number of off types is tolerated in the samples, but they do not enter into the variety descriptions.

Line varieties. In truly self fertilizing crops varieties can be fixed till they fit the model of the pure line, that is completely isogenic. But in practice the self fertilization nor the selection are so rigid, to the theory.

A blend of distinct lines however, is not capable of protection otherwise than through the description and the protection of the components.

Stock varieties. Varieties of cross fertilizing crops (excluding hybrid varieties which will be treated separately later on cannot be inbred due to the consequent loss of vigour. Each variety, therefore, consists of a heterogenic population. Even within a uniform environment the individuals form a range of phenotypes.

Population genetic considerations and investigations executed at our Institute have taught us that the heterogeneity of these cross fertilized varieties is not at all chaotic.

When a basic population is split up into sub populations through selection by both nature and man and when these subpopulations are multiplied during several generations, the resultant varieties always are in compliance with a certain model.

For each linear measurable character the phenotypes of the variety will show a frequency distribution that is near to the normal or Gausz distribution. This normal distribution can be characterized by two parameters, viz. the mean ($\bar{x}$) and the standard deviation (S.D.). The variety description is based on the mean only. The standard deviations assessed in the trials are used as a measure for the homogeneity of the varieties. A stock variety is considered to be sufficiently homogeneous when for each of the investigated characters the standard deviation does not exceed that of the established varieties grown in the same trial.

The breeding work is most effective when it is executed as follows. The existing variation is gathered and enriched by purposefull crossings. The total variation is canalized into distinct and reproduceble stocks, as many as possible, which are stored for further evaluation and selection. This breeding method can be named stock selection.

The clear separation of stock making and evaluation is essential for it enables the breeder to repeat the testing of large series of genotypes under different conditions, in different countries, etc. The multitude of purposes for which grasses are being used makes this advantage very evident, but the same holds true for any other species.

Between complete self fertilization and complete random mating many transition cases exist. The respective varieties can in majority be classified as stock varieties.

It should be stated explicitely that in all three models treated so far only one single genotype is described in its expression, registered and protected. In the case of stock varieties this is the genotype of the individual that represents the average value for all characters. During the course of the trials it is always checked whether this average individual really exists. In a population with a bimodal frequency distribution for one or more characters the average individual might be missing. In this case the variety cannot be registered on the stock variety model.

To put it briefly:

A homogeneous stock is a population reproduced by cross fertilization with limited genetic variation. The residual genetic variation is polygenic (due to many genes with small effects). The expression of the residual genetic variation is undistinguishable from the effects of modifications.

A stock variety is considered to be sufficiently homogenous for registration when, as far as can be deduced from the characters assessed, the investigated samples are in agreement with this model and the total variation found does not exceed that of the established varieties.

Clone-, line- and stock varieties remain stable during successive multiplications. This is not so with the last group.

Hybrid varieties. When for the production of certified seed a number of clones, lines or stocks are crossed and multiplied according to a definite formula, we get hybrid varieties for which at the end of each cycle a satisfactory degree of stability can be achieved irrespective of their homogeneity.

The number of male and female parents, influenced by such factors as emasculation and male sterility, the way in which the parents are combined and the number of generations are decisive for the genotype or genotypic composition of the resultant hybrid variety. This variety can be isogenic or more or less heterogenic. Often the heterogeneity is not unimodal and cannot be described with one parameter.

Lack of homogeneity limits the possibilities for distinction. It is not clear at this moment how the concept of distinctness has to be interpreted with respect to a polymodal or polymorf hybrid variety. This is a matter for further consideration.

In the Netherlands for the judgement of the homogeneity of hybrid varieties the existing and preferably the leading varieties were taken as a standard. In some cases this standard is low, but there is a shift towards higher standards. The prevailing principle remains that the level of homogeneity required for new varieties is related to that of the varieties in common use.

Plants breeders rights on a hybrid variety do not imply the protection of the basic components. Breeders are therefore recommended to seek protection for these basic components directly, when this is possible. In grasses and forage crops this possibility is limited to lines and stocks. There has not yet been developed an adequate technique for growing breeders clones in trials for plant breeders rights.

DISCUSSION

Gillet: You have spoken about lucerne. Our varieties are based on for instance four clones, which we let interpollinate. The seed harvested is then multiplied for a definite number of generations. Is this for you a hybrid variety?

Duyvendak: In France this is called a "hybrid variety on clones". In this case of lucerne you have four clones, you polycross them and then multiply in isolation for four generations. This is the formula of the "hybrid variety on clones".

Wright: In your paper you talked about stock varieties and you made the statement: "In a population with a bimodal frequency distribution for one or more characters, the average individual might be missing. I would like if you would explain that before we talk about heterogenity in a hybrid population.

Duyvendak: We have met the case. A Timothy "variety" which was made by just putting together S48 and S51 (earliest), which were two existing Aberystwyth varieties. Each as far as the date of ear emergence is concerned with a certain distribution. What we found was that the sample delivered to us showed a horizontal piece in the graphs of cumulative frequency distribution for date of ear emergence. In a non-cumulative frequency distribution we would have found a depression. This is a bimodal frequency distribution. There is no individual which has just the average date of ear emergence. Then this individual is missing.

In a population in equilibrium we find for each polygene character a normal distribution. Furthermore between characters there is no correlation. It therefore must be very easy in a population of 60 plants to find an individual, which represents the average value for all the different characters. And if this is not present the population is not normally distributed. Is this an answer to your question?

Wright: Yes. In fact that "variety" was a straight mixture.

Duyvendak: Indeed, but it had been multiplied several generations and still it showed its origin.

Wright:When it was multiplied for several generations as a mixture it would come together.

Duyvendak: Yes.

Davies, E: I was not quite in agreement with your explanation with regard to lucerne. I had thought this would have been designated as stock variety. At least in some other countries if you say hybrid variety then you have to guarantee that there is a certain percentage of crossing say 75% or 50%. In your example you multiplied from generation to generation. That could in fact become a stock variety.

Duyvendak: That is just the reason of this discussion today: this is considered as a hybrid variety in France and as a stock variety in other countries. And I think it must be very clear today how we define it. If as Mr. Gillet has said the number of generations is restricted and fixed the population is not in equilibrium. Such a variety can be called a hybrid variety. But if a variety based on clones is produced by a Dutch grass breeder who has marketing either the second, the third or the fourth generation assuming that there is no generation shift, because he has selected against the major heritable variation, we must call this a stock variety. I think we must be very clear and say if a definite generation is produced according to a definite formula we better call that a hybrid variety. Don't you agree?

Davies, E.: No, I do not quite agree, because most of the varieties that we produce are in fact generally produced to a scheme. At least we start with a specified number of clones.

Duyvendak: But also with a specified number of generations?

Davies, E.: Yes.

Duyvendak: Is it so that you assume that there is a difference between the generation you market and the former generation? I put it in another way: "Do you think that the controlling certification office can use the same variety description and the same standard sample for the basic seed as for the certified seed generation?

Davies, E.: Yes, I would say.

Duyvendak: If these are identical, I would define this for the moment as a stock variety, and if these are not identical we better speak of a hybrid variety.

If you do not agree with the words, if you want me to avoid the word hybrid variety in the case of lucerne, I am prepared to do so. But I think we must distinguish these two cases.

Davies, E.: Yes, but these are difficulties in using the word hybrid. We use the word hybrid in hybrid ryegrass to mean hybrids between perennial and Italian and we loosely describe our varieties as hybrid varieties.

Duyvendak: That is true but I want to avoid loose definitions. Let us call it a "constructed variety". How would you like to define a variety when the two last generations are not alike. I have tried to push them into the wording of a law.

Davies, E.: Synthetic variety.

Duyvendak: Well, there are synthetic varieties which are stable from one generation to another.

Ahloowalia: In corn it may be a synthetic or a composite and a hybrid. These are two distinct things.

Duyvendak: Let us than say stock varieties, versus constructed varieties.

Horne: In complementing Mr. Duyvendak on a very clear paper, I would like to take up one or two points with him. Plant variety rights and registration commend themselves as principles. But yesterday we saw the ecotypes and I suppose that 10-20% would perhaps be as much as one could possibly expect to fit into a scheme. From some of the ecotypes we saw, the problem is greater if you try to superimpose say crown rust resistence or some of this kind. I can see the convenience in putting these into a pigeon kole. But in international seed certification we have made much more use of the comparison of the breeders stock and the commercial variety and how these compared.

And finally I would like to ask if we are not in serious danger of getting into trouble over this blend, because it is nonsense as far as I know at all the common practice to go on multiplying herbage varieties, grass varieties for example in the OECD scheme. In the National Scheme normally there is one multiplication of breeders seed or basic seed and then it comes into the certification crop. And I would have thought that here we could very much overdo this idea of not being able to bring types together. I mean what about the polycross for example.

We have this concept right through.

I would like to ask you two questions about this. Where are we getting to? Are we in danger of throwing away quite a lot of very valuable material because it does not fit neatly in the right pigeon hole from the taxonomic point of view.

Duyvendak: May I just comment on this first. Everybody will agree that a model may never block reality. We must form our models to the real situation, not the other way around. And the real situation is very complicated. We are in need of models in order to be able to make variety descriptions. If we do not have models we can not make descriptions. There is no danger for plant breeding. Plant breeders must only help us to find out the models according to which we are able to describe the varieties. That is the right order of working. Do you agree with this?

Horne: Well I think there is a great danger here in being landed with an extra job of having to take some marker genes or some difference and use it for purposes, which are really not agronomic purposes at all.

Duyvendak: I did not mention marker genes.

Since this was not the problem we were talking about at this moment. The question was according to what theoretical models can we describe the varieties. Do you agree that one cannot treat crossfertilizing crops as if they were potato clones?

Horne: No, certainly not.

Duyvendak: That would bring us nowhere. We must distinguish between different cases. At first we look at the clones and the lines, but they are not useful for the cross fertilizing crops.

Two possibilities we have left now, the first I would call stock varieties, which are not restricted as far as the number of generations is concerned and which fit the legal formula: "the variety is stable in his essential characters, that is to say remains true to the describtion after repeated reproduction or propagation".

I would assume that the majority of the grass varieties is not defined as to the generation. Am I wrong supposing this? There are so many breeders present. Do you agree with me that not one definite generation is being marketed, either the third or the fourth, just according to the market situation? I would call this a stock variety. Do breeders agree with this assumption or am I completely wrong? I see many heads nodding.

Emecz: With new varieties we have to give in the U.K. the generation that will be marketed. It is a little bit difficult to expect absolute freedom as far as generations are concerned. I can expect that there will be no major shift between the third and fourth generation as you pointed out, but I would be more surprised if you could go on unlimited and you would find no shift afterwards. I do not expect to have complete freedom. So there must be some sort of description within which the breeder can or cannot market the variety.

Duyvendak: That is right. With my model, within the limits you have given and which are considered by the breeder as well, one will not go on undefinitely. It is treated as if it were a stock variety, that is what I want to define, I want to have a model for further discussion as far as homogenities and the requirements for homogenity are concerned.

To summarize these requirements briefly: The hybrid variety is stable on account of its formula. Like the clone- or line variety the stock variety is stable on account of its homogenity. Therefore the homogenity requirements must be much more severe with stock varieties than with hybrid varieties.

Zaleski: I have two questions. First of all I would like to explain that as far as hybrids between rye grasses and any species of fescue is concerned its exactly the same approach in the U.K. as in Holland. If we get on the market any hybrid between the two species, we either fit it in fescue or in ryegrass, but what I would stress now to breeders who are producing this hybrid is that it may be a completely new species and it is not our job to define the new hybrid as a new species. It is the international body, which is dealing with taxononomy and they should define the new species.

The other point is about the lucerne hybrid. I do not quite agree with Mr. Duyvendak but I think what he meant really is not a hybrid between two different species of lucerne, because there are only two, *Medicago falcacta* and *M. sativa* and everybody knows that now there is no pure species of *M. sativa*, it is more or less already a hybrid. But there is internationally a third hybrid recognized which is called in Europe *M. varia* and in America *M. media*. And when we are talking about hybrids and I am sure French breeders will support me they are producing hybrids, they are introducing falcata blood in it. And this is called *M. varia*.

Duyvendak: There has been discussion between the French registration authorities and us as far as the description of lucerne varieties is concerned. We have tried to agree that the lucerne varieties should be described as stock varieties, with a given model of normal distribution for all the polygenic characters and homogenity with regard to the qualitative characters. The French authorities and the French breeders did not agree with this because they said there is no reason for going so far. These varieties are not being multiplied further than a certain generation, so they fit into the wording of the Paris Convention: "the breeder has defined a particular cycle of multiplication and the variety is stable at the end of each cycle". We can avoid the word hybrid and call them "cycle varieties" or "formula varieties".

Badoux: I would just like to ask a short question to Mr. Duyvendak. Do you consider in your examination the cytological stability of your hybrid? You know already that in tetraploid varieties there are a lot of aneuploids, but still more in the interspecific hybrids. If you have a variety say with 56 chrom. and part of the progeny may loose a part of the chrom. or may come back to 42 or another number. Would you accept this variety for registration?

Duyvendak: I do not think so. It is instable in some characters and therefore it cannot receive plant breeders rights.

Emecz: With the describtion of your hybrid varieties, you make the distinction of different generations. Do you require to describe this variation from generation to generation? For example you have in a variety of kale five percent aberrant plants at the third and 15 percent at the fourth generation level. Is it satisfactory if you only describe one generation and do you accept that there will be differences if you depart from that generation?

Duyvendak: If it is a small percentage of admixtures not belonging to the variety and not meant to be reproduced by the breeder, it is a matter of tolerance more than a matter of description of the variety itself. We do not describe varieties on account of small admixtures.

Emecz: According to your definition a variety is a hybrid variety if it is not constant from generation to generation. Do you wish to have that actually measured and do you want the breeder to give you the difference in range?

Duyvendak: If the basic seed generation is different from the certified seed generation, the certified seed generation is being described for plant breeders rights. The seed certification authorities are interested in having a describtion of the previous generation as well. They will need two different standard samples and two different descriptions for one variety.

Gillet: You are able to certify clone varieties for certain species. I do not quite understand why you are not able to certify the parental clones in the hybrid varieties, in synthetic varieties, such is the case for grasses or lucerne for instance.

Duyvendak: In your country luzerne varieties are certified by a control system that comprises all generations from the clones onwards. This is not the case in our country. I see the possibility but it is not done yet.

Glas: Am I right in supposing that a grass breeder can in future also apply breeders rights for a hybrid variety in grasses when he can guarantee that he will always market say the fourth generation after the clones.

Duyvendak: It is the same answer as in the former case. I think it is a possibility which is not yet present in our country and would be worth considering. We are not able to have clones in our trials, because our trials are based on seedlings and not on clones. If clones have different ages, they are not comparable and we cannot describe them. But it would be very interesting to develop this model, this possibility. The hybrids for which one can get plant breeders rights so far are hybrids on lines and hybrids on stocks, but not hybrids on clones. Therefore we go over the other model and treat them as if they were stock varieties.

Zaleski: I again want to say that in the U.K. this problem of instability of a variety is so simple, by asking the breeder how long the variety will be stable. He has the opportunity to say that the variety is only stable for five generations. Then he will have to go back to his original stock seed.

Duyvendak: Then you treat the variety during the 3, 4 and 5th generation as being stable over the generations.

Zaleski: Yes, it is. And definitely after the 5th generation the multiplication must stop.

Duyvendak: This could be called a "restricted generation variety".

Zaleski: Yes.

Lackamp: I would like to return to the question posed in the previous discussion by Mr. Horne. I think it may be possible that two varieties are distinct in agricultural characters and this distinctness can be assessed by official trials and notwithstanding that it escapes these models. How will we find a solution for this problem?

Duyvendak: In what way do you think that it will escape the models?

Lackamp: Well in this way. It could be possible that two varieties are distinct in agricultural characters. But their anatomical and botanical characters could be identical and that would mean the variety would escape to the description for the registration.

Duyvendak: To take a clear example. Let persistency be the only difference between varieties which are otherwise completely alike. This is a very difficult case for plant breeders rights.

I am afraid that we than have to use persistency as a descriptive character, which can only be established by long term trials. I would advice all breeders to avoid the case and try not to select one single variety for persistency but to combine different varieties before the selection is being started.

Gillet: My question is in connection with the preceding one. This morning you have said many things about description but nothing except at this moment about characters of agricultural value. Suppose a variety is easy to recognize but has a bad agricultural value. Can it be registered in the Netherlands?

Duyvendak: Yet it can. All varieties in order to be admitted to trade must be registered first.

Gillet: Are they allowed to be commercialized?

Duyvendak: Varieties of amenity grasses yes, varieties of agricultural crops no. For the agricultural crops we have a restrictive catalogue.

Gillet: And for the catalogue the agricultural value is taken into account?

Duyvendak: Yes. On the other hand for registration the value is of no importance, but we use every character, persistency and yield included although these are the most, so to say rotten characters one can imagine. So I always hope that breeders are using starting material with other characters as well in order to help us not taking too much time for the registration. But if these difficult agronomic characters are the only ones, we have to use them. We do not throw away varieties.

Problems posed by a sterile hybrid between two types of tall fescue: European and mediterranean

by

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ABSTRACT

European and mediterranean types are fully interfertile, provided that plants flowering simultaneously are mated. Due to winter susceptibility of the mediterranean type, this is not always easy to realize.

F_1 hybrids have a range of vigour between 70% and 152% of S 170 (dry matter yield in spaced plants nurseries, in autumn 1972).

Sward yield is more difficult to obtain due to lack of seed. Their measurements is in progress.

Fertility has been restored by colchicine treatment.

Tillers are taken from whole nursery plants in autumn, and planted in potting mixture in a cold frame. After 2 weeks, old leaf sheathes are taken off, so that the young buds become visible. The tiller bases are then soaked in 0.3 - 0.4% colchicine solution for 12 hours at room temperature. Then they are washed, soaked in water for 10 hours and planted in a cold frame or in the field.

The following spring, fertile tillers are marked, then separated in the autumn.

In a trial, 5 tillers of each of 100 clones were treated in this way. 82% of the clones and 34% of the tillers gave fertile panicles.

Fertility was estimated on their C_1 progenies. Among 70 plants, 45% showed between 1 and 2 seeds per spikelet, 15% more than 2 (S 170 = 2,27).

But the vigour was strongly depressed. Hexaploid and dodecaploid parts of 26 C_0 clones were planted in microtrial and cut at the heading stage. The loss of yield ranged from 0 to 70% (38% as an average).

Their C_1 progenies varied greatly in vigour. Most were very weak but 2 of them yielded respectively 62 and 83% green matter more than S 170 (9% and 16% more dry matter) at a vegetative cut in the nursery.

Four C_1 families, which were vigorous in the nursery, were compared with S 170 in sward yield. Sown in 1970, their average yields were equal to S 170 in 1970 and in spring 1971, then fell to a 75% level during summer

and autumn 1971, and finally resumed at least the 100% level during the whole year 1972; on one cut (17/7/1972) they even reached a 113% level.

Their in-vitro digestibilities were equal to that of S 170 on 18/6 and 13/9; 6,4% more on 3/5 (heading) and 8% less on 22/7 and 22/10.

Frequency of aneuploids and their influence on a pure stand of 4x clover

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ABSTRACT

Various aspects of aneuploidy have been studied on two populations of tetraploid red clover. In the first case, vigour and fertility was studied on euploid and aneuploid plants kept in pots in a greenhouse (Ellerström and Sjödin, Hereditas 1966). Later on, the behaviour of aneuploids in a tetraploid red clover ley was studied (Ellerström and Sjödin, Zeitschrift für Pflanzenzüchtung 1973). As expected, aneuploid plants were found to be less vigorous than euploids. The total number of flower heads per plant was determined in the first mentioned study. Because of the small number of plants it was only possible to consider differences between aneuploids as a whole and euploids. The average number of flower heads per plant amounted to 47.7 for euploids and 26.7 for aneuploids. Controlled crosses were made between euploids and between aneuploids and euploids, the result in the first mentioned cross being 55 per cent and in the last mentioned crosses 43 per cent. Thus, it is obvious that aneuploid plants are inferior to euploids both with regard to vigour and fertility.

The aneuploid frequency in the second population studied was comparatively high, 35 per cent. The ley to be studied was sown as a small field plot of 5 m^2. The row distance was 15 cm and the seed rate equivalent to that normally used, i.e. 19 kg per ha. The field plot was sown at the end of June without a cover crop. Small strips of the rows were then randomly selected in the autumn of the seeding year. The plants growing on these strips were dug up and transferred to pots for root tip fixations and chromosome number determinations (aneuploid frequency 39 per cent). The same procedure was repeated in the spring of the first year ley (a.f. 30 per cent), in the autumn of the second year ley (a.f. 18 per cent) and in the spring of the third year ley (a.f. 15 per cent).

At the last occasion the stand had been so thinned, however, that all available plants were transferred to pots. However, the border rows, which had not been deprived of plants for aneuploid frequency checks and which furthermore had been to some extent sheltered by a frame surrounding the field plot, were almost intact. Plants were also taken from these border rows for chromosome determination at the last sampling occasion (a.f. 31 per cent). From this it is evident that a considerable reduction in aneuploid frequency has taken place first in the second year ley. The following winter does not affect the aneuploid frequency although the stand in the third year ley was rather heavily thinned. Much to our surprise the aneuploid frequency in the border rows was not different from that in the seed lot. With the seed rate used (19 kg/ha) there will be 121 germinating seeds per metre of row length. According to studies of Swedish clover leys the number of plants per metre will drop to about 68 already in the autumn of the seeding year. The corresponding figure in the spring of the first year ley is 30 plants. There was a normal plant density in the field plot under study both in the first year ley and in the border rows in the third year ley. Thus, roughly 75 per cent of the germinable seeds or corresponding plants have died during the period from the sowing to the spring of the first ley, this without any measurable decrease in aneuploid frequency. An even stronger reduction has probably taken place in the border rows in the third year ley, but also here with no change in aneuploid frequency. A considerable reduction in aneuploid frequency has taken place first after a much heavier thinning of the stand. This obviously implies that there has been a considerable number of euploids which have been less vigorous and compatible than the aneuploids although the aneuploids are on an average less vigorous than the euploids. Thus, the aneuploids in this population, have had an almost negligible influence on the vegetative production, although their frequency is comparatively high. There are reasons to suppose that this would have been true also with regard to seed production.

Effects of association and density on the evaluation of lucerne genotypes(x)

by

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ABSTRACT

If spaced plant nurseries are of common use in the breeding of forage crops, another approach is represented by the study of swards. In that case, it is needed to choose between a working scheme in monogenotypic culture or in association. This problem is examined in the present paper for lucerne breeding.

For that purpose, an experimental design has been laid out in order to evaluate four lucerne genotypes, in pure stands and in binary mixtures; for the monocultures, five densities of sowing are compared.
The whole set of the treatments is submitted to two water managements (normal and half hydric supplies).

This paper is concerned by some of the results of the first six cuttings, in terms of dry matter per experimental unit (boxes) and per plant. The data are expressed as plastic responses, in terms also of direct and associate effects, and analysed according to a "mechanical" diallelic model for the set of binary associations and of the corresponding monocultures. The genotype associations are compared to: the mean partner, the better partner and the best tested genotype. The density effects are described by means of the representation proposed by de WIT; the association dynamics also are studied by a model due to this author.

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The discussion of results concerns the genotype interactions and the statistical significance of the effects (Table 1), the situation evolution in the associations (Figure 1), the association interest in confront with monocultures (Figure 2). Also the stability of situations is studied.

In conclusion, when the association effects are analysed, in terms of yield performances, the constancy of the signification of type of culture (monoculture or association) effects is emphasized. The examination of these effects, partner by partner, shows chiefly situations of competition. In most cases, the density effect seems to be low. With regard to the dynamics of mixed populations it appears a trend to an alternacy between the relative replacement rates, for a same genotype, from one cutting to another.

But in order to judge of the association and density effects in the evaluation of genotypes, the total yield of the six cuttings must be considered. In a breeding program, the aim is either to develop a variety with an absolute superiority or to search for a variety association better than the best tested genotype. The first results of the described experiment show that no association is better than the best monoculture (Figure 2). Alternative testing remains possible: genotype selection in association or in monoculture. A part from the technical difficulties which have to be not neglected, the collected data in association show that to sort genotypes on the basis of direct effects in intergenotypic competition conditions leads to misestimate the yield potential of the genotypes. In every way, it is important to bring out the relative good agreement between the several rankings (Figure 3); Therefore, the monoculture test seems to give more information with the minimum of troubles.

References

EUCARPIA (1973) - Glossaire de termes et définitions - Groupe de Travail "Compétition et sélection des Plantes fourragères", Miméo, 27 pp.

Table 1. Parameter signification at each cuttings (Dry weight per box)

	Water supply											
	Normal						Reduced					
Rank of the cuttings	1	2	3	4	5	6	1	2	3	4	5	6
Simple analysis:												
Between cultures differences	11.10^{xx}	5.15^{xx}	9.48^{xx}	9.74^{xx}	10.56^{xx}	16.75^{xx}	9.01^{xx}	4.41^{xx}	8.84^{xx}	6.60^{xx}	3.35^{xx}	9.92^{xx}
Diallel analysis:												
a. general association ability	4.62^{x}	NS	NS	5.15^{x}	NS	6.19^{xx}	NS	NS	NS	NS	NS	9.76^{xx}
b. specific association ability	3.29^{x}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
c. general domination ability (1)	36.80^{xx}	20.73^{xx}	39.96^{xx}	38.84^{xx}	39.00^{xx}	69.25^{xx}	38.92^{xx}	16.71^{xx}	31.17^{xx}	26.11^{xx}	9.84^{xx}	35.13^{xx}
d. specific domination ability	7.51^{xx}	3.59^{x}	5.11^{x}	NS	6.89^{xx}	5.51^{x}	NS	3.60^{x}	5.08^{x}	5.98^{xx}	4.15^{x}	3.99
$\frac{\text{association mean}}{\text{monoculture mean}}$ (in %)	99	105	104	103	109	108	102	99	99	99	99	103

The F value is indicated when it is significant:

to the 5% level x

to the 1% level xx

(1) Differential effect of competition, measured by the analysis of the differences (EUCARPIA, 1973)

Figure 1. EVOLUTION OF THE SITUATIONS IN THE ASSOCIATIONS FOR THE SUCCESSIVE CUTTINGS (Dry weight per box). Definition of the situations according to the ratio of performances : $\frac{\text{mixture}}{\text{pure stand}}$ $\frac{Yi/j}{Yi/i} - 1$

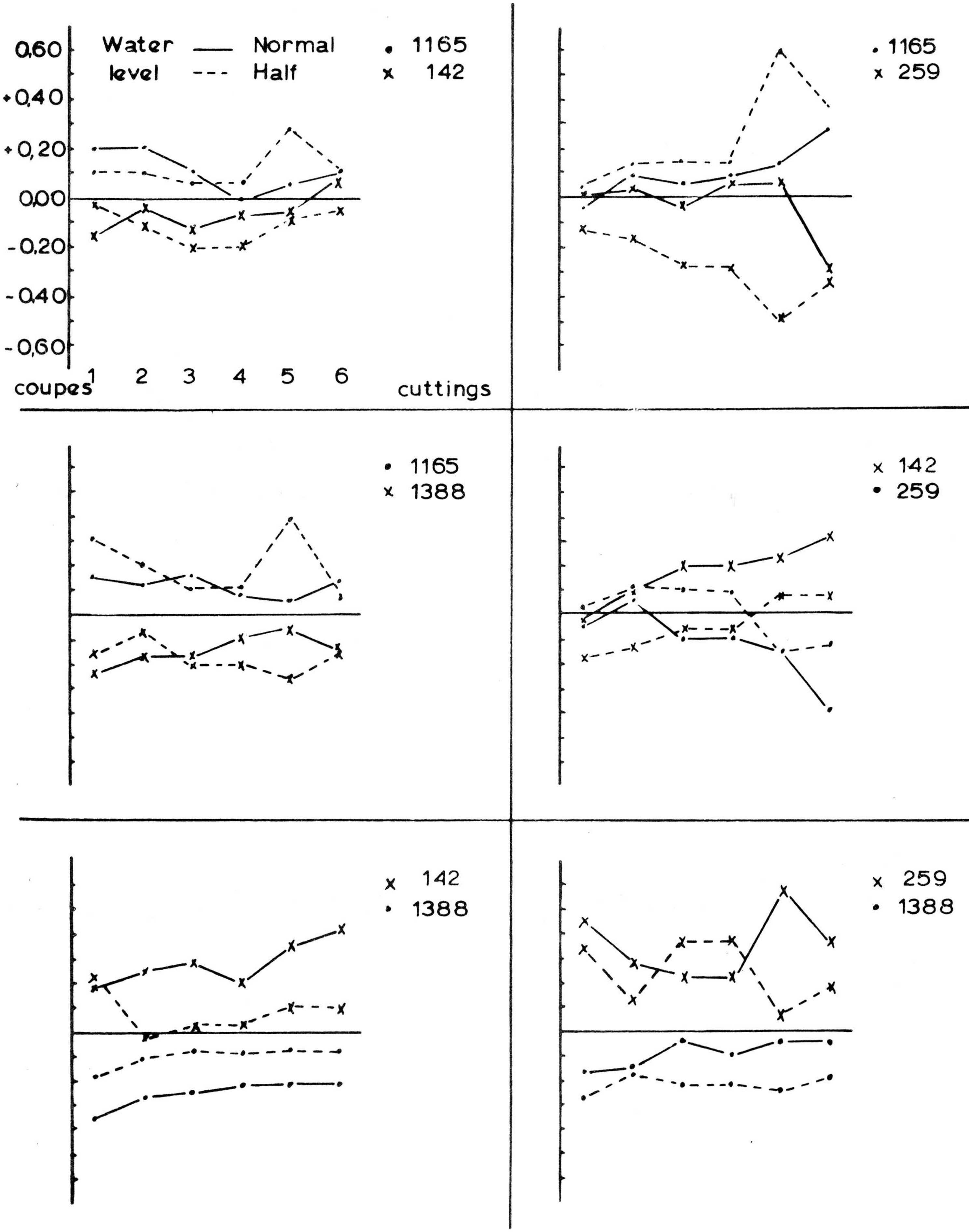


Figure 2. RELATIVE INTEREST OF THE ASSOCIATIONS IN CONFRONT WITH MONOCULTURES (Dry weight per box)

(1) $\frac{\text{Association}}{\text{Mean partner}}$ (2) $\frac{\text{Association}}{\text{Better partner}}$ (3) $\frac{\text{Association}}{\text{Best tested genotype}}$

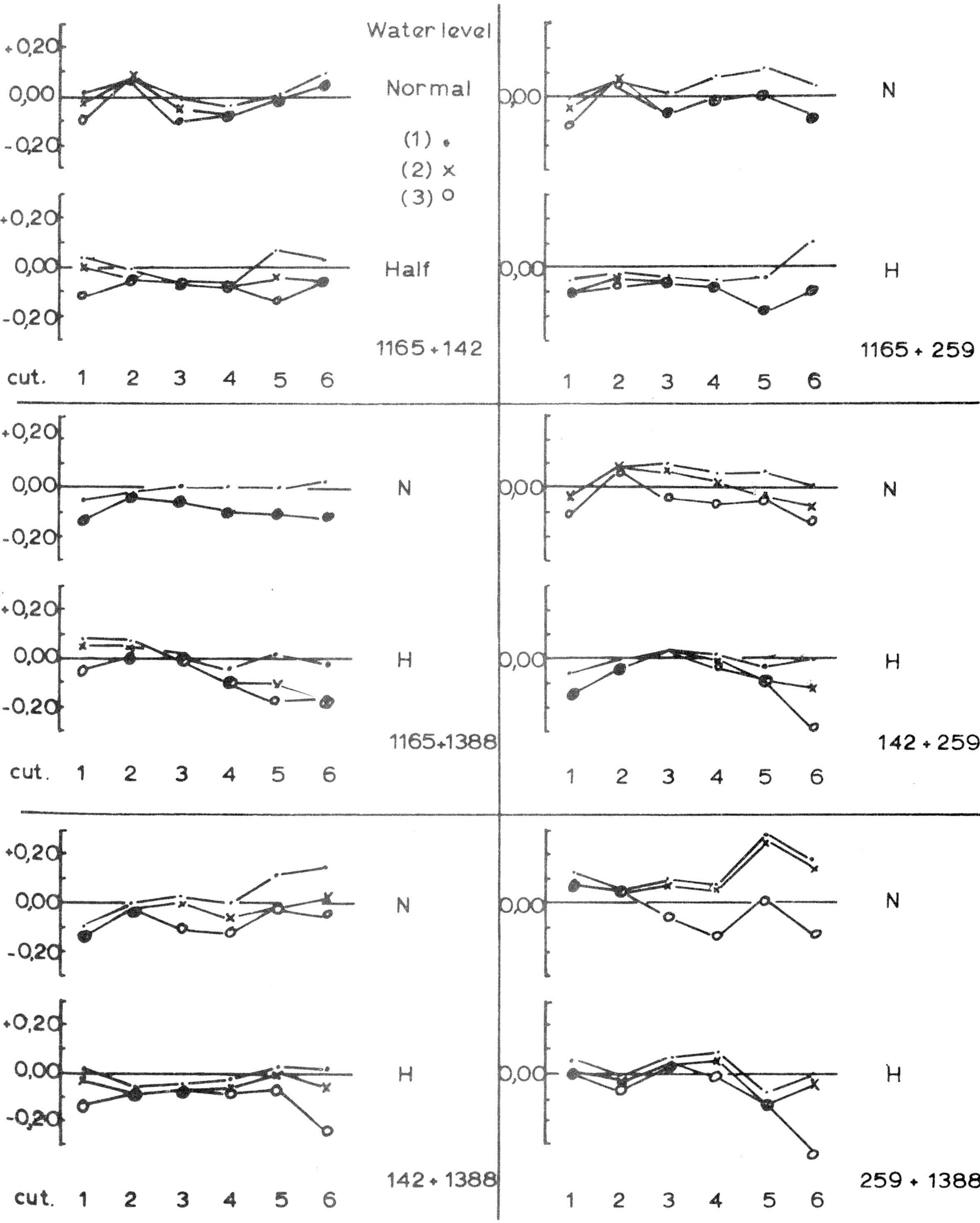
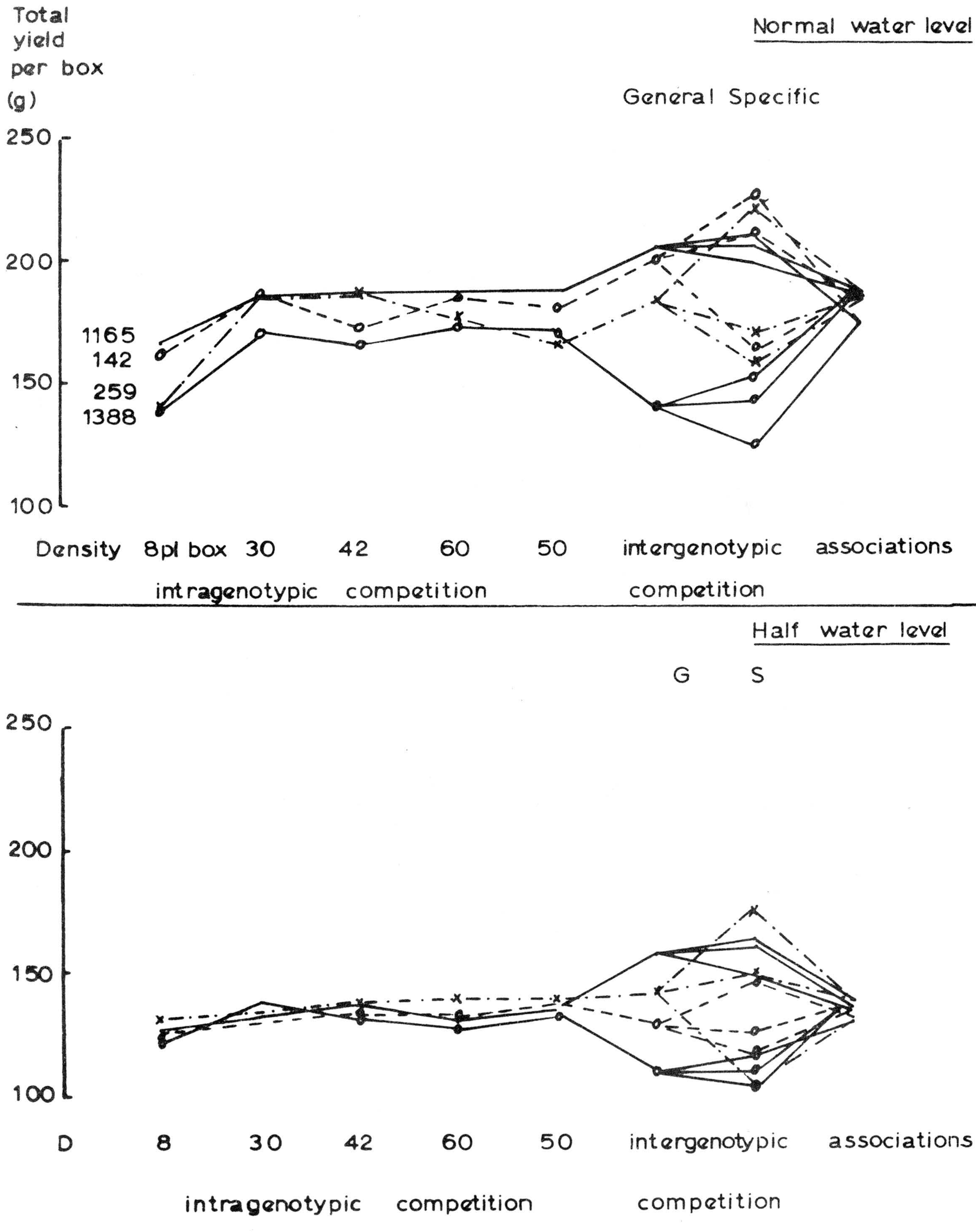


Figure 3. EFFECT OF THE CONDITIONS OF GENOTYPE EVALUATION ON THEIR RANKING



Some problems of male sterility transmission and combining ability in lucerne hybrids made with a cytoplasmic male sterility system

by

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ABSTRACT

Practical application of a cytoplasmic male sterility system in lucerne/*Medicago sativa L.*/ seems to represent an essential progress in the possibilities of yield increasing. This report is intended - on one hand - to present data on a new source of cytoplasmic-genic male sterility discovered by DAVIS - GREENBLATT 1967, and PEDERSEN - STUCKER 1969, in lucerne. On the other hand, it discusses some problems concerning the data obtained so far on the identification of steril F_1 lines with a better combining ability required for the production of hybrids. It can be stated that lucerne breeders can work with male sterile F_1 combinations in spite of the fact that the inheritance of the system has not been clarified yet.

In smaller scale investigations made between 1961 and 1967 into a possible utilization of male sterility seven clones of genic male sterility character were found in the breeding nurseries. A subjective rating in the field made of 80.000 plants in 1968 as a continuation of the investigations then a single control by acetocarmine staining resulted in 20 male-sterile plants. After a several times repeated staining - and occasionally pollen germination - control of ms plants grown in the field and in the greenhouse, respectively, as well as selection for disease resistance and other agricultural characteristics, irrespective of the environmental factors, one fully sterile and three partially (about 90%) sterile clones were left for the specific crosses. The four clones (completed by nine male-sterile clones originating from Bernburg, GDR) were crossed with 107 selected unrelated inbred (mainly S_1 and S_2) male-fertile plants in order to make an attempt to find maintainers producing male-sterile F_1 progenies.

For sterility classification by microscope F_1 lines only containing plants found male-sterile or partially sterile in the visual field rating were used. The examinations were performed repeatedly at various times with two staining techniques on 20-30 mixed or individual flowers from at least 25 plants of these lines grown first in the field then in greenhouse. In doubtful cases the classification was carried out on the basis of pollen germination. Table 1. only contains the sterility classification of F_1 lines showing an average male sterility of at least 95%.

With a further investigation into sterility transmission in view, after a repeated reproduction of male-sterile F_1 lines considered promising for the hybrid combinations 68-281 F_1 plants per line were visually classified.

Thus, when each of the 13 male-sterile clones was crossed with 107 male-fertile plants, it was only in 15 F_1 lines of the ms-19 female plant that almost all progenies were fully or partially sterile. In some lines sterility transmission was practically 100% with certain maintainer parents. The ms-19 clone originates from the Eynsford variety. By staining and pollen germination on agar medium as well as on the basis of histological and histochemical analyses it was practically found 100% sterile. The anthers are shrivelled and anthesis seldom occurs. In the 17 cross-combinations shown in Table 1. its female fertility on the basis of more than 12.000 flowers is an average of 4,7 seeds per pod.

Flowers of ms-19 female plant and its sterile F_1 progenies, as well as those of other ms plants used for crossing and of some fertile male parents were subjected to histological and histochemical analyses at different stages of development. On this basis there is a light-microscopically well demonstrable difference in structure and size between the pollen, the exosporium and endosporium, the anthera and loculamentum, respectively.

After the male-sterile phenotype had been successfully transmitted to the F_1-s, 8-13 random plants of four F_1 lines were back-crossed to the male parent. The sterility classification of a total of 842 plants of the 38 BC_1 progenies was carried out in the same way as in the F_1 plants, but plants not showing visible pollens were not later controlled by microscope. A second backcross combination was made with random plants of two BC_1 lines (Table 2).

The data of Tables 1. and 2. suggest that - in agreement with the interpretation of DAVIS - GREENBLATT (1967) and PEDERSEN - STUCKER (1969) - this new source of male sterility too can be considered as cytoplasmic-genic, on the basis of more than ten male-sterile F_1 lines obtained by crossing the male-sterile ms-19 plant with 107 pollen-fertile plants, and according to the male-sterility data of the BC progenies.

Although there are data available in the literature on the importance of both general and specific combining ability, it was assumed that the use of heterosis in true hybrids would be easier for the plant breeders if G.C.A. considered as the main source of variation (GALLAIS-GUY 1970).

By using three genetically divergent open pollinated varieties as male parents to 13 selected sterile F_1-s, G.C.A. for forage yield was estimated. For the production of three-way crosses, open pollinated populations of a 15-clone synthetic (C-I), a Hungarian local (C-II) and a variety-cross (C-III), were used as male parents. Seeds of the 39 possible hybrids were produced by hand-pollination in the greenhouse. Male-sterile single cross hybrids were produced with one cms clone and with 7 unrelated maintainer clones showing a higher seedsetting per pod in the outcrossings. Total green yield of five harvests in 1971-72 was studied in the field. Three-way crosses, single crosses and open pollinated parents were sown in a randomized complete block design with four replications, each one represented by a single row 1 m long with 100 seedlings and spaced 0,2 m between rows.

When studying the percentage values of yield increase in three-way hybrids, compared with the two parents we find the positive effect of two single crosses (cms-19 x CS-44 and cms-19 x CS-54) especially outstanding in their three-way hybrids (Table 3). Although the positive effect was find only for 57, 57 and 50% of the hybrids with C-I, C-II and C-III parents, respectively, it was suggested that heterosis did not reach maximum with the single crosses. The majority of three-way hybrids produced without using inbred lines showed, however, a positive trend.

Testing for the G.C.A. of 13 single crosses showed that the above mentioned two single crosses (cms-19 x CS-44 and cms-19 x CS-54) had a better G.C.A. compared with the other ten ones (Table 4).

Considering that in the presented data the single crosses were produced with a given cms clone only, in the result of testing for G.C.A. an important role was played by the maintainers. In the case of an adequate A-type parent (cms) the selection of B-type parents (maintainers) is thus very important. From the practical point of view of lucerne breeders the production of superior three-way hybrids seems to require the use of still more C-type tester parents (pollinators) with a wide genetic diversity. By reducing the σ_e^2 to a minimum, the AB-lines showing a stable ranking may give a good chance for the production of superior three-way hybrids.

There are field experiments with larger plots in process in which more than 30 C-parents were used (by hand pollination in the greenhouse and by leaf-cutter bees Megachile rotundata (F) under cages, respectively,) for the experimental three-way hybrids to study the combining ability of sterile AB-lines.

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Table 1. Degrees of sterility in selected 17 F_1 lines from ms-19 lucerne clone crossed to 107 male-fertile plants based on pollen staining and/or germination tests completed by visual rating.

Male parent	Number of F_1 plants per sterility class					Average % male sterility	Visual rating[++]			
	0-50 %	51-75 %	76-85 %	86-95 %	96-100 %		f	pf	ps	s
TK- 3	-	-	-	-	25	100	-	1	11	163
TK- 2	-	-	-	2	48	100	-	-	2	215
CS- 33	-	-	-	1	24	99	-	2	7	181
TK- 23	-	-	-	1	24	99	-[+]	-[+]	21	179
CS- 27	-	-	-	2	23	98	-	-	4	64
CS- 34	-	-	-	2	24	98	2	15	22	242
CS- 36	-	-	-	3	22	98	-[+]	5[+]	22	144
CS- 17	-	-	-	4	21	98	2	7	25	99
CS- 29	-	-	-	4	21	98				
TK- 16	-	-	-	5	20	98	-[+]	1[+]	31	171
CS- 8	-	-	-	5	20	97				
CS- 44	-	-	-	6	19	97	-	-	5	230
CS- 46	-	-	-	7	18	97	-	3	9	72
CS- 9	-	-	-	9	16	96	1	8	21	139
CS- 54	-	-	-	10	15	96	-	1	28	184
CS- 32	-	5	1	4	40	96	1	1	12	162
CS- 38	-	-	1	9	15	95	-[+]	5[+]	60	204
<u>Some other data:</u>										
ms-19xCS-61	1	-	2	-	7	89				
ms-19xCS-50	-	3	2	4	4	88				
ms-80208xCS-17	-	5	2	2	2	78				
ms-07xCS-17	-	3	2	2	-	76				

++ = fertile; partial fertile; partial sterile and sterile

\+ = controlled by microscope

Table 2. Degrees of sterility in backcross populations of selected lines based on pollen staining and/or germination tests or visual rating.

Pedigree of males	Population	Plants per sterility class				Total
		0-50%	51-85%	86-95%	96-100%	
TK- 2	BC_1	-	-	-	136	136
CS- 44	BC_1	-	-	4	165	169
TK- 23	BC_1	-	5	4	172	181
CS- 32	BC_1	4	24	14	314	356
TK- 2	BC_2	-	-	1	12	13
TK- 23	BC_2	-	-	1	13	14

Table 3. Percentage values of total green yield increase in three-way hybrids compared with the two parents at 1971-72

Single cross females	Male C-I.			Male C-II.			Male C-III.			Average yield of parents kg			
	$\bar{P}_1$	$\bar{P}_2$	MP	$\bar{P}_1$	$\bar{P}_2$	MP	$\bar{P}_1$	$\bar{P}_2$	MP	Single cross +/	C-I.	C-II.	C-III.
cms-19 x CS- 9	14,3	1,4	7,9	7,4	3,8	5,6	-	-	-	72,9			
cms-19 x CS-32	0,0	0,0	0,0	0,6	3,4	2,0	7,5	2,0	4,8	77,5			
cms-19 x CS-36	3,3	-1,1	1,1	0,0	0,0	0,0	0,0	0,0	0,0	78,7			
cms-19 x CS-44	16,1	11,3	13,7	20,6	26,0	23,3	11,0	7,2	9,1	78,8			
cms-19 x CS-54	53,0	6,6	29,8	46,6	11,4	29,0	51,8	6,6	29,2	57,3			
cms-19 x TK- 2	0.0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	68,0			
cms-19 x TK-23	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	74,9			
											82,2	75,4	81,6

+/ L.S.D.$_{.05}$ = 0,13

M.S. = 237,668^{x}

σ_G^2 = 39,454

σ_P^2 = 119,305

P_1 = single cross parent

P_2 = C-parent

MP = $P_1 + P_2/2$

0,0 = negative effect

\- = no 3-way hybrid

Table 4. Ranking of male-sterile single crosses obtained by cms-19 x 13 different maintainers according to its yielding ability with three tester parents at 1971-1972.

Rank	With tester parent C-I.	C-II.	C-III.	With average of 3 parents	Average yield kg
1.	CS-44	CS-44	CS-44	CS-44	0,913
2.	CS-17	CS-17	CS-54	CS-17	0,874
3.	CS-54	CS-34	CS-29	CS-54	0,862
4.	CS-36	CS-54	CS-46	CS-46	0,795
5.	CS-46	CS-33	CS-32	CS-32	0,785
6.	CS-29	CS-32	CS-17	CS-34	0,782
7.	CS-33	CS-27	CS-33	CS-33	0,771
8.	CS- 8	CS-46	CS-34	CS-29	0,758
9.	TK-23	CS-36	TK-23	CS-36	0,743
10.	CS-32	TK-23	TK- 2	TK-23	0,740
11.	CS-34	TK- 2	CS-36	CS-27	0,714
12.	CS-27	CS-29	CS-27	TK- 2	0,689
13.	TK- 2	CS- 8	CS- 8	CS- 8	0,677
M.s.	$237{,}6^{xx}$	$343{,}4^{xxx}$	$250{,}8^{xx}$		
σ^2_G	42,6	67,0	41,3		
σ^2_P	109,6	142,5	127,0		

General and specific combining ability in lucerne at different levels of inbreeding and performance of second-generation synthetics measured in competitive condition (x).

by

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Introduction

Our breeding program on lucerne is based on an initial period of selfing. The self-pollination was repeated during four years. All the trials, from the parental population to the progeny test, have been carried out in sward conditions.

With clones of plants selected in S_0 - S_1 - S_2 - S_3 - S_4 diallel crosses and Syn-2 generation synthetics have been realized in cages by hand-pollination.

In this paper are reported the results of 4 diallel crosses between 8 clones B(vigorous plants) type and of their Syn-2 generation synthetics.

Diallel Crosses

The clones of the four diallel crosses derive from two different origins: four clones are from the ecotype Friulana and four clones from the bred variety Florida.

The trial was realized at the density of 330 plants per square metre.

Dry matter weight, earliness and mortality were determined. One precutting and four cuts have been effected.

Only the dry matter weight data are reported in this paper.

Data were analyzed and the sums of squares for single crosses were partitioned into general and specific combining ability according to methods suggested by Griffing.

(x) This work was supported by funds from the Italian Research Council (C.N.R.)

a) Effects on the variances - The magnitude of the variances (Tab. 1) for the general combining ability is considerably larger than that for specific combining ability. The general combining ability is always highly significant. The specific combining ability is not significant at the levels of selfing S_0 and S_1; it is significant at the level S_2 and highly significant at the level S_4.

b) Effects on the means - According to the theoretical results on the inbreeding and the structure of synthetic varieties at the tetraploid level, the more inbred are the parents, the weaker are the single crosses. Nevertheless, the present results show a favourable effect of selfing not only on the variances but on the means as well. This appears in Fig. 1, in which the clone and single cross values are reported for each diallel cross.

On the base of the results of diallel crosses we are allowed to say that intentional selection of vigorous plants in each generation of selfing increases the frequency of favourable genes and gene combinations.

But the positive effect of selfing should be better appreciated if one keeps in mind the importance of the structure in the expression of hybrid vigour; it follows that the comparison must be done, for instance, not between single crosses S_0 and single crosses S_2, but between single crosses S_0 and second generation synthetics of the S_2 level. Such a comparison, referring to our data, shows that through two generations of selfing and selection an increase of 18% in yield is achieved.

Fig. 2 shows the performance of clones used in the four diallel crosses. Technical causes concerning the culture of plants and selection should be sufficient to explain the differential reactions at the fourth level. Nevertheless it is important to notice that the unfavourable effect of selection occurs in plants of the bred variety Florida, while the other parent variety, Friulana, an ecotype, does not feel it, but increases.

c) General and specific combining ability effects - Considering the general and specific combining ability effect, which are reported in Tab. 2, the difference between maximum and minimum values increases according to the levels of selfing.

The clones A, E and F exhibit a very good general combining ability at every level of selfing; the clone B value decreases at the S_4 level.

Syn-2 generation synthetics

With the genetic material of diallel crosses S_0, S_1 and S_2 were built 21 2-clone, 6 4-clone and 3 8-clone synthetics.

The performance of second generation synthetics is represented in Tab. 3 The 2-clone synthetics yield on average less than the 4-clone and 8-clone synthetics. That is true at every level of selfing. Each 4-clone and 8-clone synthetic is composed by Florida and Friulana fifty-fifty.

The actual best yield was obtained with a synthetic variety made with 4 clones at the second level of selfing. Such a synthetic produces 47% over the average of its two parental populations.

The inbreeding depression in Syn-2 generation synthetics generally decreases with the increasing level of selfing.

Furthermore some 4-clone and 8-clone Syn-2, at level S_1 and S_2 of selfing, yield more than their first generation.

Conclusions

1) The general combining ability is always highly significant, while the specific combining ability becomes significant only from the level of selfing S_2.
2) The efficiency of the selfing and of the subsequent selection in improving the genetic worth of parents is very important. When the selection is realized in competitive conditions. Selfing and selection should be pushed as far as possible.
3) As for the convenience of building hybrids instead of synthetics, it seems that single cross hybrids should be convenient when working with non-inbred material; but when inbred parents are used, the superiority of hybrids over the synthetics is too limited indeed to cover the increased cost involved in producing hybrid seed.
   The optimal solution in the breeding of lucerne seems as far to be in synthetic varieties based on 4-clones highly inbred.
   Nevertheless, in our experiments there is also some evidence of an advantage in using double cross hybrids made with clones at the third generation of selfing. An experiment in course is intended to prove the value of this method.

Tab. 1 - General and specific combining ability variances in diallel crosses at different levels of selfing.

	CUT	$S_0 \times S_0$	$S_1 \times S_1$	$S_2 \times S_2$	$S_4 \times S_4$
1	G.C.A.	13.31xxx	8.69xxx	40.04xxx	46.13xxx
	S.C.A.	1.53 N.S.	4.44x	8.43xxx	8.67xxx
2	G.C.A.	14.43xxx	26.42xxx	57.06xxx	40.00xxx
	S.C.A.	2.18 N.S.	4.44 N.S.	7.00xxx	7.72xxx
3	G.C.A.	16.92xxx	19.95xxx	53.26xxx	57.35xxx
	S.C.A.	2.04 N.S.	3.29 N.S.	4.32x	6.97x
4	G.C.A.	12.00xxx	16.25xxx	37.06xxx	43.59xxx
	S.C.A.	1.75 N.S.	1.51 N.S.	3.10x	5.33xxx

Tab. 2 General combining ability effects in diallel crosses among clones at different levels of selfing.
average of 4 cuts

Clones		$S_0 \times S_0$	$S_1 \times S_1$	$S_2 \times S_2$	$S_4 \times S_4$
Florida	127	1.08	1.56	3.89	2.53
	633	0.34	1.22	1.38	- 1.96
	1171	0.44	- 1.73	- 0.25	- 2.44
	1497		0.00	- 3.52	- 2.46
Friulana					
	649	1.01	0.87	2.18	4.62
	652		1.27	0.61	1.27
	940		- 1.17	- 0.69	- 0.50
	1836	- 2.87	- 2.04	- 3.60	- 1.02

Note: The $S_0 \times S_0$ values have been calculated on a restricted 5-clones diallel cross including only clones represented all in the selfing levels.

Tab. 3 - Performance of the second generation synthetics (dry matter yield in g/plot). Average of 4 cuts.

Level of selfing		Number of parental clones		
		2	4 (o)	8 (o)
$S_0 \times S_0$	MEAN	9.55	13.20	12.42
	MIN.	6.93	13.13	
	MAX.	12.12	13.27	
$S_1 \times S_1$	MEAN	12.20	14.16	14.12
	MIN.	7.14	10.41	
	MAX.	15.44	17.91	
$S_2 \times S_2$	MEAN	12.94	16.57	17.49
	MIN.	7.53	11.68	
	MAX.	17.27	21.47	

Parental population mean = 14.54

Friulana = 11.91 Florida = 17.18

(o) Synthetics built with Florida and Friulana fifty-fifty

Figure 1. EFFECT OF THE LEVEL OF SELFING ON THE MEAN AND VARIABILITY RANKED CLONES (CL) AND SINGLE CROSSES (S.C.) - AVERAGE OF 4 CUTS

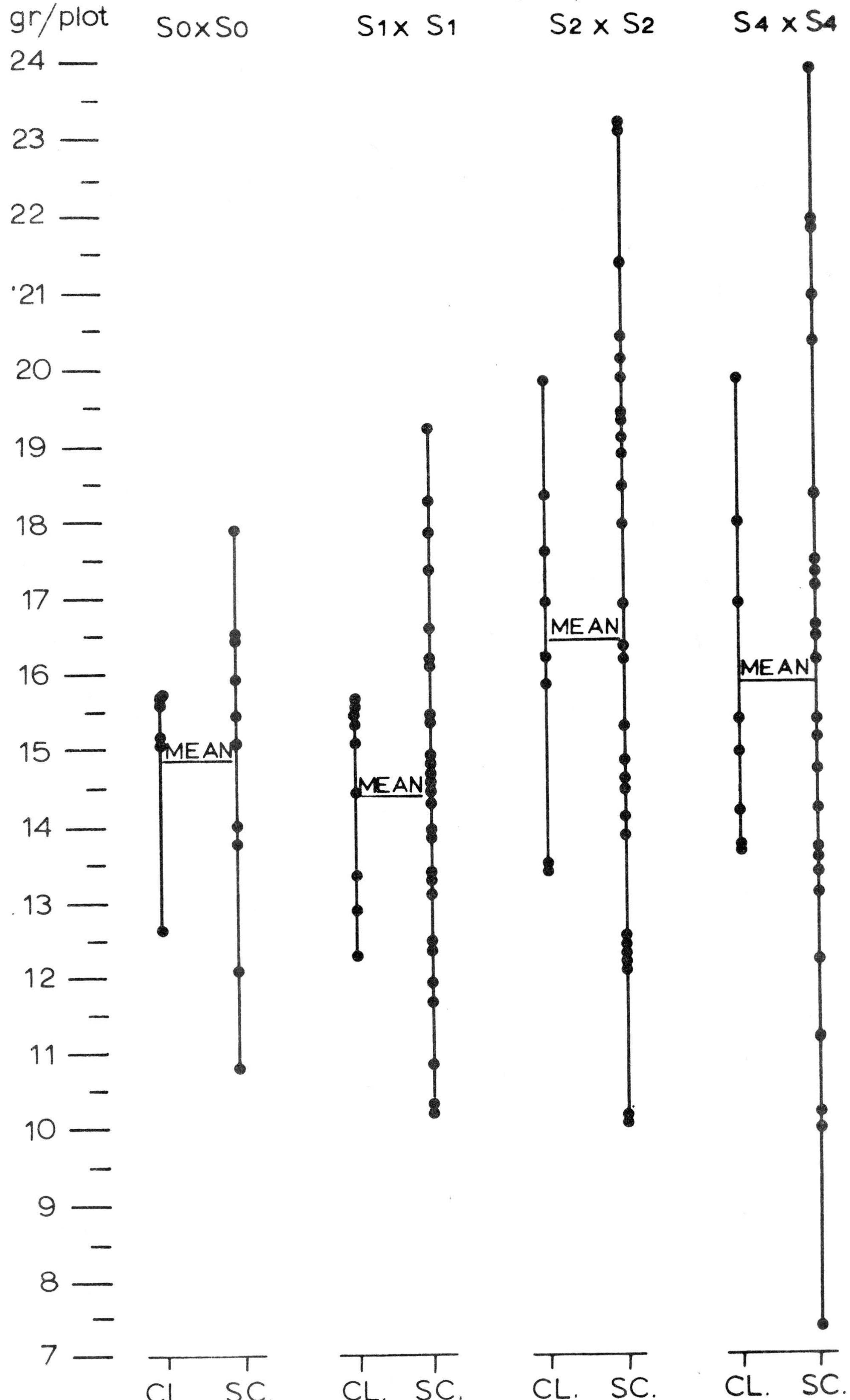
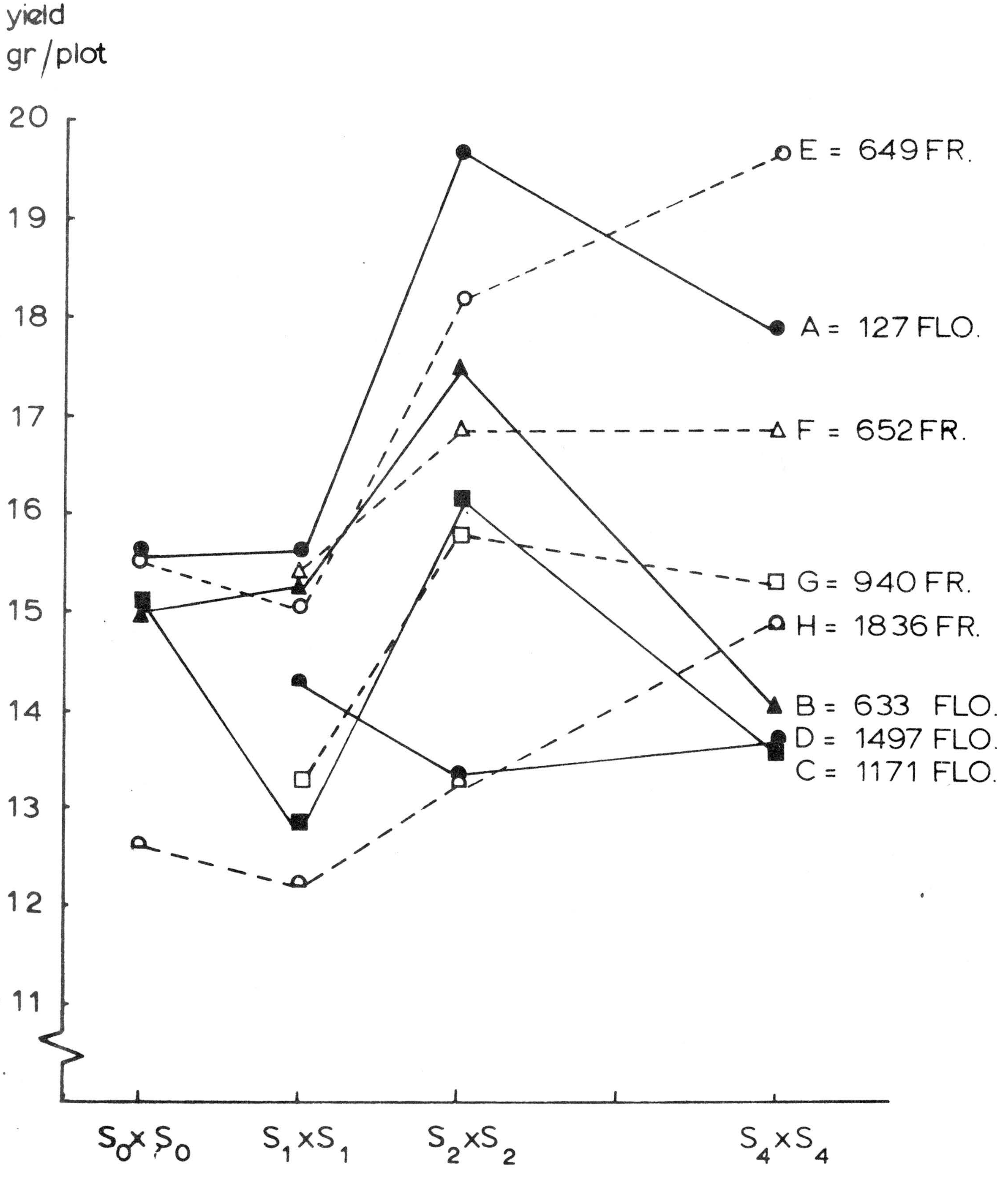


Figure 2. DRY MATTER YIELD PERFORMANCE OF CLONES IN DIALLEL CROSSES AT DIFFERENT LEVELS OF SELFING. AVERAGE OF 4 CUTS



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DISCUSSION

Chairman: Dr. Julén.

Van Bogaert: I want to ask Mr. Gillet: "What was the reason that the crosses between two types of the same species were sterile?"

Gillet: I do not know. I expect there is a way of specification. There seem to be in reality two different species, only taxonomists have not noticed it. This is my opinion.

Thomas: The cytology of hybrids between some mediterranean and indigenous tall fescue has been looked at and a number had irregular meiosis - there was a number of multivalents and univalents. The hybrids were also sterile. We are looking further at a larger number of crosses involving a whole range of lines from Northern Europe to Marocco to assess the differential that has occurred within the species.

Gillet: Did you not notice small chrom. rearrangements?

Thomas: Yes, there were some chrom. rearrangements translocations and consequently univalents. But whether this is due to differentiation or to gene-effect, we are not quite sure.

Gillet: But if colchicine-treatment restores fertility, I think we can discard the hypothesis of the gene-effect. Do you not agree with this?

The restoration is not full but interesting nevertheless.

Emecz: I would like to ask Mr. Gillet what type of infertility you have got. Are there any flower heads formed or do you have only vegetative growth?

Gillet: There is no abnormal flower formation, but there is no seed at all.

Lewis: Our cytological studies on North African x North European hybrids indicated that their sterility was chromosomal rather than genic.

Certain combinations tended to have 14 bivalents and 14 univalents indicating marked differentiation in one of the three genomes involved.

In a series of crosses, involving a geographical range of material, it was found that the degree of sterility of the hybrids reflected the degree of geographical diversity of the parents.

Our experience with chromosome doubling of Fa (to 2n=84) is that this results in agronomically undesirable material, which is very coarse in habit and lacking in vigour. It is useful only as a problem donor in our crosses with L.m.

Gillet: If this is the case for these hybrids, I think this way is hopeless.

Lewis: Well there may be differences in the material used.

Jones: I think there is one point that you must realize and that is quite evident from the work that has been done in Aberystwyth. What we call tall fescue is a very big aggregation of different elements which are engaging in the various types of specifications, which we do not fully understand

and it is quite evident that you get all sorts of strange combinations, which give pairing or lack of pairing. It is a very complex group and it will take some time before I think cytologists and taxonomists come to grips with the situation and really find out what is happening here. So the word tall fescue I think is representing quite a complicated situation.

Gillet: I agree with you.

Davies; E.: I was not quite sure from the describtion of Dr. Sjödin, what in fact was the difference between the border row and the broadcast plot. It is in fact that the plot itself was broadcast and the border was drilled with a more dense seed rate as a result?

Sjödin: No, they were drilled in the same seed rate, but the difference was that after the three years you have a very thin stand in the middle of the trial, but the border rows were intact, because we had not taken away samples there. It was completely intact. But the difference was in the density of the stand.

Nüesch: You gave the frequency of hypo- and hyperploids. Is there any influence of the weather on the frequency of hyperploids?

Sjödin: Well I did not mention any particular reason for this, because there could be many factors, especially the environment, as you say. It is considered for example, if you have an environment which is in favour of giving low chiasma frequency in the material, then you will have a higher frequency of the special univalents and it seems this forms laggards rather frequently. They will be eliminated and that increases the frequency of hypoploid gametes and by that also the frequency of hypoploid plants. That is one reason for the difference in the frequency of hypoploid plants and there are probably more.

Nüesch: Did you check the aneuploids in different generations after colchicine treatment? Is there any shift in aneuploid frequency?

Sjödin: In this case we have not done it, because this was an old population. We are not checking the aneuploid frequency every year. This is rather labourious to do so. So I have no idea of any changes. But I do not think that you can find any regular pattern for this, since environment is that factor which influence the aneuploid frequency mostly.

Glas: I got the impression that a high percentage of aneuploids in the border rows was explained by the fact that you had a more dense stand in those rows and that aneuploids were protected by euploid plants. Could you explain why we get in general higher seed yield of tetraploid red clover in a thin stand than with a higher seeding rate?

Sjödin: Generally after a few years you will have a somewhat thinned stand caused by climatically influences. In a thin stand you have a lower frequency of aneuploids and in that way you could have a higher seed yield.

Bócza: Have you in your experiments a reduction of vigour from these aneuploid plants?

Sjödin: We have not been able to measure the yield in this case. We were only interested in the aneuploid frequency. But as mentioned in the 1966 investigation we measured the vitality as the number of flower heads per plant. We did not measure the negative influence on yield directly, because that would mean that you should have one field with only euploids and another field with aneuploids.

Davies: Some people believe that the reduction you get during establishment is selective. Favourable genotypes will survive. Other people think marked reduction is in fact random. Do you think there is a random reduction in the number of plants and that in that case an equal number of presumably aneuploids and euploids were surviving?

Sjödin: We believe that the reduction of the euploids is depending on the genotypic constitution of these euploids and also of the physiological stages of these euploid plants and that the weak plants are killed first of course.

Davies: The proportion of the aneuploids remains the same.

Sjödin: Yes, the proportion of the aneuploids is the same and we know that you have a reduction in the number of plants from 121 down to 30 plants in the first spring. That must indicate that you have a reduction of as many euploids as aneuploids.

Davies: That is in fact random.

Sjödin: Yes, but then what kind of euploids are killed. That must be the weak euploids. And that must depend on the genotypic constitution of this plant.

Emecz: Do you think it would be practically possible to apply some sort of stress on the population to get away some of the aneuploids and thereby increasing seed yield potential, because one other explanation for the better survival of the aneuploids in your border rows could be the less stress. You have probably better conditions in the surrounding area. We have border effects in other crops as well. I am just wondering if it would be a very attractive idea of applying manurious stress to get rid of your aneuploids without any chrom. checks.

Sjödin: In the border rows you have less stress, because they were protected by the frame. But if it then is possible to make artificial stress in the field? May be it is.

Julén: I would be interesting to see.

Jones: To get back to the point that Mr. Davies raised about the seedling competition. I think the point that I would make as well is that in the first seedling competition the survivors might indeed be very random and that competition then really comes about when you get competition between more mature plants. Your figures do say that the frequency of aneuploids is the same and as far as we know, there is no evidence therefore that we have had a decrease in vigour, that is to say that plants which now survive were the most vigourous, but it could well be I think that we got simply the chance of fact of the early germinaters, the seedlings which manage to get ahead a little bit. And then subsequently when you are imposing some sort of stress and your plants are becoming more mature, then the competition will seem to be taken place without any reduction of the aneuploids. As far as the border rows are concerned I think Mr. Emecz then might be right in supposing there is not so much stress.

There is nothing particular of selecting the plants against the euploids. That is what I would see in the figures that you have on the board anyway.

Sjödin: Well I agree with you, but do you not consider hard thinning of the stand as a stress factor?

Jones: Thinning out must be a result of competition. You must have a removal of unfavourable genes I suppose.

Sjödin: Or even a natural thinning by winter or anything like that.

Emecz: That should not be a stress in fact. That would be the opposite. I think it is a bit contradictious here. If you have more competition then you would have a bigger stress. You remove your plants, then there is more chance to develop fully. More light more nutrients and so on. (So less stress).

Van Bogaert: It is a question a little bit outside this paper, but what is the seed yield of this tetraploid regarding to the diploid?

Sjödin: I do not know it. To fix a figure for the seed yield of a tetraploid is difficult because one year you have 1 kg and another year you have 300 kg.

Julén: I think it was the variety 059, which gave better seed yield than most of the other tetraploids. I should say under field conditions the seed yield was about 80% of the diploids in kg per ha, that would mean around 50-55% fertility.

Van Dijk: I would ask two questions to Mr. Jacquard. The first one is that I did miss the density you used in your experiment. The second is: How reliable do you think are experiments about competition in boxes in the greenhouse compared to the conditions in the field?

Jacquard: Yes, I agree with you there is a possibility of discrepance between the results obtained in the very different conditions in the boxes and in the field, but I think that it is necessary in the first steps to elucidate some problems in more controlled conditions then in the field. Now in 1973 Mr. Rotili and Mrs. Zannone have laid out a new experiment with six genotypes in the field.

Van Dijk: And the densities you used in your experiments?

Jacquard: The density is 800 plants per m^2. That is the condition of the establishment of the field crops of lucerne. It is not the condition usually observed in the yield for the subsequent years, where the density is about 150-200 pl. per m^2 in the mean of the situation.

Hill: I like to ask Dr. Jacqard a few questions, mainly on points of clarification of the data which he presents in table 1 of his paper. I wonder what he means by simple analysis. Is this a straight analysis of all the mixtures taken together?

Jacquard: It is a straight analysis of the ten types of cultures of for all taken together.

Hill: In connection with your diallel analysis. Your C item, which you call general domination ability, is this an analyse of row and column differences?

Jacquard: Yes.

Hill: These are highly significant throughout. Does this mean that there is one particular lucerne genotype which is dominant over both water regimes and densities? You can not tell this from the data, nor can you determine whether this item is interacting with those environmental variables which you are imposing in the experiment. I would suspect, for example, from the fact that the relative replacement rates were not straight lines but fluctuated up and down, that different genotypes were dominant at different stages in the experiment.

Jacquard: In fact there are two sets of genotypes, two early and two late genotypes. If we consider the mixture of the early and the late genotypes then there is a tendency to the domination of the early ones. But referring to the relative replacement rate this means that there is no exclusion of genotypes. This does not mean there is an inversion of the domination effect. That is the genotype that is advantageous at the first cut is also advantageous at the second, at the third and also at the six and so on.

Hill: But not to the same extent?

Jacquard: Yes. In the first cut if a genotype has gain then in the second and third cut the relative increase of the first genotypes is minor than the relative increase of the depressed genotypes of the precedent.

Hill: They tend to balance out?

Jacquard: Yes. It is a balance of the relation of the dynamics of the two genotypes, but there is no change of the sign of "the plastic response".

Hill: One might still expect your C-item to interact with your environmental variables because the interaction can arise by changes in the magnitude of the differences between genotypes without affecting their order.

Jacquard: Not changes in order due to the water regime.

Hill: What about densities?

Jacquard: Density is not included in this analysis.

Hill: Another question. At the end you concluded that there was no 50-50 mixture which was basically better than the better monoculture. Firstly, how do you explain this result, secondly when reading through the literature one often sees the statement that 50-50 mixtures are not better than the better monoculture. Would you agree with me from your own experiments, and also from other people experiments, that 50-50 mixtures which are better than the better monoculture are indeed hard to find? You do not come across them very often?

Jacquard: I think that this is very hard to find for two reasons. First: We have no examples in grasses or lucerne of mixtures constituted after cycles of selection for coadaptation. But if we look at the results obtained by Anson and Steen on soja (then it could be) when we conduct a selection scheme on mixing ability that you have the possibility to find at the end of the cycle that there is a result of a mixture of 8 strains higher than the better component. These 8-strains was then a choice on coadaptation for the growth in the mixtures. Another reason: It seems that it is, when we talk of monoculture in allogamous plants in fact the monocultures are polymorphic mixtures of genotypes. And I think it is not so difficult to find mixtures better than the best component with autogamous plants.

Emecz: The results of Dr. Jacquard laid to the conclusion that the evaluation in pure stand is as good as in mixtures. It is in contradiction with the theory. We all excepted some time ago that in pure stand a minimum competitive ability would be an advantage. But it is very unlikely to be an advantage in a mixture. Now it is just possible that you have as much intravarietal competition as intervarietal, but one would feel that this is a rather coincidence but one would not expect this on a large scale. So one feels it would be probably to optimistic to expect that this situation would be more the rule rather than the exception. Are you willing to agree with this interpretation.

Jaquard: Yes, I agree.

Van Bogaert: I want to ask Mr. Rotili. Did I understand quite well when he said at his conclusion at the end that he preferred a four clone synthetic? I do not see why he reached such a conclusion because one of the tables showed us that the 8 clones synthetic in the syn 2 generation had the highest production.

Rotili - Mrs. Zannone: The maximum production gives the 4 synthetic not the 8. The further depression may occur when non-inbred material is employed. But when we use inbred material the further depression in the next generation is not diminishing. It is different in the trend.

Davies: I must congratulate Dr. Rotili and Mrs. Zanone. They have a lot of material as I could see. One point that did intrique me was the different result that they obtained from the two parental varieties Friulana and Florida. Is there anything particular in the breeding of Florida to help to explain this?

Mrs. Zannone: Florida is a bred variety. We think it is starting from a more inbred level than the ecotype Friulana, which is not bred at all.

Bócza: Did you use S3 generation for the crosses?

Mrs. Zannone: Yes, we have used the S3 too, but as we had no 8 clone diallel because lack of material we had only 4 clone diallels in the S3. The S3 results are completely in agreement with the other levels.

Emecz: You have indicated there is a difference in the subsequent generations based on the number included in the synthetic. Now would you recommend then that the number of the clones should vary depending on the generation that would be the end product of your work?

Mrs. Zannone: Dr. Rotili agrees that in general the number of constituents is linked with the generation one commercializes. But the 4- and 8-clone synthetics give the same results to the 4th generation. The results are in agreement with the results of Mr. Davies who used non-inbred material.

Emecz: And you assume that the marketable generation would be a fourth?

Mrs. Zannone: Yes.

Dijkstra: The choice for a 4- or 8-clone synthetic will also depend on the variation in the clones you have. I do not know if you had a clear variation in your experiment, but when there are four clones much better than the other, you like to choose these four clones and when you compose an 8-clone synthetic you have to include in this case more bad ones. I think this variation must also be taken in consideration.

Mrs. Rotili: We agree.

Hill: In table 3 of this paper Dr. Böjtös gave values for total green yield in three-way hybrids compared with the mid-parents, and he pointed out that there were two particular crosses, I believe, that would be better than the mid-parent. Are they in fact better than the better parent, which is the way we normally express heterosis?

Böjtös, Z.: I did not mention only the deviation from the mid-parent. I compared with the single crosses and with the tester parent and the mid-parent.

Davies: Have you (Dr. Böjtös) any experience with differential activity of pollinating insects on the male sterile lines?

Böjtös: Yes. We have that seen only under cages with leaf cutter bees. With open pollination in the nurseries there were quite differences between the F_1 lines for seed yield. I can state that some ms lines have a relative very good seed setting under open pollination. In the USA they found with the same experiments also a differentiation between the seed yield in the lines. The average is about not more than 28% of the normal seed setting. And therefore the price of the hybrid seed is relative expensive. But I think we can select for this and we can solve this problem.

The readers will publish or probably publish their papers in the following periodicals:

Ahloowalia	Theoretical and Applied Genetics
Badoux	Le Recherche Agronomique en Suisse
	Landwirtschaftliche Forschung in der Schweiz
Böjtös	Zeitschrift für Pflanzenzüchtung
Van Dijk	Euphytica
Dijkstra	Euphytica
Duyvendak	Will not be published in this stage
Jadas, Hécart and Gillet	Will not be published in this stage
Rotili, Zannone and Jaquard	Euphytica
Lewis	Euphytica
Mackay	Euphytica 22(1973) 495-499
Rotili and Zannone	Euphytica
Sjödin	Aneuploids in tetraploid red clover
	Zeitschrift für Pflanzenzüchtung
	Species crosses in *Brassicaceae*
	Will not be published in this stage
Thomas	Euphytica
Wit	Will not be published in this stage

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