

EUCARPIA
FODDER CROPS SECTION

Report of meeting held at the
Station d'Amelioration des Plantes Fourrageres
LUSIGNAN

September 15th 17th 1970

I.N.R.A.

Station d'Amelioration des Plantes Fourrageres
86 - LUSIGNAN
FRANCE

Editions S.E.I.
C.N.R.A. Versailles
Novembre 1971

E U C A R P I A F O D D E R C R O P S S E C T I O N

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Lusignan, September 15th-17th 1970

PREFACE

Kindly invited by Dr. P. Mansat, Director of the I.N.R.A. Station d'Amelioration des Plantes Fourrageres, Lusignan, France, the Fodder Crops Section of Eucárpia ajourned in Lusignan in September 1970. The meeting was attended by 80 persons, including Dr. Mansat and his staff, and it took place on September 15-17.

The main subject to be discussed was :

BREEDING FOR YIELD IN CROSS FERTILIZED FORAGE PLANTS.

- 1) Various breeding methods.
- 2) Theoretical and experimental results with and without inbreeding,
Different kind of cultivars,
Choice of constituents,
Yield evolution in advanced generations of synthetics.
- 3) Value of the results according the stand density,
Components of dry-matter yield,
Interaction between genotypes.

During the meeting Dr. Mansat and his co-workers presented some of the research projects carried out at the station.

On behalf of the board of the section I wish to express our gratitude to Dr. Mansat and his staff for the very efficient way in which the meeting was prepared, for the interesting demonstrations of the work done at the institute, and for their generous hospitality.

The present report comprises the papers given during the meeting, and the board thanks the authors for their contributions.

DLF & FDB
Boelshøj, November 1970.

K.J. Frandsen,
President of the
Fodder Crops Section.

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CONTENTS

Preface
Participants
Papers

| | | |
|---------------------------------|---|-----|
| Y. Demarly | Genetic comparisons in di and tetraploids | 9 |
| E.L. Breese and M.D. Hayward | The genetic basis of present breeding methods in forage crops | 39 |
| U. Simon | Effect of diversity, number and genetic relationship of components on yield of synthetic varieties of lucerne | 55 |
| W. Ellis Davies | The yield in succeeding generations of experimental lucerne synthetics based on varying plant numbers | 63 |
| H. Toxopeus | Preliminary results of yield trials with "stubble turnips" (<i>Brassica campestris</i> var. <i>rapa</i>) in the Netherlands | 73 |
| G. du Créhu | Problem of production of double cross hybrids in kale (<i>Brassica Oleracea</i> L.) | 85 |
| F. Lorenzetti | First generation synthetics from genotypes at various levels of inbreeding in lucerne..... | 95 |
| A. Gallais and P. Guy | Use of heterosis in breeding autotetraploids | 105 |
| D.A. Bond | Breeding methods in field beans (<i>Vicia faba</i> L.) | 119 |
| N.O. Bosemark | Use of mendelian male-sterility in recurrent selection and hybrid breeding of beets | 127 |
| V. Connolly | General and specific combining abilities in white clover (<i>Trifolium repens</i>) | 137 |
| I. Sikora and D. Bošnjak | The methods of alfalfa breeding in the area of Slavonija | 151 |

| | | |
|-------------------------------|---|-----|
| G. Julén | Breeding for increased yield of dry matter and of seed in tetraploid red clover | 157 |
| P. Rotili | Selfing effects on S_2 and S_3 generations in <i>Medicago sativa</i> | 169 |
| T.I. Emezc | Methods of yield-assessment during individual performance test and subsequent realization of yield potentials in synthetics | 189 |
| M. Gillet | Morphological characteristics and expression of vigour | 207 |
| P. Rotili and Miss L. Zannone | Intra and intergenotypic competition in <i>Medicago sativa</i> | 213 |
| P. Jacquard | Variability of competition responses for 14 alfalfa "genotypes" | 237 |

GENETIC COMPARISONS IN DI AND TETRAPLOIDS

Y. Demarly

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1 - FIRST QUESTIONS

If we consider the primitive form of the genetic information in the circular chromosome of bacteria and viruses, what is the meaning of the linear arrangement divided in constant numbers of various chromosomes for most of plants and animals ?

Why does this linear stock of DNA registered in one sequence at the haploid level need such a redundancy when written two, three, four times in di, tri tetraploids ? *Aberfenn*

May I before dealing with the essentials of my subject, make an attempt to answer these questions.

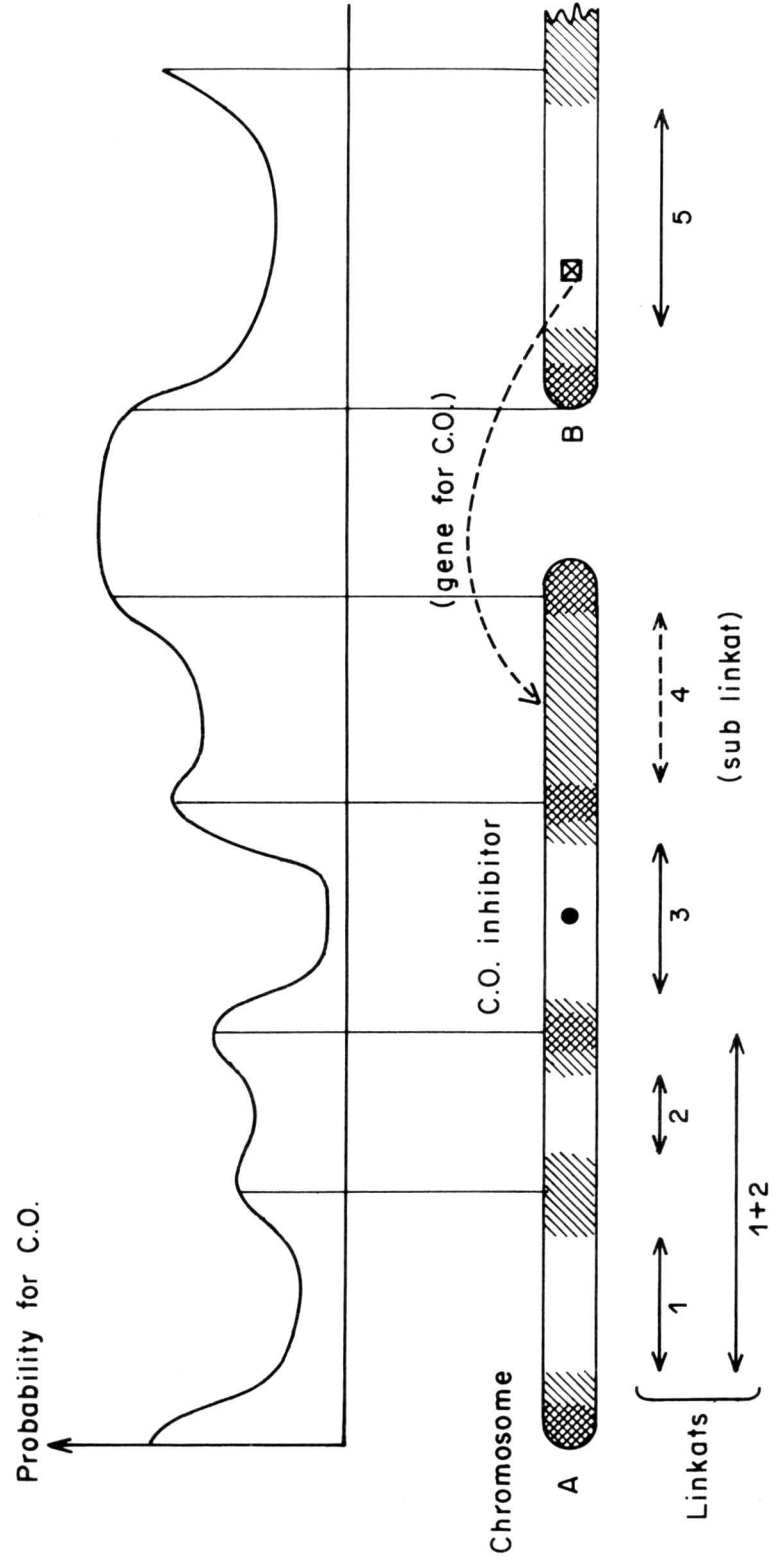
For the first one, we will consider the frequency of crossing-over. We generally assume that chiasmata occur at random. This is not so. There is evidence that crossing over may be increased by physical or chemical agents. Rhoades (1941), Burnham (1949) and Clark (1956), have shown that in maize for many chromosomes, crossing-over is considerably higher in the male organs than in the female.

The differences appear to be greatest for genes located near the centromere. Riley on wheat for chromosome VB and Dempsey and Rhoades on chromosome 10 in maize put in light that crossing-over can be lowered or enhanced by the presence of certain genes. Many authors give various examples of similar observations (in Primula, in Barley, in Cotton and amongst animals).

Consequently large blocks of genes are continuously transmitted intact from generation to generation. I proposed in 1965 the word "linkat" to represent these preferential block-units. The length of one linkat is not constant, it is defined in probability as an expected mean length. There is a weak probability for a crossing-over to occur inside a linkat. The probability is much more higher for the segment located between two such blocks. We know in most plants many sets of characteristics inherited as a whole. In such a view, the total coordinated genetic content of a linkat is protected by natural selection, whilst in order parts of the chromosome it occurs frequently many

FIGURE I

Distribution of linkats



recombinations giving random segregations and leading by breakage through evolution process to distinct chromosome numbers (fig. 1).

E. Anderson put this problem in quantitative terms and concluded that among the F_2 offspring the realizable recombination per generation is only $\frac{1}{500.000}$ of that possible if each pair of alleles were freely recombining.

Therefore we must consider that genetic information is shared in different linked sets, each of them having its own selective and functional advantage. The chromosome numbers are one of the main features of this partition (fig. 2).

Turning now to the second question it is obvious that in the multitude of kinds of living plants produced by organic evolution, a general feature is the constant decrease in importance of the gametophyte (haploid stage) in respect to the sporophyte (diploid form) arising for autogamous angiosperms to the most protected type of haploid organs. Is there a better security in this doubled information of sporophyte when organisms become more complicated? Is there, even, any selective advantage of the higher numbers of replications in polyploids?

Whatever may be the reasons there appears to be in most plant species an optimal level of polyploidy above which added chromosomes or genomes lead to depression of growth and vigour. Polyploidy takes a large place in angiosperms. Following L. Cuenot more than fifty percent of species are polyploids. G.A. Malligan analysed 151 species and found 54 % diploids and 46 % polyploids (39 % in annual weeds, 33 % in bisannual, 55 % in perennials). It is obvious that polyploidy is more common in perennials and asexually reproduced plants than in annuals, in alpine than in lowlands plants.

From my point of view we must not emphasize on the differences between auto and allopolyploids. It is often a matter of mechanical attractive forces at zygotene pairing. The discovery of a suppressor of homeologous pairing in common wheat, located on chromosome 5B which insures diploid pairing in hexaploid by preventing the pairing of chromosomes that are homeologous is significant. Homeologous chromosomes have substantially the same gene content, there must only be some structural differences (a species classed as an allopolyploid because it lacks multivalents, may actually be an autotetraploid).

The evolutionary process of genetic diversification consists then essentially of registering and selecting a certain number of variants from a primitive fundamental code (fig. 3).

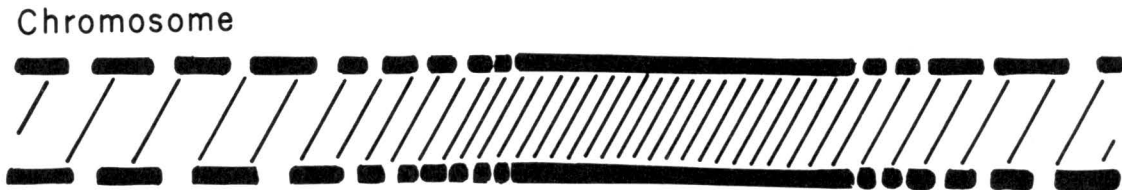
This leads to the operon structure where operator "commands" to a set of coordinated structural genes.

This leads too towards polyploidy where 4 alleles may give a better adjustment of genetic information :

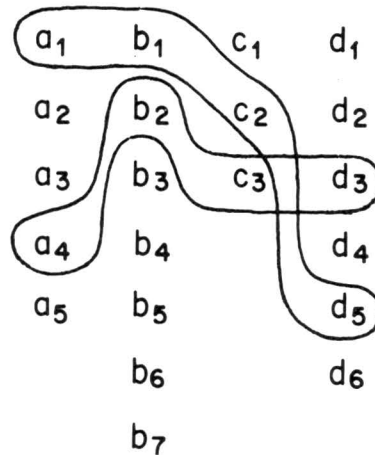
- the number of genomes,
- the number of chromosomes and its significance,

FIGURE II

Diagram of a linkat



Examples of
selected allelic
formulae



Linkat

Set of preferential
allelic arrangements

Selectively and
functionally co-ordinated

The cohesion of which is
protected by a decrease
of internal C.O.

FIGURE III

Diagram

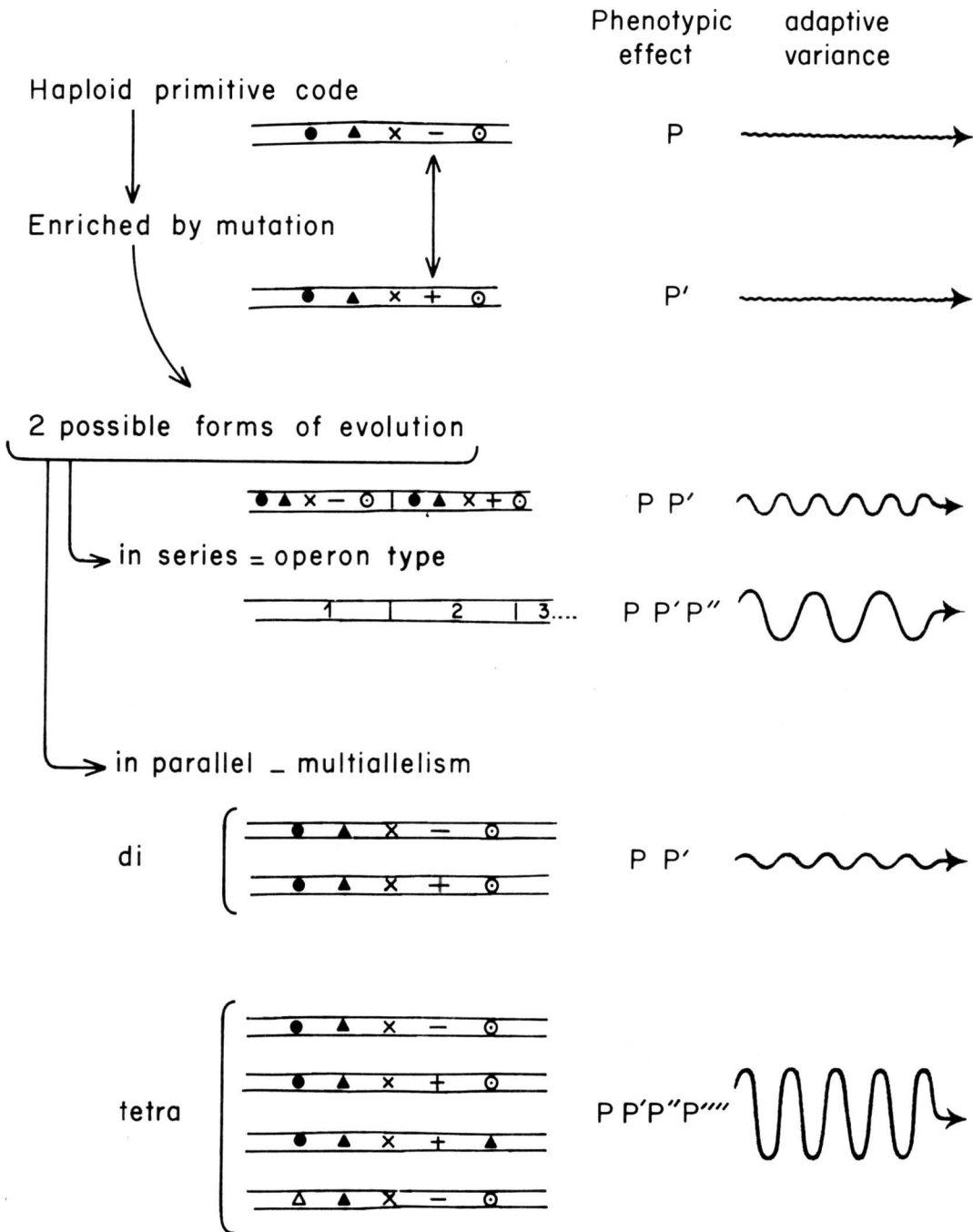


FIGURE IV

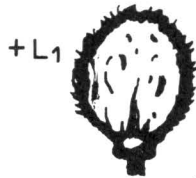
Datura Primary trisomics



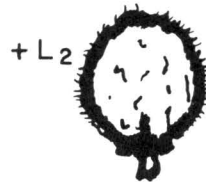
Normal



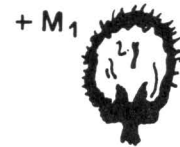
Rolled



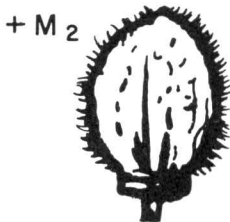
Glossy



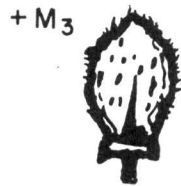
Buckling



Elongate



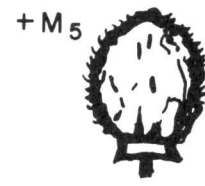
Echinus



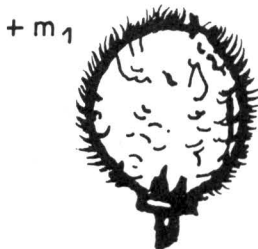
Cocklebur



Microcarpic



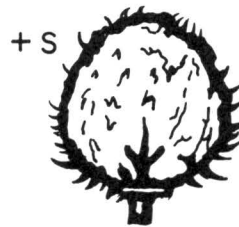
Reduced



Poinsettia



Spinach



Globe



Ilex

- the selective value of linkats,
- the functional importance of operon and the primary effect of cistron.

II - STATIC APPROACH

A - Dosage effect

The static effect of addition in supplementary genetic information has been extensively studied in trisomics where each supernumerary chromosome gives its own particular characteristic phenotype. (*Datura* with its haploid number of 12 chromosomes was the best illustration of this effect (fig. 4).

But in an allopolyploid such as wheat, the ^{redundancy} redundancy of genetic information leads to modifications of morphological aspects from Triticum monococcum to T. durum and T. vulgare.

Going down to cellular level, there appears a relationship observed by Delaunay between reduction in chromosome size and decrease of the amount of meristematic tissue. The amount of chromatin plays probably an important part on the meristematic activity of a cell. To be sure, polyploidy increases cell size in the meristematic and differentiated tissues. The whole cellular organisation is modified : number of chloroplasts, of mitochondria, of nucleolus, and so on

And this occurs as well in homozygote as in heterozygote material.

This increase of cell size depends as much on the amount of cell elongation and on the number of cells produced during growth as it does on the initial size of the cells. (In many species one may identify tetraploidy by measurements of the guard cells of the stomata and of mature pollen grains).

Moreover of the primary effect of polyploidy on cell size, the comparison of isogenic di and tetraploid cells shows a net difference in water content. As a secondary effect, polyploidy increases the water content relative to the amount of protoplasm and therefore lowers osmotic tension.

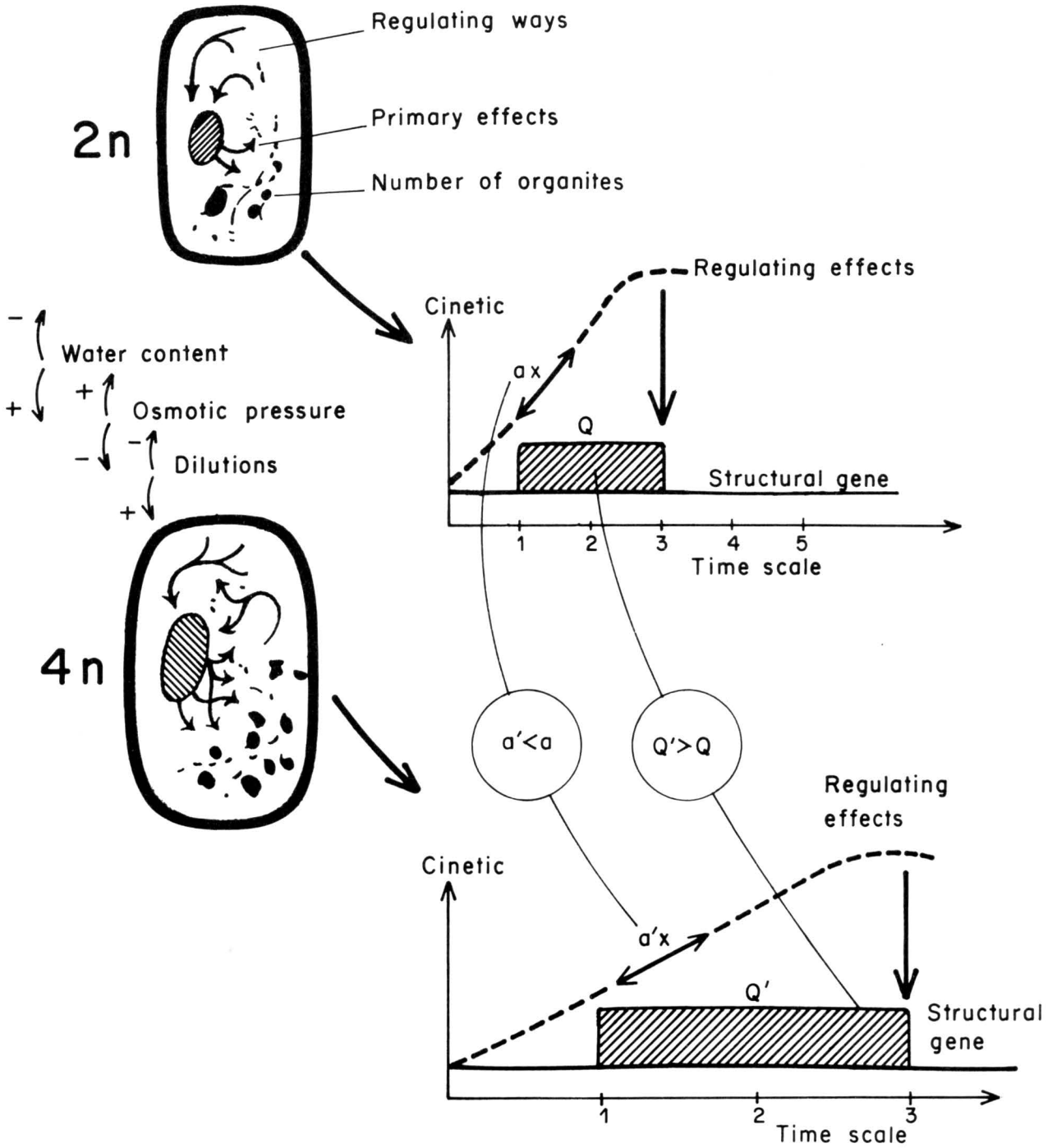
Another secondary effect may be found in the differences between diploids and autotetraploids for the content of various substances (protein, chlorophyll, mitochondria, pigments, auxins and various vitamins). These synthesis are probably in correlation with the growth rate which is slower in autotetraploids.

This causes autotetraploids to flower relatively later and in some cases to have a longer flowering period. As a consequence of this slow growth we observe a reduction in the amount of branching and of tillering.

Last effect of chromosomes doubling concerns the shape of the individual organs of the plants as interfering on coordinated morphogenesis. We can say that these effects are also consequences of cell size and of intensity in meriste-

FIGURE V

Cellular Comparison



matic activities. The most consistent of these effects is on the thickness of leaves and other appendages.

Moreover these direct actions appears in the cytoplasm of tetraploid organisms, a high density of regulatory mechanisms in the epigenic environment and consequently a better homeostasis (fig. 5).

But is it possible to find general features in the effect of chromosomes doubling ? Probably, yes for some characteristics related to cellular physiology (as thickness of leaves or earliness in a given array of genotypes, but not for remote populations and for a set of species because of compensatory mechanisms between higher cell size, slower growth, additive action of particular genes).

Plant breeders who tried to compare di- and tetraploid levels have generally used isogenic material. Most of their comparisons are somewhat biased because we must not only have the same genic material at haplo and diploid level, but have also in the same trial homozygote and heterozygote formules.

It is clear that only this complete comparison allows a share between the additional effects of various linkats and other effects such as dominance and epistasis.

B - Interactions

The actual value of a genotype depends not only on the dosage effects of homologous linkats but on the consistent interactions between the primary functional units. These interactions support the overdominance theory of heterosis which states that heterozygosity "per se" is necessary for the full expression of vigour.

Brewbaker gives the following four types of allelic interaction leading to one-gene heterosis : (fig. 6)

| genotypes : | ii | jj | ij |
|--|---------------------|--------------------------------|--|
| - supplementary action (x and y are entirely different products) | x | y | X + Y |
| - alternative pathways (where E ₁ , E ₂ are environments) | X in E ₁ | X in E ₂ | X in E ₁ and E ₂ |
| - optimal amount | 0,1 | X | 2X |
| - hybrid substance | X | Y | Z |

FIGURE VI

Overdominance

OVER DOMINANCE
according to *BREWBAKER*

| Genotypes | ii | jj | ij |
|--|------------------------|------------------------|---|
| The hybride exhibits : | | | |
| 1°) A supplementary action : (x and y are different products) | X | y | X + y |
| 2°) Alternative pathways : (where E ₁ and E ₂ are environments) | X in E ₁ | X in E ₂ | X in E ₁ and E ₂ |
| 3°) Optimal concentration : | 0,1 X | X | 2 X |
| 4°) Production of an hybrid substance | X | y | Z |

How can we imagine in a polyploid these models of inter allelic actions in connection with the different structures of the plants ?

In a diploid, for a given chromosomal region where are found 2 homologous linkats i and j, we may consider that the mean value is :

$$A = \alpha_i + \alpha_j \text{ (2 additive effects of linkats)}$$

$$+ D = \beta_{ij} \text{ (dominance interaction between i and j)}$$

In a tetraploid, I proposed in the years 60 to make the analysis in terms of structures. It was really the research of Carnahan, of Dudley, in 1966 of Busbice and Wilsie, and of Gallais and Guy in France that give a clear approach of different interactions.

As presented by Gallais this structure can be expressed in various identity patterns (situation d'identité proposed by Gillois) (fig. 7).

| In a diploid with two homologous linkats i, j | In a tetraploid with four linkats i, j, k, l |
|--|---|
| ————— | ————— |
| i = j Probability P ₀ | i = j = k = l Probability P ₀ |
| i o j Probability P ₁ | i = j = k o l Probability P ₁ |
| | i = j o k = l " P ₂ |
| | i = j o k o l " P ₃ |
| | i o j o k o l " P ₄ |

(where = means identic and o means different).

Therefore in a tetraploid with four homologous linkats i, j, k, l it appears :

$$A = i + j + k + l \quad (4 \text{ additive effects})$$

$$D = ij + ik + il + jk + j^l + kl \quad (6 \text{ possible dominance interactions})$$

$$T = ijk + ijl + \begin{matrix} j^l \\ ikl \end{matrix} + jkl \quad (4 \text{ possible interactions of 2nd order} \\ \text{which I propose to denote triminance})$$

$$Q = ijkl \quad (1 \text{ possible interaction of 3}^{\text{rd}} \text{ order :} \\ \text{tetraminance})$$

If I do not fear to introduce two new words for these effects the reason is that they represent very consistent biological effects, when the part of dominance has been substracted (fig. 8 et 9).

FIGURE VII

Identity situations

| IDENTITY SITUATIONS | |
|---|--|
| <p>In diploid \Downarrow with 2 homologous linkats i and j \Downarrow 2 identity situations</p> | <p>In tetraploid \Downarrow with 4 linkats $i, j, k, l,$ \Downarrow 5 identity situations</p> |
| <p>$i \equiv j$ probability P_0</p> <p>$i \circ j$ probability P_1</p> | <p>$i \equiv j \equiv k \equiv l$ probability P_0</p> <p>$i \equiv j \equiv k \circ l$ probability P_1</p> <p>$i \equiv j \circ k \equiv l$ probability P_2</p> <p>$i \equiv j \circ k \circ l$ probability P_3</p> <p>$i \circ j \circ k \circ l$ probability P_4</p> |
| <p>\equiv means identical \circ means different</p> | |

FIGURE VIII

Genetic effects

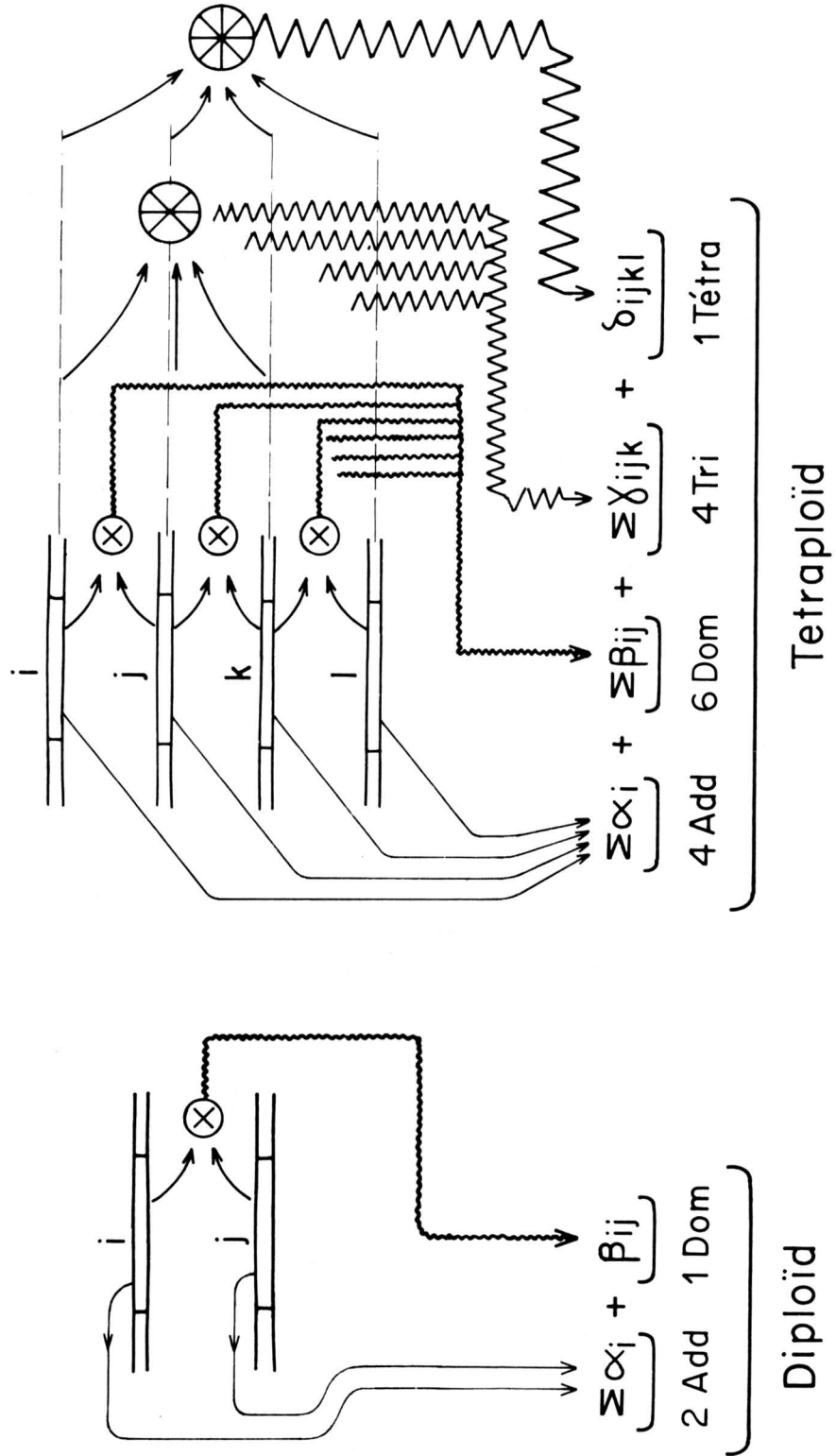
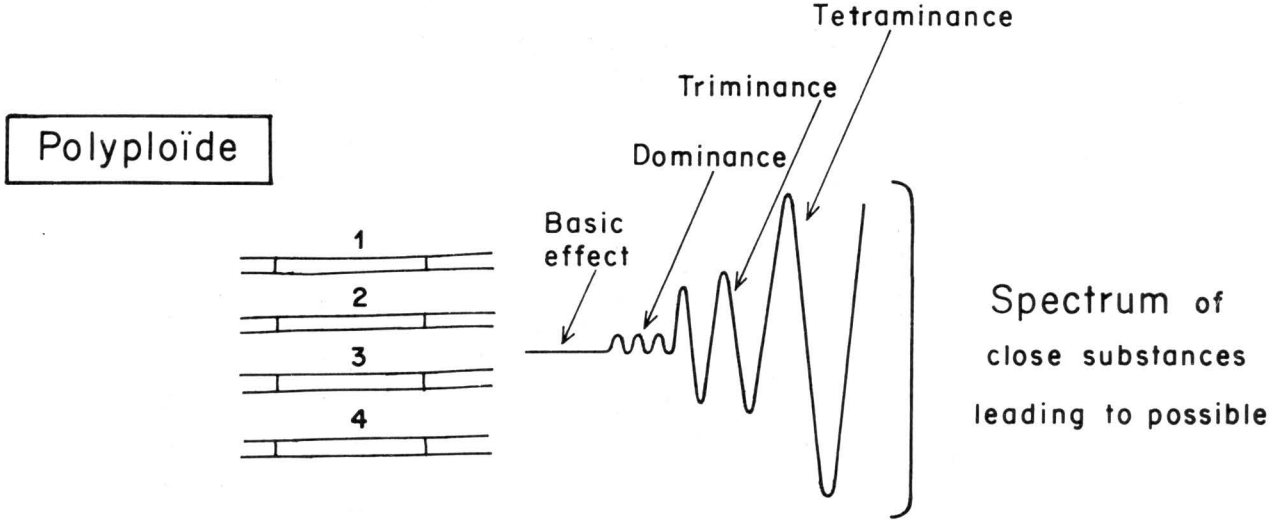
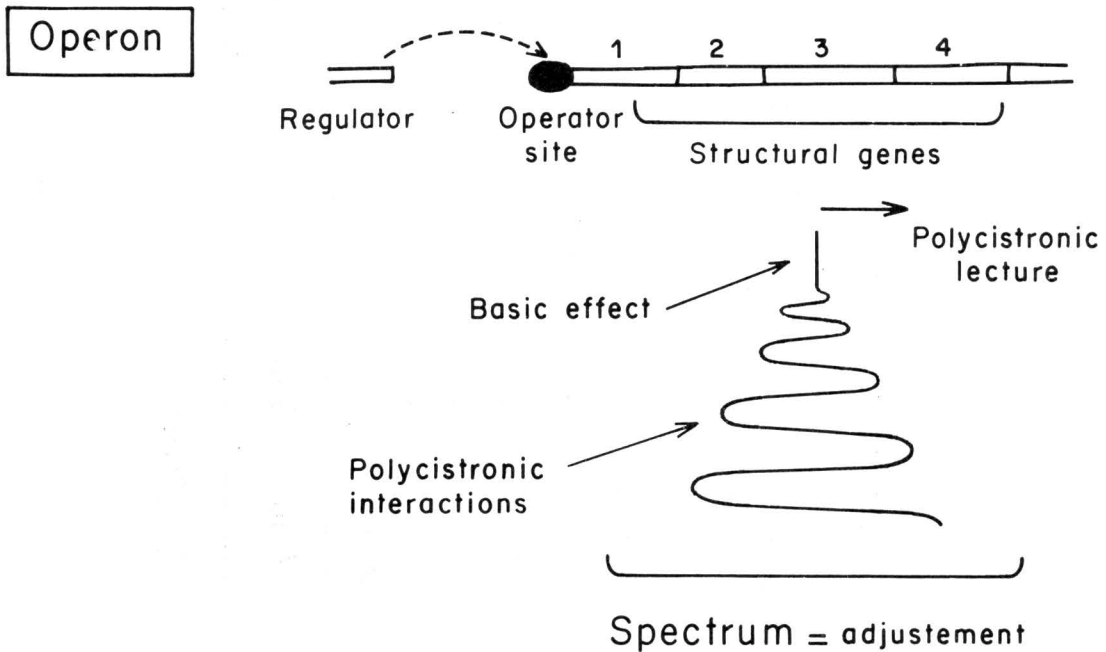


FIGURE IX

Comparison



Adjustment to different environment

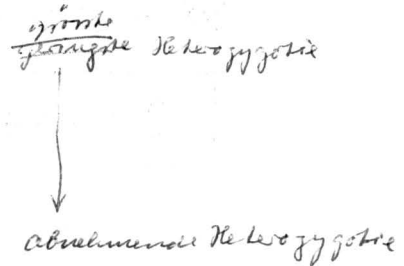


Two set of experimental results, show the important place of these kinds of interactions : fig. 10

First was the comparative yield of top crosses of lucerne between respectively S_0 , S_1 , S_2 , S_3 , S_4 , lines pollinated by a same population.

The figures are : (in tons per hectare)

| | |
|-------|--------|
| S_0 | 58, 72 |
| S_1 | 53, 78 |
| S_2 | 45, 40 |
| S_3 | 41, 86 |
| S_4 | 40, 27 |
| 1 s d | 3, 4 |



Second datas arise in a comparison between many double and single hybrids in Lucerne too :

| <u>Mean yield of double hybrid</u> | <u>of single hybrid</u> |
|------------------------------------|-------------------------|
| 112, 9 | 105, 0 |
| 109, 0 | 105, 0 |

Both experiments show that when triminance or tetraminance are proportionally reduced, the yields of the tetraploid decreases.

In tetraminance for instance, we must think in terms of a specific interaction between 4 different enzymes. It is difficult to imagine that this superiority of some tetraploids may be a consequence either of supplementary action where X, Y, W and Z would be entirely different products, or of realisation of an optimal amount of product.

The most probable explanation lies in a better homeostasis in four epigenic environmental situations or in a synergy given by a hybrid complex substance S from the four components.

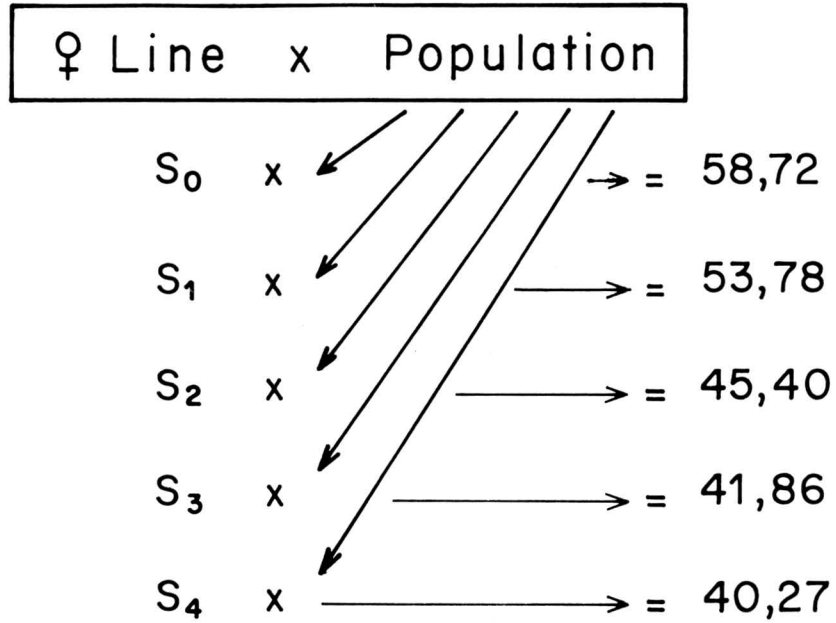
We get here, in the case of tri- and tetraminance a large diversity at biochemical level which gives rise to many possibilities of adjustment in various environments. Again we must put here the accent on the similarity with the various structural genes developed by natural selection and bound to a same operator gene.

Therefore the value of an individual in a tetraploid species lies mainly on the high order interactions contrasting with a diploid species where this possibility does not exist.

FIGURE X

Contribution of structural heterogeneity

1st experiment



2nd experiment

| | Yields |
|----------------|------------------|
| Single hybrids | (105,0 105,0 |
| Double hybrids | (112,9 109,0 |

After Guy and with some simplification the genetic value of a tetraploid plant is :

$$G = (M) Z + F (Y) J$$

where (M) is a matrix-line of weights :

(m_1 for additive effects, m_2 , m_3 , m_4 for dominance tri and tetraminance respectively).

Z is a matrix-column for the probability of the effects (additivity, dominance tri and tetraminance).

J is the set of "structural" interactions as defined by Guy ($A \times D$, $D \times T$, $D \times Q$, $T \times Q$...)

F (Y) is a function of the whole structure of various linkats in the individual plant.

C - Static of a population

In a given breeding system it is easy to characterise a diploid population.

The allelic frequencies and the inbreeding coefficient are sufficient to sketch the general pattern.

For a tetraploid species, things are more complex. A population can be perfectly determined by the ratios of 5 genotypic structures ; monogenic, digenic (simplex and duplex) tri and tetragenic. The frequencies of these 5 elements are written like a vector S denoted as generation vector :

$$S = \begin{pmatrix} aaaa \\ aaab \\ aabb \\ aabc \\ abcd \end{pmatrix}$$

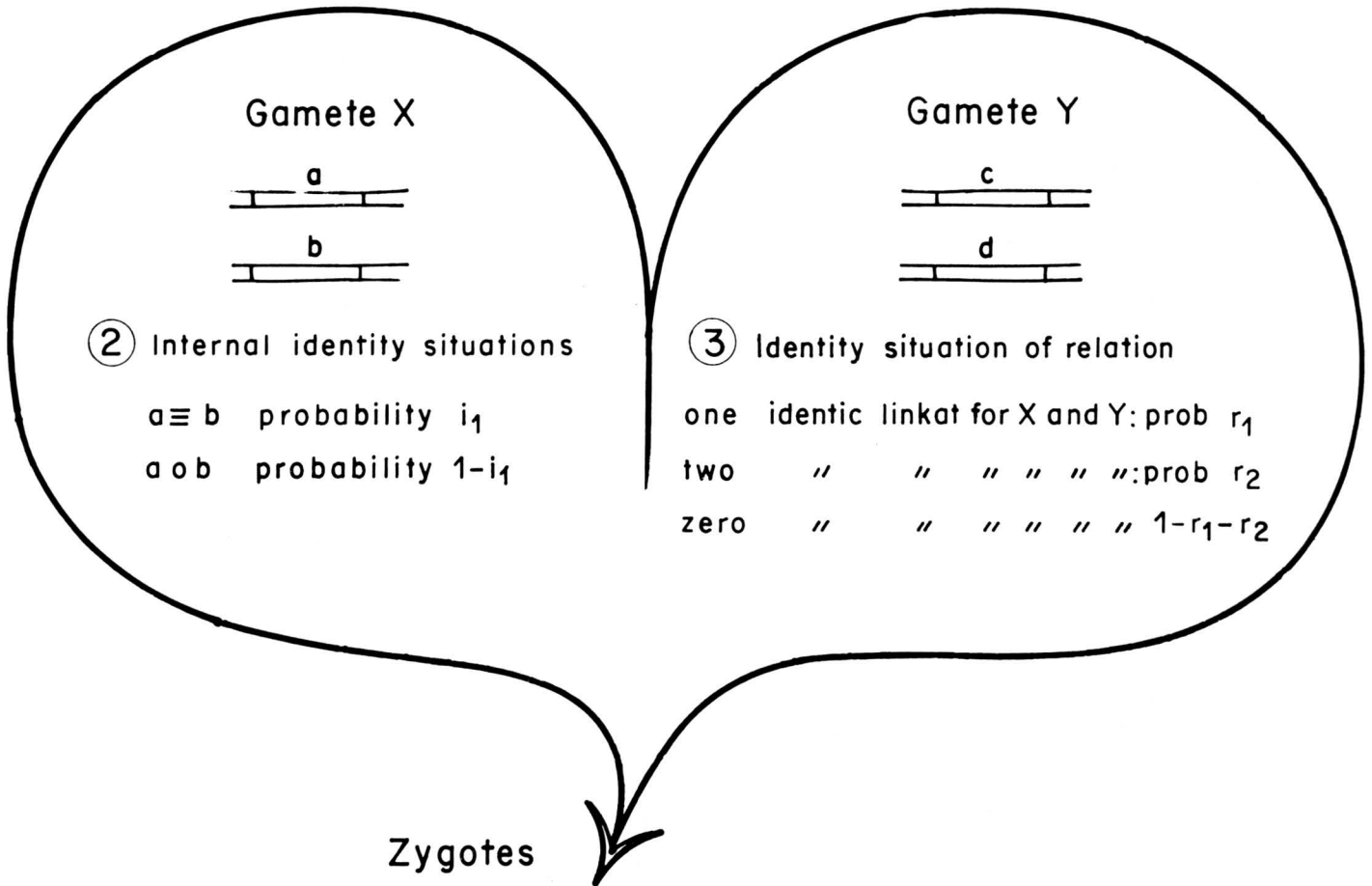
The rates of these 5 genotypes are given by the situations of identity (Gillois, Bouffette, Gallais) Such a characterisation commonly used by many authors needs parameters which reflect the internal diversity of tetraploid population.

I suggested in 1963 the use of gametic structures and frequencies to describe such a diversity.

In fact for a given locus, in a given plant, one gamete can only exist under two states : homozygote that is in a situation of internal identity, or heterozygote. Zygotic structures which result from two gametes are, at our sense,

FIGURE XI

Fertilization



| | |
|-------------------------------|---|
| aaaa (monogenic) | $i_1 i_1 r_2$ |
| aaab (simplex digenic) | $i_1 (1-i_1) r_1$ |
| aabb (duplex digenic) | $(1-i_1)^2 r_2 + i_1^2 (1-r_1-r_2)$ |
| aabc (trigenic) | $(1-i_1)^2 r_1 + i_1 (1-i_1) (1-r_1-r_2)$ |
| abcd (tetragenic) | $(1-i_1)^2 (1-r_1-r_2)$ |

conditioned by genetic diversity of the population and breeding systems. Therefore are determined situations of internal identity for each gamete and situation of relational identity characterising relationship between uniting gametes. We find here a large difference between di and polyploids.

For a given linkat, in the gametes of a tetraploid, there are two internal "situations d'identité" (fig. 11).

Where it is such gametes unit, the various situations can be described by a relational identity parameter r (fig.11).

Which is an analogue to the relationship coefficient of Malécot. Gallais uses, in the general case, seven coefficients to describe the relationship between gametes.

The two kinds of parameters proposed here are :

i which concerns the structure of the gametes
and r which describes the different patterns of gametic union

In most cases these two parameters are not independant :

They are related to the breeding system and to the mean frequency of homologous linkats in the population. Consequently we can find an expression of the different r in terms of i and p (mean number of homologous linkats).

The following table is given as an example :

for one gamete such as a a the probability of which is i the union may arise with

$$\left\{ \begin{array}{ll} \text{a a} & \text{with a probability } i \times i \cdot r_1 = i^2 r_1 \\ \text{b b} & \text{" " } 1 - r_1 - r_2 = (1 - \frac{1}{p}) i \\ \text{a b} & \text{" " } r_1 = 2 \frac{(1)}{p} (1 - \frac{1}{p}) (1 - i) \\ \text{b c} & \text{" " } 1 - r_1 - r_2 = (1 - \frac{1}{p})^2 (1 - i) \end{array} \right.$$

and so on for a gamete a b

The conclusions to be drawn from these analysis mainly concern the allelic variation in a polyploid structure.

The increase in chromosome number provides a mask or cover for deleterious recessive genes which appears at phenotypic level only with a very little probability. As a result, the genetic load of deleterious mutations can increase greatly in a polyploid population (if this deleterious mutations are maintained, we must assume that the selective value of their interactions with other alleles is sufficiently high).

Turning now to effectiveness of natural selection we shall consider one individual linkat denoted by x arising with a frequency m in a population with other homologous structures $a, b, c, d \dots$

This linkat will ^{persist} subsist under following conditions : if the species is diploid in panmixia :

- by its dominance interaction with $a, b, c, d \dots$
- by its net additive value (with frequency m^2)
- by its selective advantage at the gametic stage for such a species natural selection may really have a direct effect.

If the species is tetraploid in panmixia :

- selection applies conjointly to the interactions of dominance, tri and tetra- minance which do not obligatory play in the same sense and can balance each other. The effectiveness of selection depends here on the nature of gene combinations.
- the value of x at the homogeneous stage interferes only with a very weak probability m^4 .
- in the gametes, with a probability m^2 we shall find a similar situation as that of diploid at zygotic stage : the homozygote diploid value will be submitted to natural selection.
- with $m(1-m)$ probability there will be in the gametes interactions of dominance which will act as selective parameters.

In a tetraploid the survival of a mutant is much more balanced between various effects and the survival much more easy. It is obvious that genetic diversity in homologous linkats should be much more larger in these polyploid populations. The internal arrangements of the different linkats must be more variable.

This probably explains the high level of heterosis frequently found inside one ecotype (for instance in Flamande lucerne) of a polyploid species. Had this species been diploid, much less success would have been achieved by internal crosses in only one population.

III - DYNAMIC APPROACH

A - Meiosis

Every body knows the process of meiosis and specially the mechanical aspects in the polyploids.

1°) For the centromeres, meiosis is very regular and simple : the four homologous centromeres 1-2-3-4 have the same behaviour as four units and segregate regularly 2-2 at first Anaphase. At each pole we find at random a pair of centromeres.

Then during the second part of meiosis there is a shift : each of the two gathered centromere segregates in a different A II nucleus, and then in a different tetrad.

2°) For a gene located near the centromere, the destiny is similar because no crossing over occurs between the given locus and the centromere.

3°) After one crossing over, the sister chromatids concerned with the exchange of material repulse at different Anaphase-I poles Tetrads configurations are similar to the first case : each pair between the six possible pairing of the 4 homologous alleles has the same probability : then each of them has a probability of $\frac{1}{6}$.

4°) In some cases it occurs an attractive force which draws to the same A I pole the chromosomes which have crossed-over. This possibility allow to sister alleles being found together in the same tetrad at the end of the Anaphase-II (α parameter of Mather). For a general and schematic analysis we shall neglect these "pseudo reductionnelles" meiosis (this does not modify the most consistent conclusions).

For the different gametes produced by the meiosis we find (fig. 12).

i homogeneous and 1 - i heterogeneous. These situations are expressed in terms of the 5 probabilities of the "situations d'identité" in parental zygotes

$$i = P_0 + \frac{1}{2} P_1 + \frac{1}{3} P_2 + \frac{1}{6} P_3$$

where it is clear that

$$i \text{ concerns 1 identity class for } P_0, P_1 \text{ and } P_3 \\ \text{and 2 equiprobable classes for } P_2$$

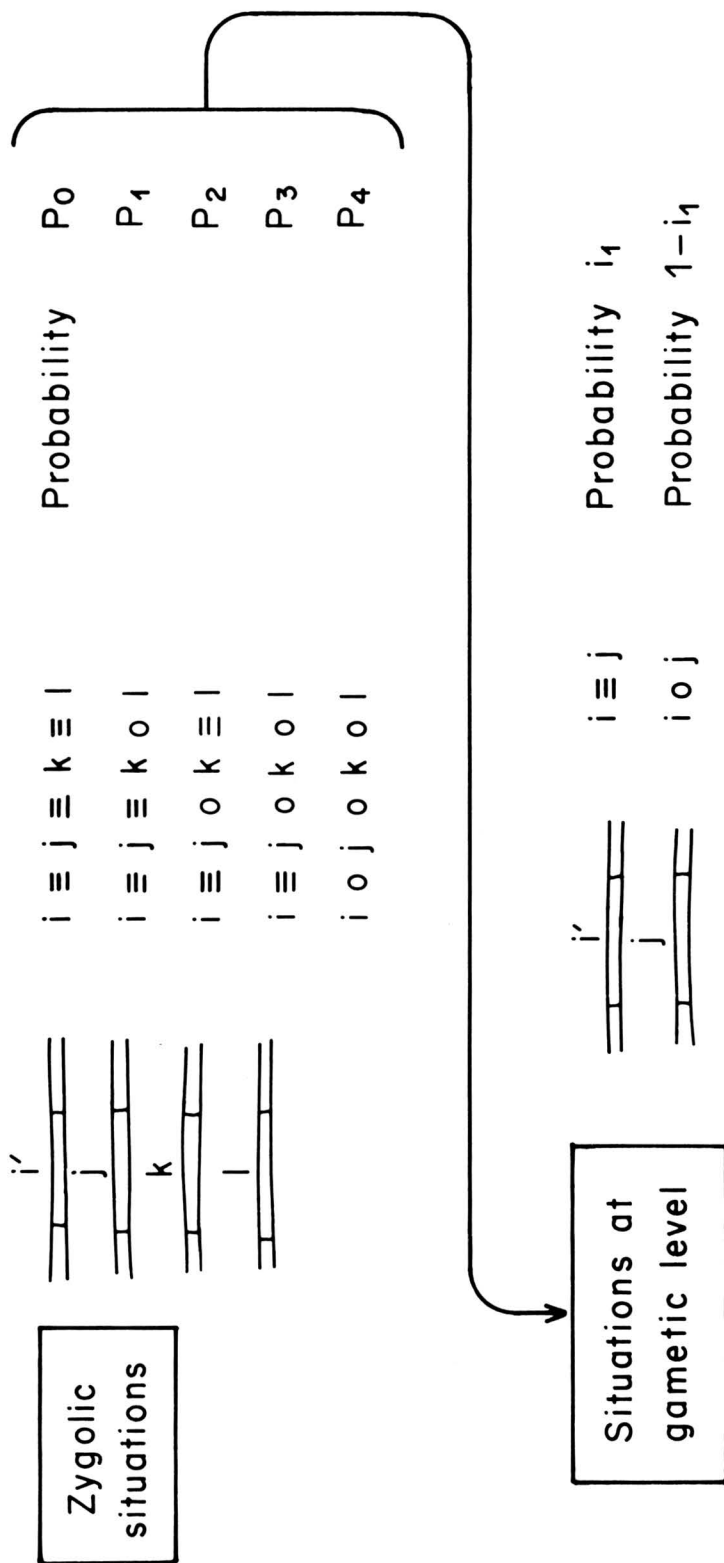
Note that in these gametes, $\frac{1}{3}$ reproduces the gametes which have given this plant that is the internal class of identity and $\frac{2}{3}$ are recombined formulae and belongs to relational identity situations.

This is specific of polyploids : the gametic output, not only contains the additive effects of the alleles of the plant as in diploids, but is able to transmit the interactions from generation to generation.

In other words if i_n , r_{1n} and r_{2n} are respectively the internal and two relational gametic probabilities of identity in the n^{th} generation, we get at the $(n + 1)^{\text{th}}$:

FIGURE XII

Gametic heterogeneity



$$i_1 = P_0 + \frac{1}{2} P_1 + \frac{1}{3} P_2 + \frac{1}{6} P_3$$

$$i_{n+1} = i_n \left[\frac{1}{3} + \frac{2}{3} \left(\frac{r_{1n}}{2} + r_{2n} \right) \right] + (1 - i_n) \frac{2}{3} \left(\frac{r_{1n}}{4} + \frac{r_{2n}}{2} \right)$$

These studies of meiosis, and the choice of a breeding system define the genetic structure of a population.

B - Selfing

Most authors have used the generation-matrix to analyse the evolution of genetic structure in selfing.

In this case of the most intensive system of inbreeding we write :

$$\{N + 1\} = \boxed{G} \{N\}$$

where $\{N\}$ and $\{N + 1\}$ are the matrix of probabilities for the situations d'identité in N and $N + 1$ generations and \boxed{G} is the operator-matrix from N to $N + 1$.

The main conclusions given by the developed formula in selfing are :

1°) Rate of fixation for an allele.

The probability to get a monogenic structure (when $\alpha = 0$) is given the following table :

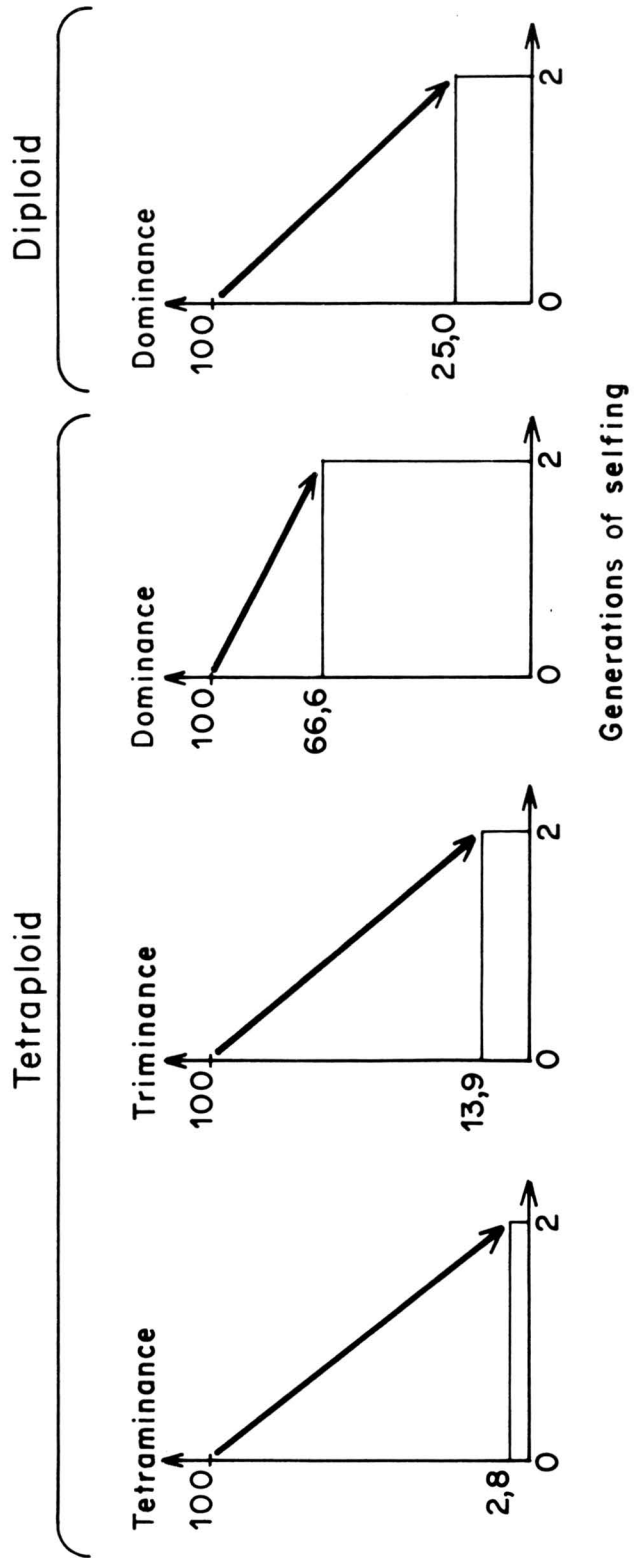
| Genotype of Mother-Plant | Probability of a quadruplex in the progeny after 1 selfing | after 10 selfings |
|----------------------------------|---|-------------------|
| tetragenic <i>a.b.c.d</i> | 0, 00 | 0, 18 |
| trigenic <i>a.a.b.c</i> | 0, 03 | 0, 40 |
| monogenic simplex <i>A.a.a.a</i> | 0, 25 | 0, 68 |

This emphasises the importance of selection a valuable parental structure (fig. 13).

In some cases, the progress towards homozygotes is very slow as relative to diploids where the rate is given by $\frac{1}{2} \left(1 - \left(\frac{1}{2}\right)^n \right)$. The ratio between heterozygosity in a tetraploid digenic duplex and in a diploid heterozygote is given by

FIGURE XIII

Destruction of interactions by two generations of selfing



$$\frac{\text{Het. } 4n}{\text{Het. } 2n} = \frac{7 \times 5^n - 1}{\frac{6 \times 6n}{\left(\frac{1}{2}\right)^n}}$$

where n is the number of selfing

| | | | | |
|-------|-----|-----|------|-------|
| n | 1 | 2 | 5 | 10 |
| ratio | 1,9 | 3,2 | 15,0 | 192,7 |

2°) Another important aspect in the inbreeding process lies on the genetic "derive" or loss of alleles. The mean number of alleles per locus has been compared in di and tetraploid by Guy (in particular he calculates for a given loss the number of plants to be kept).

$$f_{\text{pop}} = P_0 + 2(P_1 + P_2) + 3P_3 + 4P_4$$

where f_{pop} is the mean number of alleles and P_0, P_1, P_2, P_3, P_4 the probabilities of each situation d'identité.

Therefore the mean allelic frequency decreases with the loss of higher degrees in interactions (P_4 and P_3). But relative to diploid this decrease is much slower.

As an exemple we shall give the following figures :

After 2 generations of selfing :

$$f_{\text{pop}} = 2,74 \text{ starting from a tetragenic structure}$$

$$f_{\text{pop}} = 1,25 \text{ starting from the heterozygote diploid}$$

We can see that the diploid is already very poor in allelic diversity.

After 10 generations of selfing

$$\text{The progeny from the tetragenic gives } f_{\text{pop}} = 1,26$$

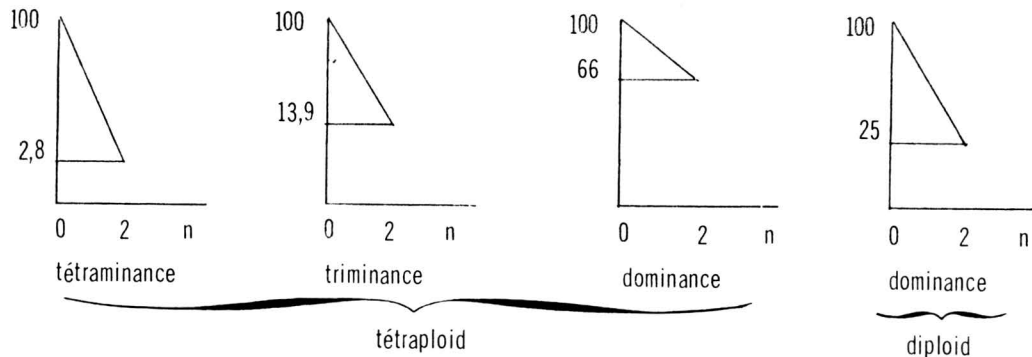
$$\text{The progeny from the diploid } f_{\text{pop}} = 1.$$

The tetraploid population keeps after 10 generations the same diversity as the diploid after 2.

3°) Relative to the loss of alleles we must point out a decrease of interactions. Carnahan first, then Dudley and more intensively Busbice drew attention on the important part played by interactions.

In fact it is not rare to observe that in autotetraploid plants : lucerne, cocksfoot, leeks, potatoes ... the loss of vigour due to inbreeding is much more higher than that which could be predicted on the basis of a progress toward homozygosity.

In the analysis we must keep in mind not only the decrease of dominance effects (where di and tetraploids behave similarly) but also the decrease of tri and tetraminance. For instance we shall note that starting from a tetragenic structure abcd, whose high vigour could be explained by tetraminance effects (which are there wholly expressed), it remains only after two generations of selfing 2,8 % of this interaction as shown in the following graphs :



4°) Another important aspect in the comparison of genetic comportment of di and tetraploids concerns the evolution of genetic variances in inbreeding systems. The analysis of J. Bouffette and A. Gallais give the formula taking in account of only additive and dominance effects.

Considering the expression of these variances, and limiting to the interactions between two alleles, Gallais concludes that the genetical variability constantly increases if it is essentially due to additive effects, but tends to a maximum and then decreases if interactions between alleles play an important part.

Here we get quite distinct conclusions from those of diploid organisms.

C - Panmixia

The behaviour of diploid in panmixia has been extensively studied since the establishment of Hardy-Weinberg law. What are the particular features for tetraploids ?

In a panmictic population where exist, for one part of the genome p structures of homologous linkats, and assuming that each has an equal frequency, we write that the internal gametic identity coefficient i_{n+1} in a $(n + 1)^{th}$ generation is equal to

$\frac{1}{3} i_n$ which represents the gametes reproducing the structure of united gametes giving the parent plant.

and $\frac{2}{3}$ resulting from a recombined structure where in the frequency $\frac{1}{2}$ the

same linkat is present.

$$i_n + 1 = \frac{1}{3} i_n + \frac{2}{3} \frac{1}{p^2}$$

$$i_n + 1 - \frac{1}{p^2} = \frac{1}{3} \left(i_n - \frac{1}{p^2} \right)$$

$$i_n - \frac{1}{p^2} = \frac{1}{3}^n \left(i_0 - \frac{1}{p^2} \right) \text{ if there are } n \text{ panmictic generations from } 0 \text{ to } n$$

In this general formula appears the limit $\frac{1}{p^2}$ for the panmictic equilibrium.

The distance to equilibrium decreases at the rate of $\frac{1}{3}$ for each generation.

Then, opposite to diploids where in the same conditions the equilibrium is obtained in two generations, we see that a tetraploid population reaches its on equilibrium very slowly. The gametic homogeneity reaches a critical value $\frac{1}{p^2}$ which in most cases is a maximum.

We can compare for one locus, the evolution in panmixia of one diploid hybrid ab with the tetraploid aabb. For the diploid in one generation the population is at equilibrium :

$$\frac{1}{4} aa + \frac{1}{2} ab + \frac{1}{4} aa$$

For the tetraploid we have then :

$$i_n - \frac{1}{4} = \frac{1}{3}^n \left(\frac{1}{3} - \frac{1}{4} \right)$$

The equilibrium is there attained very slowly. One writes the zygotic population :

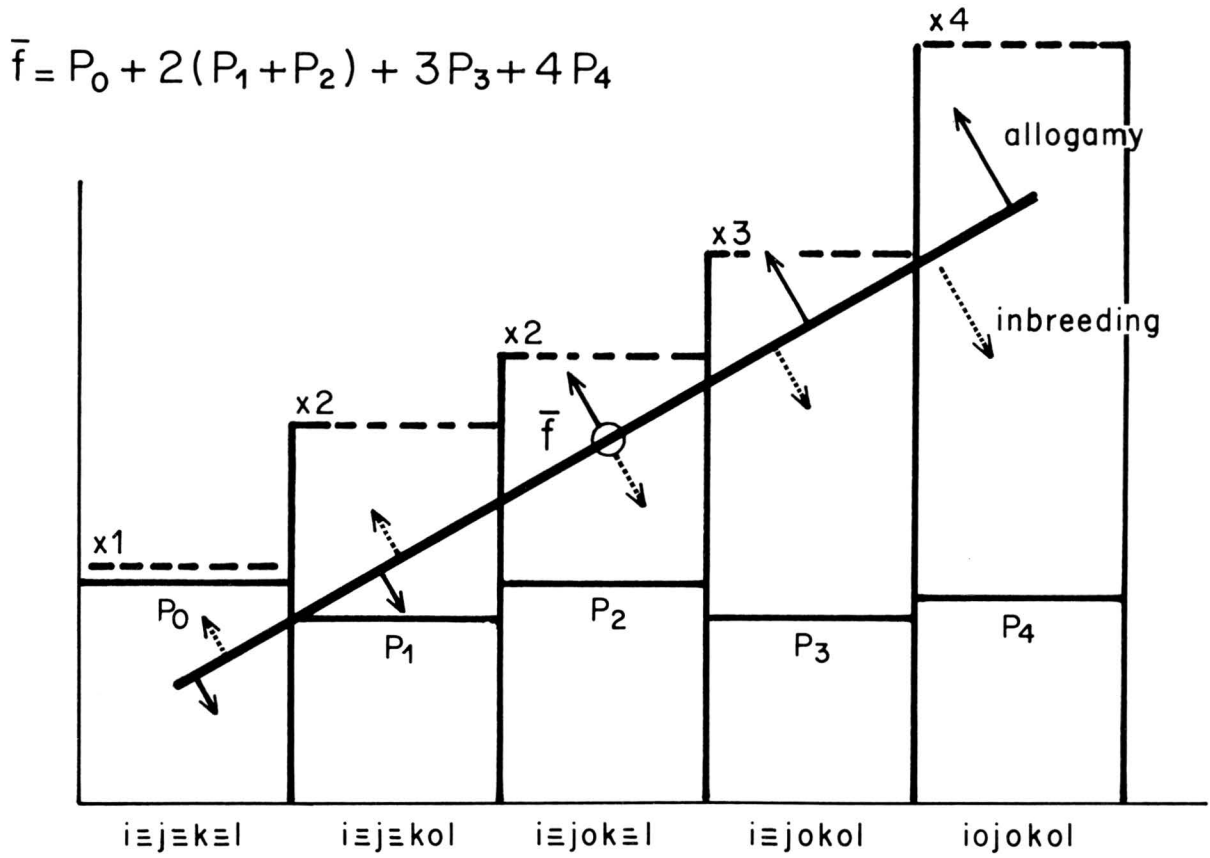
$$\frac{1}{16} aaaa + \frac{1}{4} aaab + \frac{1}{8} aabb + \frac{1}{4} abbb + \frac{1}{16} bbbb$$

Notice that i_n is of the same nature than an inbreeding coefficient.

With these hypotheses this coefficient at the equilibrium is equal to $i_e = \frac{1}{p^2}$

FIGURE XIV

Mean diversity of linkats in a population



These formulae open to the most important conclusions for panmixia :

Evolution of mean number of alleles.

Evolution of the weighted interactions (dominance, tri and tetraminance)
(fig. 14).

From these results under inbreeding as well as in panmixia we can easily infer the theoretical value of different breeding programs with tetraploids : intensity of inbreeding, single or double hybrids, synthetic varieties with their number of constituents and the number of cycles for multiplication, the efficiency of artificially induced tetraploidy

THE GENETIC BASIS OF PRESENT BREEDING METHODS
IN FORAGE CROPS

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INTRODUCTION - PRESENT EVOLUTIONARY STAGE

For any particular species, breeding methods are largely conditioned by the stage of evolution and development of that species as a crop plant. Continued selection and isolation by man are likely to have influenced the amount of genetic variability, the mating system and the type of gene action, all of which are interdependent and together determine the applicability of any particular breeding method.

Forage grasses and legumes have a relatively short history as intensively cultivated crops, and an even shorter history of directed artificial selection. As a consequence, in all the common species, there exists in the wild or semi-wild state a wide diversity of natural adaptations to climatic and edaphic conditions and to biotic systems. These vast resources of genetic variability placed the first breeders of forage crops in a fortunate position *vis a vis* their counterparts in crops which had achieved a narrow genetic base either by virtue of a long history of cultivation and selection (e.g. cereals) or, through recent introduction on too narrow a scale (e.g. rubber). Rapid improvement was possible and was achieved in forage crops by the application of sound ecological principles to the discovery and exploitation of naturally occurring ecotypes. While some advance may still be possible in this way, we now have to consider the best means of more rigorously tailoring genetic material to the demands of increasingly sophisticated farm systems.

THE BREEDING SYSTEM AND POPULATION STRUCTURE

The adjustments and adaptations to the genetic system imposed by past selection (natural or artificial) will limit and channel the responses to future selection. The genetic architecture of a species will thus determine breeding methods. A knowledge of genetic architecture is therefore a most necessary adjunct to breeding programmes and at Aberystwyth we have studied this in the diploid ryegrass species Lolium perenne, which is the most important grass in British agriculture.

Like most of the forage species, *L. perenne* is outbreeding with a fairly efficient self-incompatibility system. Past selection will have operated on heterozygotes to secure genic combinations which are relationally rather than internally balanced (Darlington and Mather, 1949). In breeding improved varieties the aim must therefore be to retain an acceptable level of heterozygosity in the production of synthetic varieties or to maximise heterozygosity as in the production of F1 hybrids. The relative advantages of these depend on a number of considerations, not the least of which is the way genetic variation is controlled by gene action and linkage.

J.P. Cooper (1961) and his colleagues have studied the variation of a number of metrical characters in different populations of ryegrass, both in seed derived from natural ecotypes and in bred varieties. The results indicate the highly heterogeneous nature of the populations and the heterozygous state of the individuals. A high degree of heritability could be demonstrated for many of the characters studied, thus suggesting mainly additive gene action. Considerable response to phenotypic selection was possible before disturbances in fertility, or other metabolic upsets, imposed limits to further advance.

Ecotypic differentiation and genetic architecture in persistent populations

A striking feature of the ryegrass species is the vast array of ecotypes. The authors of this paper have studied in some detail the genetic systems underlying this differentiation with particular reference to persistent populations. Generally ryegrasses are dominant where there is fairly close grazing by animals, and under these conditions regeneration by seed is rare, and survival of the population depends more on the ability to reproduce asexually from vegetative tillers. Thus, we have to bear in mind that the genetic isolation necessary for population differentiation may be achieved through asexual reproduction.

Genetic analysis of differences between populations has been undertaken by the application of full or partial diallel crosses (Hayward and Breese 1966, 68, Hayward and Nsowah, 1969). The salient points to emerge are, first, that for characters which may be adjudged important in adaptation genic differences are largely additive. There is little evidence of dominance and epistatic effects which, as pointed out by Breese and Mather (1960), are a means of preserving and stabilising genetic differences over sexual generations. Again this points to the importance of the asexual stage in differentiation.

A second significant feature of the analysis is the importance of maternal effects and specific reciprocal differences which we have interpreted as being most likely caused by extrachromosomal constituents (the plasmon). It would appear, therefore, that the populations may have become differentiated with respect to the plasmon.

With the plasmon implicated, the question arose as to the existence of somatic variability within a clone (single genotype) and, if so, whether this could be exploited by selection during vegetative propagation. Experiments showed that in some clones, particularly those deriving from persistent

ecotypes, response to selection for fast and slow rates of tillering could be obtained over cycles of vegetative propagation by tillers (Breese *et al*, 1965). We have again proposed that this response is most likely mediated by adaptive variability in extranuclear factors of the plasmon. We have not yet been able to demonstrate that these artificially selected differences can be transmitted over sexual generations, so the results are no more than an indication of how the reciprocal-cross differences between populations could have arisen.

The situation would appear to be that the more persistent ryegrass ecotypes are aggregates of genotypically distinct clones (we have no evidence that any particular sward is dominated by a single clone). The individuals are unified by a phenotypic expression allowing them to compete equally with each other (perhaps even co-operate) and compete successfully with other sward constituents during asexual reproduction. They are not, however, selected to transmit these characteristics with a high degree of constancy to their sexual offspring ; rather, the sexual phase is marked by maximum genetic heterogeneity mediated by additive gene action. In this sense we can see that the sexual cycle is adapted by virtue of this genetic heterogeneity to be a colonising phase, while stabilisation is achieved through asexual reproduction. Selection will initially operate to eliminate genotypes which cannot successfully compete under any specific conditions of climatic, edaphic and biotic effects. The experiments of Charles (1966) have shown that seedling elimination under such competitive conditions can be massive, reaching up to 90% in sown swards after a few months. Subsequently surviving genotypes will by vegetative propagation be represented by independent tillers and at this stage selection may operate on a second dimension of variability the plasmon. As discussed by Hayward and Breese (1968), alternating generations of sexual and asexual production may thus involve the plasmon in a dynamic role, and force a special co-adaptation of nucleus and plasmon. However, our knowledge of such possible interactions is presently very limited and we require further study of the inter-relationships of the nucleus, plasmon and all cell inclusions, including viruses, before these special extranuclear effects can be considered profitably in a plant breeding context.

Variation in Genetic System

Within the species, inherent persistency (longevity of genotypes) varies greatly, although this depends on the management. The relative importance of sexual and asexual reproduction will therefore alter and this will be accompanied by changes in the genetic system.

The physiological consequences of conscious or unconscious selection for excellence in sexual reproduction (i.e. where emphasis is placed on high seed yields) are now a part of plant breeding history. Early attempts to produce commercial quantities of seed by an unrestricted number of generations from pastures with a high reputation for animal production, led to a loss of the leafiness and high tillering capacities which characterised the original plants, and ultimately to the production of stemmy, short-lived "commercial" varieties (Beddows, 1953). The change in genetic control is likely to be

no less striking.

By analogy with experiments using laboratory organisms (Breese and Mather 1960), we can expect the uniformity of characters associated with high seed production to be reinforced by the selection of dominant and epistatic genes controlling their expression. Our evidence on this in ryegrass is scarce, but an experiment reported by Breese (1961) shows an increase in dominance variance following selection over sexual cycles. Again, the cytological studies of Rees and Ahmad (1963) show that the linkage relationships may change. The shorter lived populations have a relatively high chiasma frequency and less localisation of chiasmata. We may assume therefore that selection for efficiency in sexual reproduction has resulted in higher recombination within chromosomes leading to a finer adjustment of genic balance, but ultimately to a restricted amount of genetic variability. By contrast, the longer-lived ecotypes have a relatively low chiasma frequency with chiasmata localised at the distal ends. Large lengths of the chromosome are thus kept distinct and act as single units of inheritance (effective factors) of relatively major effect. In consequence, accumulated differences within these chromosome segments effectively segregate as multiple alleles and can maintain large numbers of polymorphisms within the populations. This may incidentally explain the very rapid response to selection for date of ear emergence obtained by Cooper (1959) in some populations.

We can now see that in this relatively undeveloped crop, the systems governing genetic variation are themselves variable. We have the contrast of highly heterozygous and heterogeneous, polymorphic and panmictic groups at one extreme, with additive genetic control and emphasis on asexual reproduction ; at the other extreme are the shorter lived sexual populations with (putatively) higher dominance and epistasis, but with reduced genetic variation. These systems are easily (perhaps too easily) adjustable by selection, but we have to be warned that movement towards the latter system - towards reduced variability - tends to be a one-way process. We must view our breeding aims accordingly and guard against methods which may be too wasteful of existing variation.

We have so far dealt with the flexibility of the species in relation to its genetic heterogeneity. Of no less importance in meeting changing demands in the environment is the phenotypic plasticity of the individual.

Genotype - environment interactions:- Adaptive responses or unpredictable instabilities ?

The ability of individuals to show plastic responses to varying environments is of special interest in herbage plants, since perhaps no other crop is expected to yield under such a diversity of climatic, edaphic and management conditions. Until recently we had to rely on the analysis of variance as means of recognising differential responses by genotypes or varieties to varying environments (Comstock and Robinson, 1952). Over the past few years interest in the form of regression analysis first proposed by Yates and Cochran has been revived by the studies of Finlay and Wilkinson (1963) and

the Birmingham School of Genetics (e.g. Alanis and Hill, 1966 ; Perkins and Jinks, 1968).

Briefly, the technique involves quantifying each environment in terms of the plant character being measured, which is normally given as the mean performance of all genotypes (varieties) under trial for each environment under test. The performance of individual genotypes may then be expressed by the regression of their individual values on this mean. In a number of cases for a number of crops, it has now been shown that significant proportions of genotype-environment interactions can be explained as differences in the slope of linear regressions so calculated. In grasses these regression analysis have been used to show that large genotype-environment interactions for the production of fresh material by spaced plants of cocksfoot have a high degree of predictability over a wide range of soil, seasonal and management effects (Breese 1969) ; they have also been successfully applied to combine yield results from a number of experiments covering a wide range of sward and spaced plant conditions for ryegrasses, again with a high degree of predictability from one set of conditions to another, including the possibility of extrapolating from spaced plants to swards (Samuel et al 1970). Information on its applicability continues to accumulate with varying degrees of success (Breese, unpublished, Jacquard 1970) but generally to proclaim its value.

Just as importantly the plastic responses which are measured by these regressions appear to be highly heritable and in the grasses are controlled chiefly by genes which are predominantly additive in action (Breese 1969, Hill and Samuel, pers. com. ; Hayward and Lawrence, 1970).

Although we clearly need to know more of the extent to which genotype-environment interactions can be explained in this way, there is no doubt that in many instances changes in genotypic values, even changes in genetic variances and components, from location to location and from year to year, may be explained by simply expressed and simply inherited linear responses. This lends hope that we shall be able not only to specify the range of conditions over which the variety may be used, but also breed for a specified range of conditions in a more determinate manner.

BREEDING METHODS IN RELATION TO GENETIC ARCHITECTURE

Sward Establishment and Population Improvement

In herbage plant breeding we have the near paradoxical situation that although we are often seeking improvement of properties associated with asexual reproduction (vegetative growth and regrowth), we attempt this by selection over sexual (seed) generations. Bearing in mind the contrasting genetic organisations that these two reproductive systems incur, as well as the conflicting physiological demands, we have to be specially careful of the breeding methods we adopt.

Perhaps, in time, improved techniques will make it possible to lay down swards

by the vegetative propagation of selected clones, but at present it is economically more feasible to establish swards from seed. Seed rates are determined by the necessity to obtain a rapid soil cover and so to keep in check weed species. The experiments of Charles (1966) have shown that these rates are such that there is a rapid elimination of a very high proportion of the germinated seedling which is particularly marked in the more persistent, high tillering varieties. Only a small percentage of the sown genotypes survive to form the sward, the actual percentage depending on the duration of the ley, but is less than 10% after a few months. Under present cultural systems, therefore, the competitive conditions of the sward involves a high genetic wastage which can be tolerated (and is indeed made necessary) by the asexual reproductive capacities of the survivors. The type of these survivors depends on the management. Thus establishing through seed at high rates we are recreating the conditions for ecotype evolution on a miniature scale, and the consequences of subsequent management are an extension of the ecological principles governing this process.

As breeders, we have to be concerned with improving the potential of a population with respect to a specific management. Technically, we only require to achieve a proportion of desirable genotypes which are also competitively superior ; the actual proportion would of course depend on the stochastic processes governing sward establishment under specific management, but it could be a very low percentage. Breeding methods designed to raise the proportion of genotypes above this critical level would confer no certain advantage under present practice. The value of a variety thus depends less on the mean value for all genotypes than on achieving a minimum frequency of desirable genotypes, so that uniformity per se has no particular value.

Other arguments are advanced for heterogeneity as opposed to uniformity, particularly that this confers a degree of flexibility on the variety under varying managements and conditions. Too often this become an excuse for not precisely defining conditions for use. Another aspect, however, is that heterogeneity may allow the selection of favourable co-adapted genotypes (Allard and Adams 1968) which complement each other under sward conditions. This aspect, which has been termed ecological combining ability, has of course received considerable attention in the studies of mixtures, and recent work (e.g. Rhodes, 1968) lends hope that we may more precisely describe these interactions and thus be able to breed specifically for them.

With these considerations in mind, our choice of breeding methods may profitably be guided by a recognition of the way breeding systems are associated with different ecotypes as discussed earlier.

The Synthetic Variety

In herbage plants the most common breeding method is by population improvement through the use of synthetic varieties. There is often confusion as to what distinguishes a synthetic variety from other open pollinated varieties. Often the differences are only of academic importance, and we will define the synthetic variety as a population that is produced by hybridizing in all

possible combinations a number of selected genotypes, and is thereafter maintained and multiplied by random mating in isolation (over a limited number of generations). The stated aims are to increase the frequency of genes with favourable expression in particular characters while avoiding inbreeding depression.

Selection on Phenotype

The success of phenotypic selection depends on the accuracy with which we can recognise the breeding value of a genotype from its phenotypic expression. Dominance and epistasis, or environmental effects including genotype-environment interactions, all hamper this recognition. This has led to the concept of "narrow sense" heritability which is expressed as the proportion of the additive variance to the total variance observed, and which can be measured in a number of ways. From the experiments referred to earlier, many characters in ryegrass are controlled by genes which are largely additive in action, with dominance and epistasis of lesser importance. This is particularly so for the persistent types, and we might perhaps infer that many vegetative features associated with asexual reproduction may be controlled in this way. For these characters phenotypic selection should be effective.

For quantitative characters controlled by many genes, considerations in this type of selection are the size of the initial population to give a wide selection differential, and the ultimate reduction in population size following strict selection, leading to reduced variability and inbreeding depression. The latter depends on the intensity of selection together with the degree of pollination control, and the number of selection generations. Mass selection (with no control of the pollen parent) effectively maintains population size, thus allows reassortment of genes, and should result in gradual advance over generations. At Aberystwyth we have intensively and successfully selected for high and low water-soluble carbohydrates in ryegrass over three generations. Here individual male and female parents were selected and pair-crossed, but population size was effectively maintained and inbreeding avoided by maintaining a number of high and low lines and cyclically mating between them. Successful selections for high and low digestibilities were carried out over three generations of sib-mating in cocksfoot ; again, multiple lines were carried at each selection level and the best lines have been finally composited to form synthetic varieties.

Many other cases of successful phenotypic selection may be cited. A commonly found phenomenon, however, is that reported by Edwards and Cooper (1963) where selection for one component of yield evoked negative responses in other components, so that yield itself was little affected. Reports of correlated responses are of course legion. Following the lead given by the experiments of Mather and Harrison (1949) it is now widely accepted that many of these correlations represent a co-ordination of physiological processes which is often achieved through linkage of the genes controlling the different processes, and that readjustment can be obtained through recombination and selection. At the same time it must be recognised that some traits are inevitable concomitants of the same physiological process (pleiotropic effects).

Since the breeder is rarely concerned with improvement in a single trait in the absence of consideration for other characters, a knowledge of the strength and type of such associations is invaluable to the formulation of breeding objectives and in deciding on the best means of achieving these, whether by the use of selection indexes or other methods.

Selection by progeny testing

Resort to progeny tests is made when for any reason the heritability of a particular phenotypic expression is low. This of course leads to an increase in the time required for each selection cycle and Morley and Heinrichs (1960) have estimated that heritability has to be as low as 10% before the inclusion of a progeny test will give better results in annual improvement compared with phenotypic (individual) selection alone. Before we consider further some reasons for low heritability estimates and possible ways of correcting these, we may perhaps consider possible progeny testing methods and their genetical implications and consequences.

First we have to be aware that certain assessments of progeny may run the danger of giving undue prominence to sexual reproduction which is out of proportion to its evolutionary duality with asexual reproduction.

The aim of a progeny test is to distinguish superior parents. In domestic animals and most cultivated crops the value of each individual is high and uniformity is at a premium, because genetic wastage cannot be tolerated. For outbreeders the breeding value of an individual is usually determined by out-crossing methods and this results in selecting parents which are prepotent largely because of genes showing dominance and epistasis in the required direction. The value of these tests may be questioned in the relatively undeveloped herbage plants on two counts. First, asexual reproduction allows genetic wastage during sward establishment and thus, provided this wastage can be directed, does not call for uniformity. Second, putting a selective bias on genes showing dominance and epistasis in one direction may sacrifice large amounts of the additive variance which we find in natural populations, and will almost certainly be at the expense of genes showing dominance and epistasis in the opposite direction (i.e. we shall miss useful recessives). In an undeveloped crop this latter effect could be important in populations which have not previously been subjected to directional selection since here dominance and epistasis are likely to be ambi-directional (Breese and Mather, 1960). In other words we shall be selecting for only part of the full spectrum of gene differences.

We shall consequently consider selection methods based on progeny testing (recurrent selection) in this light. Recurrent selection may be classified into four types ; simple recurrent selection, recurrent selection for general combining ability, recurrent selection for specific combining ability and reciprocal recurrent selection. All these are generally discussed by Allard (1960) and the genetic implications are to some extent considered by Sprague (1966). Consequently, they will only briefly be reviewed here.

Before we consider the genetic basis of these progeny tests, further, it may be apposite to digress and consider the variously designated components of variation and how these relate to general (GCA) and specific combining ability (SCA). Confusion as to the precise meaning of these terms arises from the various approaches of statistical geneticists, population geneticists and plant or animal breeders. Strictly speaking, genetic variance is additive when the effects of gene substitutions are linear ; i.e. there is an absence of interaction between alleles (dominance) or between loci (epistasis). However, because of the systems of component analysis developed by Comstock and Robinson (1948), Mather (1949) and others, additive variance (D or σ_A^2) has come to connote fixable genetic variation, being measured as differences between homozygotes : dominance variance (H or σ_D^2) is then measured as deviations due to interactions of heterozygous loci, and epistatic variance (I or $\sigma_{AA}^2 = \text{fixable}$, J and L or σ_{DD}^2 and $\sigma_{AD}^2 = \text{unfixable}$) as deviations due to non-allelic interactions. The first of these notations in brackets is that of Mather (loc cit) and the second of Comstock and Robinson (loc cit). Thus additivity may be used variously to describe a type of gene action or to indicate the average effects of homozygous versus heterozygous contributions to variability. In the absence of overdominance or special interaction between heterozygous loci (and there is no good evidence to indicate that either of these are important generally) all phenotypic expressions to which these various types of gene action subscribe are fixable in homozygotes. By population theory dominance and epistasis arise not so much as a means of obtaining a particular genotypic value, but of securing a high frequency of these values in the sexual offspring of outbreeders.

A further confusion is sometimes provoked by the use of plant breeding terms in too loose a genetic context. Thus GCA was originally coined to describe the breeding value of an individual parent in hybrid combinations with other plants. Intuitively we can see (and it can be algebraically shown) that this breeding value will be enhanced where a parent is homozygous for genes exhibiting dominance and epistasis in the favoured direction. The term has on occasion been transposed to genetic analysis, particularly of diallel crosses, to designate differences between arrays and so has ultimately assumed equivalence with additive (homozygous) genetic variance. In the same way specific combining ability has been equated with dominance and epistatic variance, whereas it was originally coined to describe the special properties of an individual cross. It is important to realise that both GCA and SCA may be associated with dominance and epistasis : they refer to properties of individuals as parents in particular cross combinations and not necessarily to distinguish between properties of the genes controlling variation.

Of the recurrent selection methods cited, simple recurrent selection usually depends on progeny tests involving a degree of inbreeding, including selfed, full-sib or half-sib (e.g. certain forms of top crosses to a recessive tester) families. They give better estimates of homozygous variation than do outbreeding tests and so these may be particularly advantageous in relatively unselected populations such as forage plants with a high degree of additive variation or where recessive genes may be favourable (i.e. under systems of balanced dominance). Recurrent selection for GCA involves outbreeding progeny tests,

including open pollination and the more sophisticated polycross technique. Gene combinations are largely tested therefore in the heterozygous state and, as we have seen, this may provide a bias towards those genes showing dominance and epistasis in the required direction to the detriment of other favourable genes with differing gene action. Indeed, a variety produced by the polycross method at the W.P.B.S. has demonstrated a higher frequency of dominant genes than natural populations (Breese 1961). A more detailed discussion of the relative merits of inbreeding versus outbreeding progeny tests is given by Latter (1964).

Both other forms of recurrent selection have been associated with schemes designed to obtain improved complementation between populations (SCA) in the production of hybrid varieties. Neither practice (Sprague, 1966) nor mathematical models and computer simulation (Cress, 1966) show that they are particularly effective in obtaining this end, nor does reciprocal recurrent selection prove to be as effective as selection for general combining ability.

In this brief discussion of progeny tests we have attempted to raise problems which are specific to the relatively undeveloped herbage crops. Thus, although dominance and epistasis may not initially be important, we may make them so by selection over sexual cycles, particularly by placing emphasis on combining ability. In the process we may squander genes which are additive in action or useful recessives. We have argued this only in general terms ; there is clearly need for a closer study of the precise consequences of different types of selection with different gene systems. Indeed, as Sprague (1966) notes, selection theory is generally inadequate in predicting from changes in gene frequency the associated changes in genotypic values, genetic correlations or population parameters. We still have to work by intuitive reasoning based on general genetic knowledge rather than with mathematical precision.

There does, however, seem to be a powerful argument for selecting on phenotypic expression in these undeveloped crops wherever this is possible, for genetical reasons as well as for reasons of time scale. It is interesting to note in this context that Sprague (1966) considers in retrospect that significant advances could have been made by mass (phenotypic) selection in maize, given better techniques such as more precise measurement and better isolation ; indeed he estimates that this would have been more effective than any recurrent selection scheme and that the advances could have more than matched improvements through the use of hybrid varieties on the same time scale.

We have to bear in mind, however, that many of the reasons for establishing progeny tests in herbage crops are the difficulties of obtaining measurements on individual plants which can be related to yield under the competitive conditions of swards under different managements. In this respect heritability is reduced by virtue of the high environmental components and genotype-environment interactions, rather than by dominance or epistatic effects. Before resorting to progenies, however, we can consider first the value of repetitive measurements on the same plant and secondly the possibilities of establishing clonal replicates. Both of these can be used to furnish information on environmental effects and genotype-environmental interactions (see also Latter, 1964). With

the development of the regression techniques considered earlier, genotype-environment interactions may no longer hamper the estimation of the breeding value of an individual as in the past, since the response coefficients will prove to be inherited in an additive fashion and so heritability estimates may be improved by including part of the genotype-environment component in the numerator (as discussed by Breese, 1969).

Before we can fully appreciate the scope for phenotypic selection, however, we must place more emphasis on physiological, nutritional and competition studies leading to more precise measurements of plant characteristics which relate to ultimate yield. At present we are too often forced to adopt relatively slow and inefficient methods of breeding because we have to attempt evaluation of yield and competitive value through the use of progeny in sward simulation techniques; techniques which are often woefully inadequate in encompassing all required management, locational and seasonal conditions. This is a far cry from the type of biological engineering which we hope multi-discipline studies will ultimately make possible.

Hybrid Varieties

Although we have sought to emphasize the potentiality for population improvement through phenotypic selection, with special reference to the more persistent varieties, it would be a mistake to ignore completely the possibility of exploiting F1 heterosis under certain circumstances. As we have seen, the genetic organisation, including the proportions of dominance and epistatic variance relative to additive variance, can vary and indeed can easily be adjusted within the species. In the shorter-lived material in particular there may be utilisable amounts of F1 heterosis which would be useful in the production of short-duration varieties for conservation systems. Under these conditions asexual reproduction (vegetative tillering) may be less important than features associated with sexual reproduction (formation of reproductive tillers). Again, with advancing farm systems, we may seek reduced heterogeneity in the varieties. Although we shall undoubtedly achieve a degree of this by the intensive selection of synthetic varieties, it is expedient to consider the possibilities for hybrid varieties produced by seed, where heterozygosity is maximised and heterogeneity correspondingly reduced.

The conditions necessary for a hybrid programme are (1) the existence of significant amounts of heterosis (defined as superiority over the best parent) and (2) some means of easily obtaining hybrid seed, such as incompatibility or male sterility. The aims are (1) the achievement of maximal expression by specific heterozygous combinations and (2) a high degree of uniformity in the final crop. The motives are variously determined. First, there is the consideration whether desirable gene combinations can be fixed by selection, and at what rate this can be achieved. If no true overdominance exists, fixation is possible even where dominance and epistasis are present, but this may be significantly retarded by linkage in disequilibrium. Second, a hybrid programme may be motivated for commercial reasons such as control of source seed. Thus, hybrid programmes are more often expediciencies rather than necessities.

At Aberystwyth we are assessing the potentialities for hybrid programmes through a survey of the occurrence of heterosis in crosses between geographically isolated races through inbreeding studies. C.A. Foster (1968) has considered not only the incidence but also the ways in which this may be utilised. The approach which he is investigating is the formation of "mixed varieties" whereby two populations which exhibit F1 heterosis are intersown to produce commercial quantities of up to 50% interpopulation hybrids. Reliance is then placed on the competitive superiority of the hybrid under suitably managed sward conditions to ultimately dominate the crop. An extension would be the use of inbred populations as parents so that the non-hybrid fraction of the mixed variety would be further reduced in competitive vigour. Burton (1956) and Mc William (1962) have used self-incompatibility to produce commercial quantities of hybrid seed from crosses between two selected clones. Ultimately there is no doubt that male sterility could be used in this context.

In any hybrid scheme we are still faced in herbage crops with the problem of specifying and measuring useful yield parameters for specific farm systems, and so have the difficulty of recognising useful heterosis. Again heterosis may be present in one environment and not in another. Foster, for instance, finds that heterosis in spaced plants may not be apparent under sward conditions. The estimation of responses by regression analysis may help clarify this situation (Breese, 1969).

In this paper we have avoided discussion of the special problems associated with the type of heterosis achieved by the combination of characters from widely differentiated races or species, where considerations are more likely to be cytogenetic, involving manipulation of the chromosomal complement.

CONCLUSION

We have sought to relate breeding methods to the reproductive strategy and consequent genetic architecture of the crop species, with special reference to the dual sexual/asexual reproductive systems. We have indicated the high degree of additive genetic variation for many physiological characters and the possibilities for exploiting this through phenotypic selection in improving synthetic varieties. Success here will very much depend on our ability to translate the requirements of nutritional value and agronomic performance into identifiable plant characteristics, and to measure plastic responses of the genotype to the environment. The need for including progeny testing in the selection processes for any particular programme has to be carefully examined, bearing in mind (i) the genetical consequences of this as compared with the natural sexual/asexual reproductive strategy of the crop, (ii) the increased time span per selection cycle, and (iii) the possibilities for improving heritability estimates by the analysis of significant yield factors and better measurements of genotype-environment interactions.

Breeding methods also have to be closely geared to the practical usage of the crop. Under present cultural conditions only a proportion of the sown crop survives through asexual reproduction and we only have to ensure that a high enough proportion of superior genotypes are available for exploitation

by appropriate management. With advancing farm systems, greater homogeneity may become desirable and within this context there may be a use for considering the advantages of hybrid varieties. These advantages will however have to be carefully weighed against the advances which may be expected from continued selection in synthetic varieties.

REFERENCES

- Allard, R.W., 1960. Principles of plant breeding. John Wiley and Sons, inc. New York. p. 485.
- Allard, R.W., and Adams, J., 1968. The role of intergenotypic interactions in plant breeding. Proc.XII Intern. Congr. Genetics, 3, 349-370.
- Alanis, L.B. and Hill, J., 1966. Environmental and genotype-environmental components of variability. II Heterozygotes. Heredity 21, 399-405.
- Beddows, A.R., 1953. The ryegrasses in British Agriculture : A Survey. Welsh Plt Breed. Stn. Bull. Ser. H. 17.
- Breese, E.L., 1961. The genetic assessment of breeding material. Proceedings Eighth International Grassld. Congr. 1960, 45-49.
- Breese, E.L., 1969. The measurement and significance of genotype-environment interactions in grasses. Heredity, 24, 27-44.
- Breese, E.L., and Mather, K., 1960. The organisation of polygenic activity within a chromosome in *Drosophila*. II. Viability. Heredity, 14, 375-401.
- Breese, E.L., Hayward, M.D. and Thomas, A.C., 1965. Somatic selection in perennial ryegrass. Heredity, 20, 367-379.
- Burton, G.W., 1956. Utilization of heterosis in pasture plant breeding. Proceedings Seventh International Grassld. Congr. 1956, 439-449.
- Charles, A.H., 1966. Variation in grass and clover populations in response to agronomic selection pressures. Proceedings Tenth International Grassld. Congr. 1966. 626-629.
- Cooper, J.P., 1959. Selection and population structure in *Lolium*. III. Selection for date of ear emergence. Heredity, 13, 461-479.
- Cooper, J.P., 1961. Selection for production characters in ryegrass. Proceedings Eighth International Grassld. Congr. 1960, 41-44.
- Comstock, R.E., and Robinson, H.F., 1948. The components of genetic variation in populations of biparental progenies and their use in estimating average degree of dominance. Biometrics 4, 254-266.

- Comstock, R.E., and Robinson, H.F., 1952. Genetic parameters; their estimation and significance. Proceedings Sixth International Grassld. Congr., 1952, 284-291.
- Cress, C.E., 1966. A comparison of recurrent selection systems. Genetics, 54, 1371-1379.
- Darlington, C.D., and Mather, K., 1949. The elements of Genetics. Allen and Unwin Ltd. London, pp. 446.
- Edwards, K.J.R., and Cooper, J.P., 1963. The genetic control of leaf development in Lolium. II. Responses to selection. Heredity, 18, 307-317.
- Finlay, K.W., and Wilkinson, G.N., 1963. The analysis of adaptation in a plant breeding programme. Aust. J. Agric. Res., 14, 742-754.
- Foster, C.A., 1968. Intervarietal heterosis in Lolium perenne L. Ph. D. Thesis, Queen's University, Belfast.
- Hayward, M.D., and Breese, E.L., 1966. The genetic organisation of natural populations of Lolium perenne L. I. Seed and seedling characters. Heredity, 21, 287-304.
- Hayward, M.D., and Breese, E.L., 1968. The genetic organisation of natural populations of Lolium perenne L. III. Productivity. Heredity 23, 357-368.
- Hayward, M.D., and Nsowah, G.F., 1969. The genetic organisation of natural populations of Lolium perenne L. IV. Variation within populations. Heredity, 24, 521-528.
- Hayward, M.D., and Lawrence, T., 1970. Genetic control of variation in a selected population of Lolium perenne. Can. J. Genet. and Cytol. (in press).
- Jacquard, P., 1970. Study of the social relations between seven forage species at two trophic levels. Paper to Eleventh International Grassld. Congr. (in press).
- Latter, B.D.H., 1964. Selection methods in the breeding of cross fertilized pasture species. In Grasses and Grassland, Ed. C. Barnard. MacMillan and Co. Ltd. London.
- Mather, K., 1949. Biometrical Genetics. Methuen and Co. Ltd., London. pp. 162.
- Mather, K., and Harrison, B.J., 1949. The manifold effect of selection. Heredity, 3, 1-52 and 131-162.

- McWilliam, J.R., 1962. Interspecific hybrids in Phalaris : hybrids between P. tuberosa and the hexaploid race of P. arundinacea. Aust. J. Agric. Res., 13, 585-598.
- Morley, F.H.W., and Heinrichs, D.H., 1960. Breeding for creeping root in alfalfa. Can. J. Plt. Sci., 40, 424-433.
- Perkins, J., and Jinks, J.L., 1968. Environment and genotype-environmental components of variability. III. Multiple lines and crosses. Heredity, 23, 339- 356.
- Rees, H., and Ahmad, K., 1963. Chiasma frequencies in Lolium populations. Evolution, 13, 575-579.
- Rhodes, I., 1968. Yield of contrasting ryegrass varieties in monoculture and mixed culture. J. Brit. Grassld. Soc., 23, 156-158.
- Samuel, C.J.A., Hill, J., Breese, E.L., and Davies, A.G., 1970. Assessing and predicting environmental response in Lolium perenne. J. Ag. Sci., 75, 1-9.
- Sprague, G.F., 1966. Quantitative genetics in plant improvement. In Plant Breeding, Ed. K. J. Frey. Iowa State University Press, Ames, Iowa.

**EFFECT OF DIVERSITY, NUMBER AND GENETIC RELATIONSHIP OF
COMPONENTS ON YIELD OF SYNTHETIC VARIETIES OF LUCERNE**

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INTRODUCTION

The creation of a synthetic variety is the ultimate goal of the polycross breeding method. A synthetic variety consists according to the definition by Kehr (1964) of advanced generation progenies derived and reconstituted from the same set of selected clones or seed lines. In lucerne usually a certain number of selected clones with good general combining ability is used to form the basis for a synthetic variety.

There are, among others, three questions to be answered before the breeder reaches his decision.

a) Diversity vs. homogeneity

Some research workers (Sachs 1953, Demarly, Guy and Chesneau 1964 and Guy 1966) put forward the idea that diversity of the genotypes would favour agronomic performance of the variety more than homogeneity. This simplifies to the question : Which variety is expected to yield more, one that is composed of a mixture of contrasting but complementary components, or one that consists of single highly productive genotype ?

b) Large vs. small number of components

If one accepts the hypothesis expressed in (a) it would be logical to select a relatively large number of clones in order to achieve the desired diversity which is supposed to result in superior performance.

Contrary to such a conclusion Bolton (1948) and Graumann (1952) suggested that a synthetic made up of only a few highly combining clones would yield more than one composed of many clones.

c) Genetic related vs. unrelated components

A number of clones of high general combining ability may be desirable to form a synthetic variety. Do we have to pay any attention to the genetic relationship of the clones when making the decision which clones to choose ?

A number of field experiments was initiated to investigate these questions.

RESULTS

a) Diversity of components

The objective of the first experiment was to find out whether the mixture of contrasting clones with complementary characteristics would yield more than the mean of the pure stands of the contributing clones, or, in other words, if one could expect a positive interaction among clones.

We know, for example, that both stemmy, upright growing types of lucerne as well as leafy, prostrate types are able to give high yields. The hypothesis was that the yield of the mixture of the contrasting types would exceed the mean yield of the components.

However, such an interaction might occur only if the clones were mixed in certain proportions. Therefore, mixtures of various proportions viz. 3:1, 1:1, and 1:3, were chosen. Three sets of clones were used to establish such mechanical mixtures of contrasting plants.

In practice, of course, more than two types contribute to a synthetic variety. Therefore, two more complex mixtures consisting of three and eight clones respectively, differing in various agronomic characteristics, were added. There was a total number of eleven different mixtures.

The clone combinations were planted in 1963 as a five times replicated randomized block design, each plot being 1 x 1 m large, with plants being spaced 12,5 cm apart. Such a spacing simulates a natural lucerne field stand according to Davies (1963).

Green matter yields were determined in 1963 and 1964. A typical example of the results obtained is presented in table 1.

Table 1. Total yield 1963 of lucerne clone combination set no. 1

| | Clone A 100% | 3:1 | 1:1 | 1:3 | Clone B 100% |
|------------------------------------|-----------------|-------|-------|-------|-----------------|
| Yield kg/m ² weighed | 3.94 | 4.14 | 4.32 | 4.77 | 5.06 |
| Yield kg/m ² calculated | - | 4.22 | 4.45 | 4.78 | - |
| Difference | - | -0.07 | -0.13 | -0.01 | - |

The difference in green matter yield between clone A and clone B is highly significant. However, none of the mixtures of this set exceeded the expected yields, and never did any particular mixture exceed the yield of the better component.

The result of the F-tests for clone combinations are summarized in table 2.

Table 2. Significance of F-tests for clone combinations

| Set of Clones | Year and Cut | | | | | | | | |
|--------------------------------------|--------------|------|------|-------|------|----|-----|----|-------|
| | No. | 1963 | | | 1964 | | | | |
| | | I | II | Total | I | II | III | IV | Total |
| 1 two clones linear non linear | * | | *** | | | | | | * |
| | ** | ** | *** | | | | | | |
| 2 two clones linear non linear | | | ** | | | | | | |
| | | *1) | **1) | | | | | | *1) |
| 3 two clones linear non linear | | | ** | | | | | | |
| | | * | *** | | | | | | |
| 4 three clones | | | | | | | | | |
| 5 eight clones | | | | | | | | | |

* P < 0.05 ; ** P < 0.01 ; *** P < 0.001

1) cubic

Significant deviations of the mixtures from the calculated mean yield of the components were recorded in only three comparisons. This occurred in clone combination set no. 2 where the admixture of a less productive type to the more productive type resulted in a significant yield depression and vice versa.

Also, neither the three clone nor the eight clone combination exceeded the mean of its components.

Our conclusion from these results is that there is no reason to believe that a synthetic variety composed of contrasting types with supplementary agronomic characteristics would yield more than one consisting of a uniform type.

b) Number of components

The next experiment was designed to test the effect of the number of components on the yield of a synthetic. Seed of five experimental synthetics, consisting of 2, 3, 4, 12 and 16 clones, respectively, was produced in 1964. The composition of the synthetics is shown in table 3.

Table 3. Composition of five experimental synthetics of lucerne

| Clone No. | Synthetic No. | | | | |
|------------------------|---------------|---|---|----|----|
| | 1 | 2 | 3 | 4 | 5 |
| 1602 1/8 | + | + | + | + | + |
| 1659 4/1 | + | | + | + | + |
| 1643 4/8 | | + | + | + | + |
| 1615 3/1 | | + | | + | + |
| 1663 2/6 | | | + | | |
| + 8 additional clones | | | | + | |
| + 12 additional clones | | | | | + |
| Number of components | 2 | 3 | 4 | 12 | 16 |

Green matter yields of the synthetics were determined in 1966 and 1967. Results are presented in table 4.

Table 4. Green matter yield of five experimental synthetics of lucerne, first generation, at Weihenstephan 1966 and 1967

| No. of Components | 1966 | 1967 | Sum |
|---------------------------|--------------------|--------------------|---------------------|
| Du Puits t/ha relative | 62.5 <u>100</u> | 63.4 <u>100</u> | 125.9 <u>100</u> |
| 2 | 122 | 115 | 119 |
| 3 | <u>111</u> | 110 | 111 |
| 4 | <u>112</u> | <u>106</u> | <u>109</u> |
| 12 | 113 | <u>104</u> | <u>109</u> |
| 16 | 113 | <u>101</u> | <u>107</u> |
| LSD 5% t/ha | | | 11.3 |

Significant deviations from 2 clone synthetic yield are underlined.

It is evident that yield decreases with increasing number of clones. The two clone synthetic is consistently highest yielding, the sixteen clone synthetic is the least productive.

Table 5. Green matter yields of five experimental synthetics of lucerne, first generation, at two locations 1967

| No. of components | Weihenstephan | Gnodstadt | Mean |
|---------------------------|--------------------|--------------------|--------------------|
| Du Puits t/ha relative | 63.4 <u>100</u> | 35.7 <u>100</u> | 49.6 <u>100</u> |
| 2 | 115 | 112 | 114 |
| 3 | 110 | 115 | 112 |
| 4 | <u>106</u> | 111 | 108 |
| 12 | <u>104</u> | <u>103</u> | 103 |
| 16 | <u>101</u> | <u>103</u> | 101 |
| LSD 5% t/ha | 5.7 | 3.2 | |

Significant deviations from 2 clone synthetic yield are underlined.

Adaptability to different environmental conditions is a major requirement for a crop like lucerne. A better adaptability of the multiple clone synthetics as compared with the two clone synthetic might be anticipated. The experimental data given in tables 4 and 5 lead to the conclusion that different years and locations notwithstanding the yield relationships remain essentially the same. The 2 clone synthetic is always at the top, the 16 clone synthetic at the lower end of the yield array. The genetic variability of only two clones is apparently of such a magnitude as to award the variety with sufficient adaptability to different environmental conditions.

c) Genetic relationship of components

The effect of close genetic relationship among components on the yield of synthetic varieties was investigated in an experiment the result of which is presented in table 6.

Table 6. Effect of close genetic relationship among components on green matter yield of synthetics varieties of lucerne - Weihenstephan 11/2 1966 and 1967

| Variety No. | Composition ¹⁾ | 1966 | 1967 | Total |
|----------------|---|--------------------------|------------|------------|
| Du Puits | t/ha | 62.5 | 63.4 | 125.9 |
| | relative | 100 | 100 | 100 |
| 1 | A ₁ A ₂ A ₃ | 101 | 90 | 95 |
| 2 | A ₁ A ₂ A ₃ A ₄ | 92 | 89 | 91 |
| 3 | B ₁ B ₂ B ₃ | 108 | 98 | 103 |
| 4 | C ₁ C ₂ C ₃ | <u>112</u> ²⁾ | 103 | 107 |
| ∅ 1 - 4 | | 104 | 95 | 99 |
| 5 | A ₁ B ₃ C ₂ F | <u>110</u> | 95 | 103 |
| 6 | A ₁ A ₃ C ₃ D | <u>112</u> | 106 | <u>109</u> |
| 7 | C ₃ D E | <u>111</u> | <u>110</u> | <u>111</u> |
| 8 | A ₁ D | <u>122</u> | <u>115</u> | <u>119</u> |
| ∅ 5 - 8 | | <u>114</u> | <u>107</u> | <u>110</u> |
| LSD 5% tons/ha | | 6.3 | 5.7 | 11.3 |

1) Components indicated by same capital letter are closely genetically related.

2) Significant deviations from Du Puits are underlined

In table 6, each of the first four varieties consists of closely related clones of high general combining ability. The yield except of variety no. 4 do not exceed the yield of the standard variety. Yet combining unrelated clones as in varieties no. 5 to 8 results in substantial yield increases. A good example offers clone group A. The related clones A₁ and A₃ combined with the relatives A₂, or A₂ and A₄, although all of them expressed good general combining ability in the polycross progeny test, did not come up to the yield expectations. However clones A₁ and A₃ combined with C₃ and D result in a superior synthetic variety (no. 6). The highest yielding varieties are those containing the unrelated clones A₁, C₃ and D. Again, the top yielding variety is composed of only two clones.

The conclusion from this and other experiments is that clones in spite of good general combining ability may not give a high yielding variety if they are closely related. It is necessary, therefore, to pay attention to the genetic relationship when selecting components for synthetic lucerne varieties.

SUMMARY

From the results of field experiments it is concluded that using a minimum number of unrelated clones of high combining ability offers a good chance to obtain a high yielding and sufficiently adapted synthetic variety. There is no evidence that the performance and adaptation of a lucerne synthetic can be improved by increasing the number or diversity of the components.

REFERENCES

- Bolton, J.L. (1948) : A study of combining ability of alfalfa in relation to certain methods of selection. *Sci. Agr.* 28, 97-126.
- Davies, W.E. (1963) : The assessment of breeder's material : Spacing lucerne. *Rep. Welsh Plant Breeding Sta.* 1962, 31-33.
- Demarly, Y. (1967) : La mejora genética de la alfalfa. *Jornadas Nacionales sobre la Alfalfa Conferencias* 1, 36-48.
- Graumann, H.O. (1952) : The polycross method of breeding in relation to synthetic varieties and recurrent selection of new clones. *Proc. VI Intern. Grassld. Congr.* 1, 314-319.
- Guy, P. (1966) : Intraspecific competition in forage plants. *Proc. XI Intern. Grassld. Congr.* 1, 183-189.
- Kehr, W.R. (1964) : Genetics and breeding nomenclature. *Rep. Nineteenth Alfalfa Improvement Conference* 1964, 62-64.
- Sachs, E. (1953) : Einige Ergebnisse unserer Luzernezüchtung. *Landw. Jahrb. f. Bayern* 30, Sdheft, 151-162.

THE YIELD IN SUCCEEDING GENERATIONS OF EXPERIMENTAL LUCERNE SYNTHETICS
BASED ON VARYING PLANT NUMBERS

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Although forage breeders have always wished to use the techniques that have so successfully exploited hybrid vigour in the maize crop, successes in this direction have been few and far between. The floral biology of lucerne severely limits the use of similar techniques because it is not possible to control pollination on a sufficiently large scale. In addition, the rate of multiplication or seed increase is slow so that it is necessary to multiply a new variety through 3, 4 or more generations in order to get sufficient quantities of seed for commercial use.

Faced with these difficulties, breeders have generally adopted some variation of the synthetic variety in order to carry through projected improvements. Such varieties have been formed from a number of progeny tested parent plants which are allowed to inter-pollinate, and multiplication of the bulked seed then takes place and is continued through the requisite number of generations. A question that soon arises is the number of basic plants to be used which will maintain optimum vigour and varietal characteristics without too much change during multiplication.

The early work of Kirk (1927), Tysdal et al (1942) demonstrated the extremely deleterious effect of inbreeding and, in general, it can be said that breeders have avoided this danger by including a large number of plants in their varieties. In the absence of detailed information, this was a practical necessity, but much of the recent information indicates that the number of basic plants can be considerably reduced. Thus Kehr et al (1961) and Nielsen and Andreasen (1970) found two-clone synthetics to be as high yielding as four-clone synthetics over four generations.

The theoretical basis of inbreeding and the structure of synthetic varieties at the tetraploid level have been discussed by several authors recently (Busbice and Wilsie, 1966 ; Gallais, 1967, 1968a, 1968b ; Guy, 1968 ; Busbice, 1969). In most of these studies it has been necessary to make many simplifying assumptions, e.g. chromosome segregations, complete panmixis, no selection from generation to generation of mating and no differential fertility. These assumptions will not invariably be satisfied in practice, but they will provide an

expected value against which practical results can be tested.

The experiment reported was set up in 1956, long before the results of experimental and theoretical studies had been published.

MATERIALS AND METHODS

Fourteen experimental synthetic varieties were built up from combinations of 2, 3, 4, 5, 6, and 8-parent plants from among eight plants which were selected from among breeding material. Six of the plants were from the variety Grimm, one from Provence and the other from Turkish material. The fourteen varieties were constructed as follows :

| Parent plant | Experimental synthetic variety number | | | | | |
|--------------------------------------|---------------------------------------|---|---|----|----|-----|
| A B C D E F G H | 1 | 5 | 8 | 10 | 12 | 14 |
| | 2 | 6 | 9 | 11 | 13 | |
| | 3 | 7 | | | | |
| | 4 | | | | | |
| No. of F ₁ fams/var. | 1 | 3 | 6 | 10 | 15 | 24† |
| Plants per F ₁ included | 7 | 6 | 5 | 4 | 3 | 2 |
| † four crosses not available | | | | | | |

The eight parent plants were crossed in a diallel fashion without emasculation and F₁ plants were placed in bee-proof cages as per plan. Thus, synthetic variety No. 1 was made up of seven F₁ plants resulting from A x B cross ; variety No. 5 from 6 plants from each of the F₁'s of A x B, A x C, B x C. Bees of the Bombus spp were introduced to effect pollination. Seeds from this pollination formed the second generation from the parent plants (G₂). One hundred seeds of G₂ were germinated, and 50 random plants were placed in bee-proof cages for further multiplication. This process was continued until fifth generation (G₅) seed had been produced. Seed not used for multiplication was stored at ± 0°C until the second phase of the experiment.

Seeds of all generations (G₂-G₅) of the 14 synthetic varieties, along with

Table 1. Analysis of variance for dry matter yields of synthetic varieties *

| | °F | M.S. | V.R. |
|-----------------------------|-----|-----------|------------------------|
| Blocks | 3 | 2,582.62 | 10.64 ^{***} |
| Synthetic varieties overall | 12 | 3,352.70 | 13.81 ^{***} |
| - Between parental groups | 5 | 6644.68 | 6.64 [*] |
| . 2 & 3 v 4, 5, 6 & 8 p. | 1 | 31,007.67 | 30.97 ^{***} |
| . 2 v 3 | 1 | 1,736.11 | 1.73 |
| . 4 v 5, 6 & 8 p | 1 | 406.22 | 1 < |
| . 5 v 6 & 8 p | 1 | 14.01 | 1 < |
| . 6 v 8 p | 1 | 59.58 | 1 < |
| - Within parental groups | 7 | 1001.29 | 4.12 ^{**} |
| . within 2-parents synth. | 3 | 1 072.58 | 4.42 ^{**} |
| . " 3 " " | 1 | 404.26 | 1.66 |
| . " 4 " " | 1 | 368.16 | 1.52 |
| . " 5 " " | 1 | 937.49 | 3.86 |
| . " 6 " " | 1 | 2 081.35 | 8.57 ^{**} |
| Error (a) | 36 | 242.82 | |
| Generations | 3 | 5636.30 | 27.83 ^{***} |
| Generation × synth. vars. | 36 | 336.84 | 1.66 [*] |
| . Gen × between p.g. | 15 | 352.85 | 1.74 [*] |
| . " × within p.g. | 21 | 325.41 | 1.61 |
| Error (b) pooled remainder | 117 | 202.51 | 2.79 ^{***} |
| Years | 2 | 92042.43 | 1265.88 ^{***} |
| Years × synth. vars. | 24 | 358.81 | 4.93 ^{***} |
| Years × generations | 6 | 112.61 | 1.55 |
| Years × blocks | 6 | 617.64 | 8.49 ^{***} |
| Error (c) pooled remainder | 378 | 72.71 | |

* P = 0.05

** P = 0.01

*** P = 0.001

* Omitting synthetic variety N°5 because of low yields in G2, and two control varieties and 6 F₁ families

six F_1 families and two control lots were sown in boxes and subsequently transplanted in the field. The layout was a lattice design with 64 entries and 4 replicates but the subsequent analyses were made as for a randomized block design. Each plot was composed of two drills 3.66 m long, 0.3 m apart with 0.076 m between the plants in the row. Experiments have shown that this spacing provides as near similar results to the broadcast plot condition as is practicable.

Two cuts were obtained in the first harvest year and in the subsequent two years three cuttings were obtained annually. Samples of 400 g were taken and oven dried to provide figures for dry matter yields.

It will be noted that G1 seed as such was not used in the final tests, because there was insufficient seed. The six F_1 families (= G1) available were insufficient to provide comparable data. The results for synthetic variety No. 5, G2 were abnormally low, and yield for this was estimated by the missing plot technique (Table 2 and 3) although original data were used in the analysis of variance (Table 1).

RESULTS

The overall analysis of variance of dry matter yields indicates significant mean squares for blocks, years, entries and the interaction of years x entries and years x blocks. In order to adopt a more detailed analysis it was necessary to omit the data for the two control varieties and the six F_1 families, since they could not be represented in 'generation' groupings. Synthetic variety No. 5 was also left out of the analysis since the yields of G2 were abnormally low (Table 1).

Synthetic variety No. 14 was then used as the control variety, as it was based on eight plants and showed comparatively little change during multiplication from the second to the fifth generation (Table 2)

Table 2. Relative dry matter yields of synthetic varieties based on varying numbers of plants. (Control No. 14 = 100 = 10,346 kg/ha)

| Synthetic vars No. | No. of parent plants | Generation | | | | Mean |
|--------------------|----------------------|------------|-----|-----|-----|------|
| | | 2 | 3 | 4 | 5 | |
| 1-4 | 2 | 94 | 83 | 82 | 79 | 84 |
| 5-7 | 3 | 108† | 93 | 89 | 86 | 94 |
| 8-9 | 4 | 111 | 103 | 101 | 99 | 103 |
| 10-11 | 5 | 110 | 95 | 106 | 91 | 100 |
| 12-13 | 6 | 108 | 103 | 105 | 89 | 101 |
| 14 | 8 (control) | 98 | 101 | 102 | 100 | 100 |

† = estimated by missing plot technique since yield of No. 5 was abnormally low

The analysis indicates highly significant mean squares for years, the highest yield being obtained in the second year, followed by the third and then by the first year. The interaction of years x synthetic varieties was also highly significant indicating that the varieties based on different parental numbers did not react the same in each harvest year.

Of greater interest are the differences between synthetic varieties, which are highly significant (Table 1). This item was partitioned first into between and within parental groups and then into its orthogonal comparisons, each with a single degree of freedom. Synthetics based on two and three plants were significantly lower yielding than those based on four or more plants (Table 2). There were no significant differences between varieties based on 4, 5, 6 and 8 plants.

The within-parental group sums of squares was significant at the 1% level and was likewise partitioned. Significant differences were only observed within the 2- and 6-plant synthetics. This latter difference is difficult to explain since four out of the six plants were common to both synthetic varieties (No. 12 and No. 13). The mean of the 2-plant synthetics varied from 79-91% of control (Table 3).

Table 3. Relative yields of dry matter of synthetic varieties. (Control No. 14 = 100 = 10,346 kg/ha)

| Synthetic variety | No. of parent plants | Generations | | | | Mean |
|-------------------|----------------------|------------------|-----|-----|-----|------|
| | | 2 | 3 | 4 | 5 | |
| 1 | 2 | 91 | 77 | 73 | 74 | 79 |
| 2 | 2 | 92 | 88 | 84 | 76 | 85 |
| 3 | 2 | 85 | 80 | 85 | 79 | 82 |
| 4 | 2 | 107 | 85 | 87 | 86 | 91 |
| 5 | 3 | 117 [†] | 105 | 92 | 98 | 103 |
| 6 | 3 | 110 | 88 | 83 | 87 | 92 |
| 7 | 3 | 97 | 87 | 93 | 73 | 88 |
| 8 | 4 | 108 | 111 | 107 | 96 | 106 |
| 9 | 4 | 114 | 94 | 96 | 101 | 101 |
| 10 | 5 | 112 | 99 | 106 | 98 | 104 |
| 11 | 5 | 107 | 90 | 106 | 85 | 97 |
| 12 | 6 | 102 | 97 | 99 | 87 | 96 |
| 13 | 6 | 115 | 108 | 112 | 91 | 107 |
| 14 | 8 | 98 | 101 | 102 | 100 | 100 |
| Mean | | 99 | 94 | 95 | 87 | - |

[†] estimated by missing plot technique because of abnormally low yield.

The overall generation mean square was highly significant, indicating a change in yield from generation to generation. A more detailed enquiry indicated that all synthetics, with the exception of the control variety No. 14, dropped in vigour from the second to the fifth generation. This decrease was not uniform from generation to generation, as the relative mean yield fell from 99 to 94 from the second to third generation ; it remained steady at this level in the fourth generation and subsequently there was another sharp decrease in the fifth generation to 87.

This trend was not true for all synthetics, however, since the remainder item of the analysis which represents the interaction between parental groups x generations, was significant when tested against the experimental error (b). Clearly, therefore, not all the varieties exhibit the same overall trend in yield between successive generation, and in particular varieties No. 5, 6 and 9 increased their yields in the fifth generation as compared to the preceding generation.

DISCUSSION

Since this lucerne experiment was initiated, the results of three other studies which have several features in common have been published. Kehr *et al* (1961) compared the performance of synthetic varieties based on two (= 2-clone synthetics), and four or six plants (= multiple clone synthetics). Average yields were almost identical in the two groups and there was a decrease in yield of 6% from the first to the fourth generation. This trend was common to both groups, but the main part of the change occurred between the first and second generation. Interactions between generations x synthetics were, however, significant, one 2-clone synthetic increasing in yield from Syn-1 to Syn-4.

Recently Nielsen and Andreasen (1970) published data on the yield of two 2-clone synthetics and the corresponding 4-clone synthetic over three generations, and also line-synthetics after inbreeding. The 4-clone synthetic yielded significantly less than the double cross hybrids, while the 2-clone synthetics were the lowest yielders. Theurer and Elling (1964) studied single and second generation synthetics of all combinations of five clones, and found that on the average synthetics based on the largest number, i.e. 4 and 5 clones, produced more hay than those based on 2-clones.

The present results are in agreement with these latter studies in that synthetic varieties based on 2 and 3 plants were inferior to those founded on 4-8 plants. The 8-plant variety yielded equally well from the second to the fifth generation, while the remainder showed a drop in yield over the same period. This was particularly marked between the second and third generation. The 2-plant synthetics were yielding 74-86% of control in the fifth generation.

The parent plants used were not inbred and probably not closely related, although they were derived from the same breeding population. It would not be unreasonable to assume an inbreeding coefficient (F) of zero. Gallais (1968b)

and Busbice (1969) have indicated that for an unrelated 2-plant synthetic at the tetraploid level, F at equilibrium will be 0.125, and this should be reached asymptotically by the fifth generation. Since the multiplication of G_1 generation under panmictic conditions represents brother/sister mating, this corresponds to an inbreeding regime, and in such a situation yield will decrease with advance in generation (Gallais, 1968b).

A feature of the present results is the fluctuation that occurs from generation to generation and the differential behaviour of synthetics based on the same number of plants. In one extreme instance this can be seen even though four of the parent plants were common to the 6-plant synthetics 12 and 13. These two synthetics had similar yields in the fifth generation, but No. 13 was much higher yielding at other times. A similar situation has been observed by Kehr *et al* (1961), Nielsen and Andreasen (1970), and was explained on the basis of differential synthesis, i.e. basing the variety on a different sample of the gene pool. In addition, the seed in both studies was produced in different years which could result in seed of different quality, so that part of the generation effect could be due to year effects.

Guy (1968) has shown that 10-20 plants are sufficient to maintain the allelic complement of a hybrid. Only seven plants were used for the synthesis of the second generation in the 2-plant synthetics, but the performance of these synthetics shows less fluctuation from generation to generation than the others based on 18-45 plants.

From these results, and using the present material, it is concluded that at least four plants are necessary to form the basis of a new lucerne synthetic variety. Since such a variety may still show a decrease in yield during multiplication, the generation used for assessment tests should not be earlier than the third.

REFERENCES

- Busbice, T.H. and Wilsie, C.P. 1966. Inbreeding depression and heterosis in autotetraploids with application to Medicago sativa L. *Euphytica* 15, 52-67.
- Busbice, T.H. 1969. Inbreeding in synthetic varieties. *Crop Sci*, 9, 601-604.
- Gallais, A. 1967. Moyennes des populations tétraploïdes. *Ann. Amélior. Plantes*, 17 (3), 215-228.
- Gallais, A. 1968a. Evolution de la vigueur des variétés synthétiques tétraploïdes au cours des générations de multiplication. I. En régime de panmixie. *Ibid*, 18 (1), 5-15.
- Gallais, A. 1968b. Etude théorique et comparée de la vigueur de différentes structures variétales chez les plantes allogames autotétraploïdes. *Ibid*, 18 (2), 99-124.

- Guy, P. 1968. Nombre de plantes nécessaires à la multiplication d'un hybride autotétraploïde. *Ibid*, 18 (4), 367-379.
- Kirk, L.E. 1927. Self-fertilization in relation to forage crop improvement. *Sci. Agric.* 8, 1-35.
- Kehr, W.R., Graumann, H.O., Lowe, C.C. and Gardner, C.O. 1961. The performance of alfalfa synthetics in the first and advanced generations. *Univ. Nebraska Res. Bull. No. 200*, 1-42.
- Nielsen, H.J. M. and Andreasen, B. 1970. Performance of synthetics and related inbreds, single- and double-crosses from four clones of lucerne, Medicago sativa L. *Kgl. Vet. -og Landbohjsk. Arsskr.*, 153-169.
- Theurer, J.C. and Elling, L.J. 1964. Comparative performance of diallel crosses and related second-generation synthetics of alfalfa, Medicago sativa L. III. Forage yield. *Crop Sci.* 4, 25-28.
- Tysdal, H.M., Kieselbach, T.A. and Westover, H.L. 1942. Alfalfa Breeding. *Univ. Nebraska Agr. Exp. Sta. Res. Bull.* 124, 1-46.

PRELIMINARY RESULTS OF YIELD TRIALS WITH "STUBBLE
TURNIPS" (BRASSICA CAMPESTRIS VAR. RAPA) IN THE NETHERLANDS

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INTRODUCTION

"Stubble turnips" is an important fodder crop in the Netherlands. Grown mainly on the sandy soils it is sown after the rye or early potato crop has been harvested, just before the middle of August. As from late October plants are lifted daily for cattle feeding according to need, and depending on the onset of frost this can go on until well into January. If left on the field the crop usually gets killed off in the course of the winter and in any case is ploughed under in the following spring as a green manure.

Present day varieties yield 40-60 tons fresh weight per ha with a dry matter content of 8-11%. Until recently the area grown was 90.000-100.000 ha annually but in the course of the early 1960's dropped to the present level of 70.000-80.000 ha.

The remarkable ability of this species to grow so rapidly under short and declining daylength conditions that it can be used as a second crop makes it an interesting proposition possibly with wider applications.

Sowing in the second week of August makes the growing season short and marginal. Success of the crop therefore becomes more than usually dependent on rapid establishment and this is mainly a function of mid-August weather conditions. The fact that rye for mechanical harvesting usually stays on the field until middle August does not improve this situation. Except for a few relatively minor insect pests the main disease problem is clubroot. Resistance to the disease has been built into most Dutch cultivars and clubroot is not a problem at present but a menace to watch. Liberal nitrogen fertilizer applications are vital for good production, however accumulation of N in the leaves can reach poisonous levels for the animals. Feeding of turnips has to be phased with milking procedure for occurrence of a bad turnip taste to the milk (Scheygrond/Vos 1958).

Breeding work at the S.V.P. was initiated by Veenstra in 1948 and strongly followed up by Lamberts in 1953 who started to develop inbred lines as a base

Table 1. Information on trials reported.

| Code | | Purpose | Number of : | | | |
|-----------|---------------------|--|-------------|------------|-----------|--------------------|
| Year sown | trial | | sites | replicates | progenies | Plot size |
| 1960 | PC | polycross test of inbred lines | 1 | 2 | 100 | 7.6 m ² |
| 1961 | RSM | reciprocal recurrent selection Meetjeslander* | 1 | 3 | 90 | 9.4 |
| 1962 | K1 | single and double crosses progeny trial | 1 | 3 | 64 | 8.0 |
| 1962 | PC | polycross test | 2 | 3 | 42 | 8.0 |
| 1963 | PC | polycross test | 1 | 3 | 16 | 10.0 |
| 1963 | K | single and double crosses progeny trial | 1 | 3 | 30 | 10.0 |
| 1963 | S ₁ | synthetic varieties trial 1 | 2 | 3 | 16 | 10.0 |
| 1963 | S ₂ | " " " 2 | 1 | 3 | 30 | 10.0 |
| 1964 | S ₁ | " " " 1 | 2 | 4 | 25 | 10.0 |
| 1964 | S ₂ | " " " 2 | 2 | 4 | 49 | 10.0 |
| 1966 | DH + S ₁ | double hybrids and synthetic varieties progeny trial | 1 | 3 | 45 | 10.0 |
| 1967 | S | old synthetic varieties trial | 1 | 3 | 12 | 10.0 |
| 1967 | DH | double hybrids progeny trial | 1 | 3 | 49 | 10.0 |

* First stage : Meetjeslander tubers were allowed to flower within a field of unrelated variety « Jobe » the seed from 90 Meetjeslander plants were sown in this trial.

for the breeding programme.

Screening programmes for clubroot resistance, high dry matter content and low mustardoil levels were carried out. In 1959 Hoen took over and began to use the available inbred lines in polycross combination and for the production of single crosses and synthetic varieties. He also initiated a recurrent reciprocal selection programme. Results of a study on heritabilities and correlations of fresh weight and measurements of the size of the roots with dry matter content revealed a significant negative correlation (-0.31) between root fresh weight and dry matter content (Hoen 1968). Hoen left in 1961 and Wit followed up the programme part time giving emphasis on the development of synthetic varieties on various numbers of inbred lines and double crosses, and the study of clubroot resistance (Wit 1966). Since 1959 large statistically laid out field trials were planted every year and fully recorded. This massive body of information is now being studied in its entirety.

MATERIAL AND METHODS

In polycross isolation the inbred lines produce an adequate amount of seed for field experimentation. This and the development of a special sowing machine to plant field trials with a plot size of about 10 m² made the execution of large uniform field experiments possible. With few exceptions the lay out was a complete randomized block design with three or four replications, sometimes a trial was duplicated on two sites. On a few occasions only were the same or similar progenies tested for more than one year. The main object was to select the inbred lines producing the highest total dry matter yielding polycross progeny and to combine these inbreds into synthetics or single or double crosses for further observation. However total fresh weight, weight of roots and leaves and their respective dry matter contents were also recorded. From these records the following standard figures are being computed and analysed for each trial.

Total Fresh Yield (FY)

Total Dry Matter Yield (DY)

% Dry Matter Leaves (DML)

% Dry Matter Roots (DMR)

Root Ratio : the ratio of weight of root over total weight (RR)

The various variances, heritabilities and relations between the characters are in the process of being calculated.

Table 1 gives information on the trials the data of which have been analysed and the figures of which are reported here. Table 2 shows how variances were estimated and heritabilities calculated. The trials with 3 major effects (sites, replications and progenies for instance) always have one of these "fixed", the variance of a fixed effect is denominated k^2 .

Table 2. Formula used to calculate variance and heritability estimates, (according to Snedecor and Cochran : Statistical Methods 6 th ed. p. 368)

- Three factor experiment with A fixed.

| Progenies | df + 1 | Variance components |
|-------------|--------|--|
| A fixed | a | $MS(A) = \sigma^2 e + c. \sigma^2_{AB} + b. \sigma^2_{AC} + b. c. k^2 A$ |
| B random | b | $MS(B) = \sigma^2 e + a. \sigma^2_{BC} + a. c. \sigma^2_B$ |
| C random | c | $MS(C) = \sigma^2 e + a. \sigma^2_{BC} + a. b. \sigma^2_c$ |
| AB | ab | $MS(AB) = \sigma^2 e + c. \sigma^2_{AB}$ |
| AC | ac | $MS(AC) = \sigma^2 e + b. \sigma^2_{AC}$ |
| BC | bc | $MS(BC) = \sigma^2 e + a. \sigma^2_{BC}$ |
| error + ABC | | $= \sigma^2 e.$ |

$$k^2_A = \frac{MS(A) - \{\sigma^2 e + c. \sigma^2_{AB} + b. \sigma^2_{AC}\}}{b \times c}$$

$$\sigma^2_B = \frac{MS(B) - (\sigma^2 e + a. \sigma^2_{BC})}{a \times c}$$

$$\sigma^2_C = \frac{MS(C) - (\sigma^2 e + a. \sigma^2_{BC})}{a \times b}$$

$$h^2 = \frac{\sigma^2_B}{\sigma^2 e + \sigma^2_B}$$

- Simple two factor design.

| Progenies | df + 1 | Variance components |
|------------|--------|--------------------------------------|
| A | a | $MS(A) = \sigma^2 e + b. \sigma^2_A$ |
| B | b | $MS(B) = \sigma^2 e + a. \sigma^2_B$ |
| Error + AB | | $= \sigma^2 e$ |

RESULTS

1. Error variance and heritability

Error variance and heritability estimates are reported in tables 3 and 4. Only those from field trials with many progenies have been quoted here so that there is some basis for cautious comparison. As concerns the error variance estimates the differences between trials (years) are rather big in contrast to the differences between characters recorded. The latter are relatively small, also within trials (years) except for the 1962 PC. The heritability figures quoted in table 5 to show larger variation between years (trials) than between characters. Root Ratio and % DM roots have the highest heritabilities with % DM roots consistent and Root Ratio perhaps remarkably variable. Close third and fourth are Fresh Yield and Dry Yield heritabilities, the former higher in every trial (year) except 1960 and 1966 and both have similar ups and downs. Percentage dry matter leaves has by far the lowest heritability figure.

2. Relationship between the characters studied

Figure 1 shows the relations of Fresh Yield with Dry Matter Yield and Fresh Yield with Dry Matter content of the Roots. The first is close and positive as is to be expected and strikingly linear. The second relation is very loose and negative. These relations have been found more or less clearly defined in each trial studied and Table 5 provides a binary table of correlation coefficients computed for three of the trials between the five characters studied.

| | |
|---------------------|-----|
| Fresh Yield | FY |
| Dry matter Yield | DY |
| % Dry Matter Leaves | DML |
| % Dry Matter Roots | DMR |
| Root weight Ratio | RR |

As expected Fresh and Dry matter Yield are very closely correlated although considerably less so in the 1961 trial. Considered as sets of three, all other correlation coefficients are much lower than the former but often highly significant. Out of 12 correlation coefficients Fresh Yield has 10 significant, and this series is closely followed by % Dry Matter Roots with 9.

Well known amongst plant breeders is the observation that yield is negatively correlated with percentage dry matter, the correlation coefficients and of course figure 1 confirm this. However this quantification reveals how loose the relation is and indeed is more often than not "ns" in relation to Dry Matter Yield. In all three trials Root Ratio is negatively correlated with root dry matter content, and in the 1960 trial it is positively related with dry matter content of the leaves. The former relation probably reflects the fact also quantified by Hoen (1968) that root size is negatively correlated with dry matter content.

Table 3. Error variance estimates expressed as percentage of total variance.

| | FY | DY | % Dry Matter | | Root Ratio | mean |
|---------------------|----|----|--------------|--------|------------|------|
| | | | Roots | Leaves | | |
| 1960 PC | 69 | 77 | 47 | 33 | 69 | 59 |
| 1961 RSM | 48 | 59 | 51 | 81 | 65 | 61 |
| 1962 PC | 21 | 40 | 5 | 5 | 19 | 29 |
| 1963 S ₂ | 25 | 46 | 19 | 22 | 35 | 29 |
| 1964 S ₂ | 54 | 52 | 46 | 60 | 59 | 54 |
| 1966 DH + S | 78 | 73 | 61 | 88 | 54 | 71 |
| 1967 DH | 20 | 24 | 13 | 26 | 24 | 21 |
| mean | 45 | 53 | 35 | 45 | 46 | 46 |

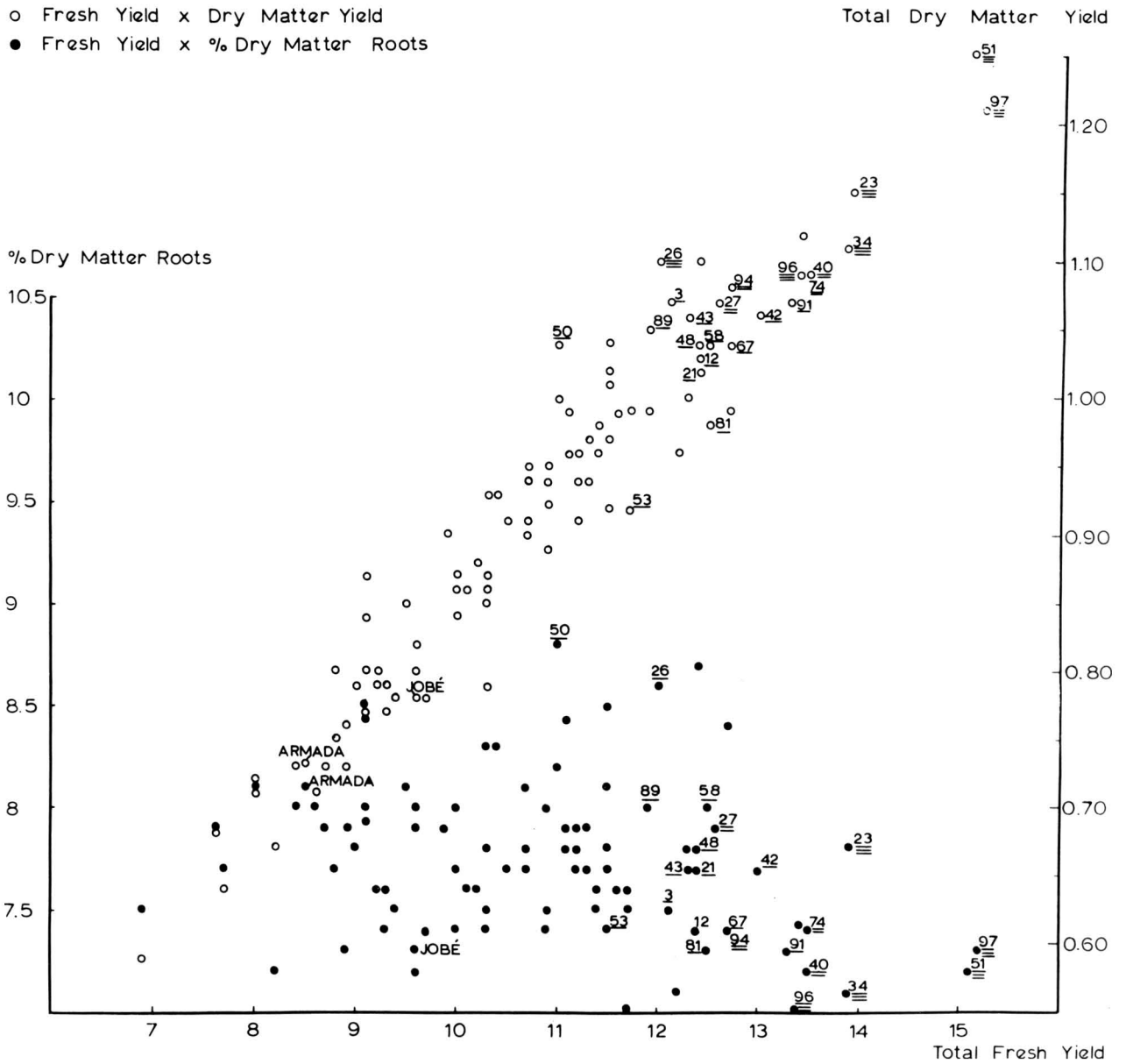
Table 4. Heritability estimates

| | FY | DY | % Dry Matter | | Root Ratio | mean |
|----------------------|----|----|--------------|--------|------------|------|
| | | | Roots | Leaves | | |
| 1960 PC | 17 | 23 | 38 | 23 | 26 | 25 |
| 1961 RSM | 46 | 37 | 48 | 19 | 35 | 37 |
| 1962 PC ₁ | 47 | 46 | 41 | 28 | 62 | 45 |
| 1963 S ₂ | 28 | 16 | 44 | 15 | 64 | 33 |
| 1964 S ₂ | 43 | 32 | 36 | 12 | 36 | 32 |
| 1966 DH + S | 22 | 27 | 38 | 13 | 46 | 29 |
| 1967 DH | 79 | 76 | 78 | 52 | 62 | 69 |
| mean | 40 | 37 | 46 | 23 | 47 | 39 |

Table 5. Correlation matrix of the five characters studied.
 ns = not significant, other figures significant at least at P 5 % level.

| | | FY | DY | DMR | DML | RR |
|-----|-------------------------------|--------------------------|----------------------------|--------------------------|--------------------------|----------------------------|
| FY | 1960 PC 1961 RSM 1962 K | | + .96 + .75 + .97 | -.27 -.24 -.53 | -.36 -.35 -.75 | -.13ns +.24 -.04ns |
| DY | 1960 PC 1961 RSM 1962 K | + .96 + .75 + .97 | | -.05ns +.02ns -.42 | -.16ns +.10ns -.64 | -.17ns +.11ns -.10ns |
| DMR | 1960 PC 1961 RSM 1962 K | -.27 -.24 -.53 | -.05ns +.02ns -.42 | | +.36 +.18ns +.42 | -.34 -.55 -.65 |
| DML | 1960 PC 1961 RSM 1962 K | -.36 -.35 -.75 | -.16ns +.10ns -.64 | +.36 +.18ns +.42 | | +.32 +.09ns +.20ns |
| RR | 1960 PC 1961 RSM 1962 K | -.13ns +.24 -.04ns | -.17ns +.11ns -.10ns | -.34 -.55 -.65 | +.32 +.09ns +.20ns | |

FIGURE I
Relations between Fresh and Dry Matter Yields and Fresh
Yield and Dry Matter content of Roots of stubble turnips (1960 PC)



3. Set up and results of Synthetic varieties experiment

Based on the results of the 1960 Polycross trial the 23 best performing polycross families were selected for blending in three combinations into synthetic varieties. The polycross families numbered in figure 1 are the ones selected. Three synthetic varieties were made, A was based on all 23 selected polycross families, B was based on the 10 indicated with two dashes underneath the figure, C was based on the 6 indicated with three dashes. Well developed good looking tubers from these polycross families were kept through the 1960/61 winter and allowed to flower in isolations in spring 1961. The varieties were coded A I'61, B I'61 and C I'61. The seed was partly sown in a field trial planted in August 1961 and the remainder stored. From each of the varieties well developed good looking tubers were kept through the 1961/62 winter and allowed to flower in isolations in spring 1962, these multiplications were coded A II'62, B II'62 and C II'62. Again part of the seed was sown in August in a field trial, together with spare seed from the I-generation. Tubers from both generations were kept through the 1962/63 winter and allowed to flower in the spring of 1963 giving a II generation but produced in 1963 (A II 63, B II 63, C II 63) and a III generation : A III 63, B III 63, C III 63. Once more seed was stored and trials to compare the various sets of generations planted in 1963, 1964, 1965 (failed), 1966 and 1967. Because the II 62 generation was not included in all years its performance has been left out in the case presented here.

Table 6 presents the fresh yield data and the analysis of variance of the three synthetic varieties in first, second and third generation from the polycrosses. Unfortunately the inbred lines could not be tested every year as polycross progeny. The commercial varieties used as standards were not the same in the various trials and in any case are likely to have changed genetically by continuing breeding work.

Comparison of the fresh yield records of the Synthetics with the cultivars as tested in various trials shows that the synthetics yield as well as the best cultivars.

The analysis of variance in table 6 shows that the year effect is particularly large. Generation effect and its interaction with year are also significant. Closer inspection of the data shows that the differences responsible for the significance of the last two effects are the (unexpected) poor yields of the I generation varieties in the years 1966 and 1967. Keeping in mind that the seed of the I-generation was harvested in 1961 these poor yields should probably be attributed to loss of growth power of the seed. The seed of the other two generations was harvested two years later in 1963. Analyses of the Dry Matter Yield show the same picture, and analyses of the other characters : Root Ratio, % Dry Matter roots and leaves only reveal highly significant effect of Years.

Table 6, Mean Fresh Yield of synthetic varieties in various field trials and analysis of variance.

1) mean Fresh Yield per plot of 10 m²

| | ' 63 1963 S ₁ | ' 64 1964 S ₁ | ' 66 1966 DH + S ₁ | ' 67 1967 S | means |
|------------|-----------------------------|-----------------------------|----------------------------------|----------------|-------|
| syn A I 61 | 36.2 | 60.2 | 44.3 | 46.1 | 46.7 |
| B I 61 | 40.5 | 60.1 | 43.6 | 42.6 | 46.7 |
| C I 61 | 39.0 | 60.7 | 43.3 | 40.6 | 45.9 |
| A II 63 | 42.1 | 60.4 | 52.6 | 55.9 | 52.8 |
| B II 63 | 39.5 | 60.5 | 47.5 | 54.0 | 50.4 |
| C II 63 | 38.4 | 58.1 | 47.8 | 52.9 | 49.3 |
| A III 63 | 40.4 | 61.2 | 46.5 | 52.4 | 50.1 |
| B III 63 | 39.9 | 59.0 | 52.5 | 52.6 | 51.0 |
| C III 63 | 37.1 | 57.9 | 51.0 | 55.2 | 50.3 |

2) Varieties × Years

| | ' 63 1963 S ₁ | ' 64 1964 S ₁ | ' 66 1966 DH + S ₁ | ' 67 1967 S | means |
|-------|-----------------------------|-----------------------------|----------------------------------|----------------|-------|
| A | 39.6 | 60.6 | 47.8 | 51.5 | 49.9 |
| B | 40.0 | 59.9 | 47.9 | 49.7 | 49.4 |
| C | 38.2 | 58.9 | 47.4 | 49.6 | 48.5 |
| means | 39.2 | 59.8 | 47.7 | 50.3 | |

3) Generations × Years

| | ' 63 1963 S ₁ | ' 64 1964 S ₁ | ' 66 1966 DH + S ₁ | ' 67 1967 S | means |
|-----|-----------------------------|-----------------------------|----------------------------------|----------------|-------|
| I | 38.6 | 60.3 | 43.7 | 43.1 | 46.4 |
| II | 40.0 | 59.7 | 49.3 | 54.3 | 50.8 |
| III | 39.1 | 59.4 | 50.0 | 53.4 | 50.5 |

4) Varieties × Generations

| | I | II | III | | |
|---|------|------|------|--|--|
| A | 46.7 | 52.8 | 50.1 | | |
| B | 46.7 | 50.4 | 51.0 | | |
| C | 45.9 | 49.3 | 50.3 | | |

5) Analysis of variance and variance estimates as percentage of total variance

| Source | df | MS | F | variance estimate | |
|--------------------|----|----------|--------|-------------------|--|
| A B C | 2 | 5.6636 | 1.13ns | 0.0 | |
| Years | 3 | 644.6530 | 123.56 | 81.0 % | |
| I, II, III | 2 | 71.1736 | 14.19 | 4.3 % | |
| A B C × Years | 6 | 0.9133 | .18ns | 0.0 | |
| I, II, III × Years | 6 | 27.4221 | 5.47 | 8.8 % | |
| A B C × I, II, III | 4 | 4.7566 | .95ns | 0.0 | |
| error | 12 | 5.0144 | | 5.9 % | |

REFERENCES

- Hoen, K. 1968. Heritabilities and genetic correlations in Turnips (Brassica campestris L. var. rapa). Euphytica 17 (3) p. 352-7.
- Scheygrond, W. and Vos, H. 1958. The effect of stubble turnips and other crucifers on the taste of milk. Meded. I.V.R.O. no. 39. Also in : Landb. Voorl. 15 (1) p. 37-44.
- Wit, F. 1966. The use of inbred lines in turnip breeding. Acta Agr. Scandinavia, Suppl. 16 p. 65-7.

PROBLEM OF PRODUCTION OF DOUBLE CROSS HYBRIDS

IN KALE (*Brassica Oleracea* L.)

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INTRODUCTION

Recent investigations showed that the production of hybrid varieties could be able to bring a great improvement in the breeding of kale, an important forage crop ; as shown by Johnston's (1963 - 1964) and our own (1969) papers, new varieties obtained by crossing may substantially outstand those grown at present by mass selection : Kale, indeed, is a cross pollinated plant for which controlled crossing is the best procedure to make the most of heterosis.

Nevertheless, the development of hybrid varieties of kale on a commercial scale raises many problems with regard to the control of pollination and the various steps in the procedure involved.

I - THE CONTROL OF POLLINATION

For that purpose, self-incompatibility is the essential characteristic of *Brassica Oleracea* having to be taken in account : this is explained by its mode of action and its inheritance.

1) Mode of action

The majority of kale plants set only an occasional seed or few seeds when mature flowers are pollinated with their own pollen because the germination of pollen on the stigma, or penetration of pollen tubes in the style are prevented. In kale crops the insects lay on the stigmas a mixture of self and allo-pollen, hence nearly a hundred per cent of cross pollination is possible even if the plant is not entirely self-incompatible.

This "self-sterility" only appears in the later stages of bud growth, one or two days at least before the bud opens, the conductor tissues of stigma and style being functional for several days ; hence simultaneous pollination of more or less numerous buds on the same inflorescence and, therefore many buds

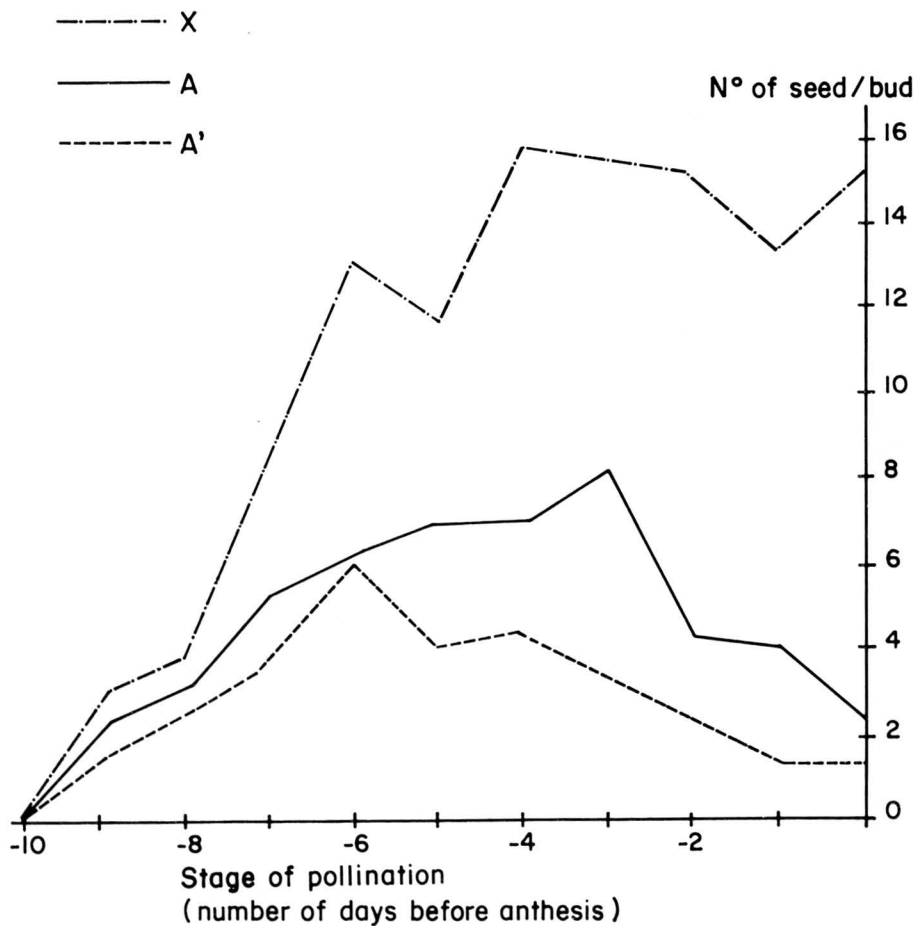
on the same plant may be successful ; bud pollination is a reliable method of obtaining selfed seed, and then inbred lines of kale.

An illustration of these facts is made in the figure 1 ; the average behaviour, in an experience made at Rennes (1964) of seven more or less self-incompatible plants is summarized by means of three curves which can be explained as follows :

- Self-pollination : number of seeds per bud (A) .
- Pollination with a mixture of self -and allo- pollen :
Total number of seeds per bud (X)
- Number of selfed seeds per bud (A')

FIGURE I

Evolution of self and cross fertility in kale (Rennes 1964)



2) The inheritance of self-incompatibility

Several authors, namely Thompson (1957-1968) gave useful informations in that respect :

Pollen and style reactions are sporophytically controlled by a series of incompatibility alleles (S alleles), the different S alleles being more or less active ; the S alleles are numerous (at best thirty) and frequency of "weak alleles" is rather low, hence the most of kale plants are highly self-incompatible but cross-incompatible.

These data explain that, under existing circumstances, the use of self-incompatibility is the most rational method for the control of pollination in a breeding programm.

Selfing -by bud pollination- of an heterozygous for S alleles mother plant leads, as nearly as the first progeny, to fifty per cent homozygous for one or other S allele plants :

$$(S_a S_b) \rightarrow 25 \% S_a S_a + 50 \% S_a S_b + 25 \% S_b S_b$$

Crossing (in natural conditions of pollination) of two inbred lines, each being homozygous for a different and active S allele, leads to a single cross hybrid.

Crossing of two single cross hybrids, including four different S alleles, leads to a double cross hybrid, a kind of hybrid variety more economically feasible for kale than single cross hybrid, since one generation more is allowed and commercial seeds are set on vigorous individual plants (single cross hybrids).

II - PROCEDURE AIMING AT DEVELOPING DOUBLE CROSS HYBRIDS

Two main points must be taken in account : the development of inbred lines, then the investigations and tests for the choice of inbred lines to be introduced into double cross hybrids.

1) The development of inbred lines

According to many experimental data it is necessary to develop a wide range of inbred lines from various kinds and origins with a view to collect an important gene stock and provide for the various farming wants.

In practice this programm is not easily feasible for the following causes :

a - The method of selfing

Bud pollination by hand requires much work and its success fairly depends on the environmental conditions and the individual plants themselves.

b - The biennial character of kale

Consequently in natural conditions of growth, two years are required to get one generation ; besides damage during winter may be very important.

c - The inbreeding depression on vigour and fertility

It is generally very pronounced but at various degrees according to strains, a complete sterility occasionally occurring in fairly vigorous inbred lines.

The percentage of material lost may be high even for kale strains more hardy than marrowstem (table 1).

Table 1 . Inbreeding effect on vigor and fertility
(Variety Demi-Moëllier - Rennes 1960-1962)

| | Year | Percentage of living plants at flowering | | Fertility | | | |
|---------------------------------|------|--|----------|----------------|----------|---------------|----------|
| | | Average | Extremes | N° flow/inflor | | N° seeds/flow | |
| | | | | Average | Extremes | Average | Extremes |
| Free pollination | 1960 | 70,0 | | | | | |
| Mother plants (I ₀) | 1960 | | | 33,7 | 11 - 72 | 15,4 | 11 - 24 |
| I ₁ | 1960 | 46,7 | 0 - 90 | 26,1 | 15 - 49 | 10,3 | 5 - 20 |
| I ₂ | 1960 | 41,8 | 0 - 85 | 20,2 | 10 - 49 | 7,2 | 0 - 18 |
| I ₃ | 1962 | 13,8 | 0 - 30 | 18,6 | 14 - 39 | 5,0 | 0 - 20 |

Unfortunately this natural selection is not closely related with the breeding value of inbred lines, namely when caused by sterility or even frost damage if frost hardiness is not a breeding purpose (for ex. Marrow stem kale).

Hence developing inbred lines in a glass house seems a reliable and feasible procedure : the mother plants chosen in nursery are carried into moderately on or after 15th november in our country. Two methods are available : Heel cutting a practice more suitable to carry inbreeding on many lines, and

transplanting the whole plants a practice which seems more suitable for the maintenance and propagation of parent lines of hybrids.

This procedure is of a great interest at three points :

- the wintering problem is cleared up.
- flowering and seed maturation occur early enough to ensure one generation per year.
- the yield of bud pollination is fairly higher than in field (table 2).

Table 2 . Selfing by bud pollination in glass-house and in fields
(Rennes - 1967)

| Inbred lines tested | Glass-house | | | Field | | |
|---------------------|-------------|-------|----------|-------|-------|----------|
| | buds | seeds | seed/bud | buds | seeds | seed/bud |
| A - 1 ₄ | 97 | 649 | 6,69 | 86 | 25 | 0,28 |
| B - 1 ₅ | 220 | 1929 | 8,77 | 336 | 692 | 2,06 |
| C - 1 ₆ | 112 | 813 | 7,26 | 215 | 322 | 1,47 |

2) Investigations and tests

The criteria useful for the choice of inbred lines to be introduced into double cross hybrids can be divided into two groups : those regarding the inbred lines themselves and those regarding their behaviour and performances when crossing.

a - Characters necessary for inbred lines

These are mainly the following :

- sib-incompatibility

The inbred lines or, in practice, at least three of the four inbred in a double cross hybrid must be homozygous for active and strong different S alleles : hence early testing of self-incompatibility and further testing of sib-incompatibility are necessary.

- vigour and fertility (for each flower and for the whole plant) sufficient to make bud pollination and production of single cross economically feasible.

Practically the breeder is led to remove early many inbred lines and often, for the others, to use, after four or five generations of selfing, sib-crossing or vegetative propagation for the maintenance of parent lines of hybrids.

b - Predicting behaviour and performances of inbred lines into double cross hybrids

The aim for the breeder, is to develop double cross hybrids with well definite characteristics and of an outstanding value.

Two conditions are a priori laid down upon inbred lines so that crossing may be successful at each step (single and double cross) :

- cross compatibility
- coincidence flowering time, an important factor to obtain the maximum percentage of crossing and yield of seeds.

With respect to other factors involved, those making the agronomical value of varieties, predicting of characteristics and performance of double cross hybrids can be more or less soon and easily effective according to their mode of inheritance ; they can be divided into two main groups in that respect :

- those under major gene control, among which many deleterious characters : their mode of inheritance is simple and easily predictable during the process of inbreeding.
- those of a continuously variable nature under the control of a large number of gene of minor effect among which are many important agronomical traits, namely height, hardiness, quality and, above all, yield. Their assessment can only be carried out on hybrids. Nevertheless it is useful to know that, as shown by Johnston (1964) a preliminary selection can be made for some of them which can be correlated between inbred and hybrids : disease resistance, hardiness, and two yield components, leaf number for which inheritance a simple additive genetic system is present, and stem length for which inheritance a low heterotypic effect is observed.

Consequently it is useful for the breeder, to start his work with a very large number of mother plants, then to develop many l_1 and l_2 progenies and also to carry out a careful programm of investigations description and preliminary selection during the process of inbreeding.

Nevertheless testing of fairly numerous inbred lines by means of crossing is necessary, and yet, the programm of tests should be economically feasible. The programm that is now proposed takes rests on various data, mainly those obtained by our investigations from 1964 to 1968 , which can be summarized as follows :

- a) - top-cross tests were carried on many inbred lines from various origins and at various stages of inbreeding, crossed by different commercial strains ; these tests enabled inbred lines to be early divided into different classes as regards to their general combining ability and also, their fitness of a

given kind of cultivation and utilization.

b) - diallel and half diallel test crosses proved the efficiency of top cross test, and then, according to Johnston (1964) that the general combining ability is always very important in kale as in maize ; nevertheless several data showed that specific combining ability should be taken into account also.

c) - some experimental field trials including double cross and alternative single cross hybrids showed that, according to Jenkin's investigations with maize the behaviour of alternative single cross hybrids was very useful in giving quite accurate estimates of total yield and -also its components- of the double cross hybrid (Table 3).

From these data, we can conclude that, in a first step of the breeding programm two kinds of tests will be successively carried on inbred lines :

a) - Early (as early as I_2) testing of their general combining ability by means of top-cross test with an adequate and genetically diversified strain as a tester.

Crossing can be made either by hand or in natural conditions of free pollination, inbred lines growing inside a pure culture of the tester, a procedure more useful for testing many lines and producing an amount of seeds suitable for several field trials.

b) - Testing the specific combining ability of the most promising inbred lines able to be introduced into a double cross hybrid variety. This will be done by means of diallel crosses or as often as not in practice, half-diallel crosses between two series of inbred lines, with the aim of providing an adequate uniformity to the commercial variety.

In further steps of the breeding programm it will be possible to more and more reduce the complexity of combining ability tests by means of three way test using a single cross hybrid as a common testor for the inbred lines among which the two partners are to be chosen.

DISCUSSION

The development and production of double-cross hybrid varieties of kale are easily conceivable, but much less easy to carry out on a large scale. The first cause of that lies on the pollination control system itself that is to say self-incompatibility. First it imposes the breeder restrictive conditions and successive tests relating to inbred lines. On the other hand developing inbred lines by bud pollination requires much manual labour. Besides self-incompatibility is not an infailing system for controlling pollination. Nevertheless since preventing its action seems to be impossible the use of any other system as male sterility is not allowed in practice.

Other restrictive conditions about selection of inbred lines and their maintenance are imposed, namely by the biennial habit of kale growth and

Table 3. Predicting double cross performance

(Trials carrying on six double cross hybrids between inbred lines of Chou demi-Moëllier)
Rennes 1965-1967 (1)

a) - Differences between double cross and the average of alternative crosses (2)

| | Fresh weight | | | dry weight | | | stem | | Leaves Number |
|---------|--------------|------|--------|------------|------|--------|--------|------|------------------|
| | Plant | stem | Leaves | Plant | stem | Leaves | length | ∅ 2 | |
| min. | 0,6 | 2,3 | 0,1 | 2,1 | 1,3 | 1,3 | 0,4 | 0,2 | 0,3 |
| max. | 10,1 | 15,8 | 8,9 | 11,0 | 18,3 | 7,7 | 13,9 | 12,6 | 12,2 |
| average | 5,0 | 7,7 | 4,2 | 5,9 | 8,2 | 4,4 | 3,8 | 5,7 | 5,2 |

b) - Differences between the four alternative single crosses (2)

| | | | | | | | | | |
|-----------------|------|------|------|------|------|------|------|------|------|
| min. | 11,6 | 11,7 | 12,4 | 4,0 | 7,9 | 3,2 | 19,1 | 14,2 | 10,9 |
| max. | 38,9 | 38,7 | 39,1 | 40,6 | 43,8 | 38,3 | 31,7 | 29,0 | 32,4 |
| average | 21,0 | 27,3 | 21,3 | 20,6 | 31,0 | 14,7 | 25,7 | 24,1 | 18,6 |
| L.s.d (P =0,05) | 11,8 | 11,7 | 13,3 | 7,9 | 14,6 | 19,0 | - | - | - |

(1) all data are given in relative values (% Proteor variety as control) ;

(2) the alternative single crosses are the four single crosses not used in the production of double cross ; thus in the double-cross hybrid (A×B) (C×D) the four alternative single crosses are A×C A×D, B×C, B×D.

inbreeding depression on vigour and fertility, with a view to ensure a sufficient yielding of bud pollinations and the success of crops for producing single-cross hybrid seed, which often will be made in very propitious environmental conditions.

With respect to combining ability tests those are not as easy to apply than for several species as Maïze. Crossings by hand require much manual labour ; when pollinations are made by means of insects, hundred per cent of crossing is not warranted owing to possible sib-compatibility of inbred lines and besides for top-cross, cross pollination between the nearest inbred lines which rarely occurs in practice, the tester being much higher and vigorous than inbred lines. Besides two factors are a priori propitious to the success and efficiency of combining ability tests : the mode of inheritance of S alleles, and the few number of individual plant necessary to set an adequate amount of seed for field trials.

That leads us to emphasize the principal factor making the production at a large scale of double cross hybrids to be economically feasible : the high rate of multiplication, that is to say the high level of seed setting of each individual plant and possibly also, of each individual bud or flower. With rather propitious conditions indeed, self-pollination of 4000 buds (1000 buds on each of the four inbred lines), which requires 15 to 20 hours of manual labour, allows to obtain at least 20 000 seeds ; this amount is sufficient for growing 50 ares of crops being to produce the two single cross hybrid seed ; even with a low yielding of these two crops, a total amount of 50 Kg of seed can be expected. With that stock, it is possible to grow according to the mode of cultivation from 40 to more than 100 ha for the production of double cross hybrid seed and then to expect from 300 to 800 qx of commercial seeds.

In conclusion, the production at a large scale of double cross hybrids of kale may not allow, for the breeders, to make the most of potential heterosis in that species. Nevertheless it offers many advantages justifying the means used to make it successful. The procedure involved indeed, allows to develop new kinds of kale varieties and to get improvement not only in yielding as a whole, but also in other characters, such as uniformity and quality of which the agronomical interest is of importance.

REFERENCES

- Crehu (du) G. (1964). - La Biologie Florale du Chou-fourrager, ses conséquences pour la sélection. Ann. Amél. Plantes. 14 (1), 5-37 - (2) 157-201.
- Crehu (du) G. (1969). - Biologie Florale et exploitation de la vigueur hybride chez le Chou-fourrager. C.R. Acad. Agric. 10, 736-41.
- Johnston T.D. (1963). - Inbreeding and hybrid production in marrow-stem kale Brassica oleracea L. var. acephala D.C.

- 1 - The effects of inbreeding and performance of F_1 hybrids.
Euphytica 12 - 198-204.
- Johnston T.D. (1964). - Inbreeding and hybrid production in marrow-stem kale Brassica oleracea L. var. acephala D.C.
2 - The prediction of double cross performance.
Euphytica 13 - 147-152.
- Thompson K.F. (1957). - Self-incompatibility in marrow-stem kale.1 demonstration of a sporophytic system. J. Genet. 55, 45-60.
- Thompson K.F. (1968). - Classified S alleles for Brassica breeders, Brassica Meeting of Eucarpia Hort. Sect - Proceeding 24-28.

FIRST GENERATION SYNTHETICS FROM GENOTYPES
AT VARIOUS LEVEL OF INBREEDING IN LUCERNE *

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The vigour of lucerne plants is strongly influenced by inbreeding ; the depression of vigour, on the basis of yield, reaches 30% in S_1 (Tysdal et al., 1942 ; Wilsie, 1958 ; Panella and Lorenzetti, 1966 ; and increases in the next selfed generation (S_2). Tysdal et al. (1942) and Panella and Lorenzetti (1966) have observed a partial restoration of vigour in S_3 , they explain with a selection for autofertility and vigour in the previous generation (S_2).

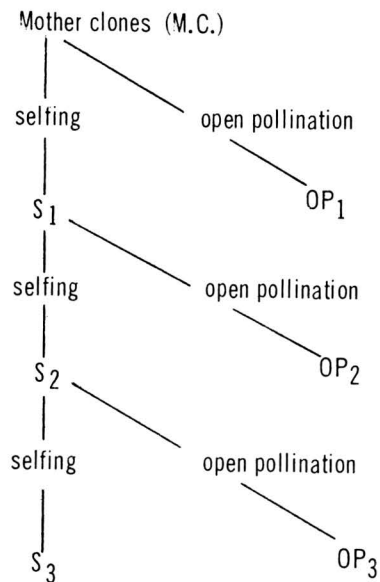
Data on advanced selfed generations give very interesting information on the theoretical aspects of the inbreeding effects and can contribute to evaluate the mother plants ; as a matter of fact the best S_1 's tend to give the best S_2 's and S_3 's (Lorenzetti, 1964).

A problem which until now does not seem to have called a sufficient attention is that of the vigour of the progenies from S materials fertilized by pollen from plants at various levels of inbreeding. In the diploid plants, e.g. maize, random crossing among lines, or crossing among lines and parental population, permit to attain the vigour of the parental population (Allard, 1960). For lucerne, experimental data on this point are scarce in literature, but theoretical considerations indicate that this species must behave in a different way, due to its autotetraploid origin. It is already known that a generation of open pollination of the S plants brings about a restoration of vigour which is not complete (Demarly, 1963 ; Panella and Lorenzetti, 1966). In the present work the vigour of seven first generation synthetics from genotypes at various level of inbreeding will be considered. The work is the first step of a long term program carried out to ascertain the value of the selection among and within inbred families when the aim of the breeding work is to build up synthetic varieties.

*Work supported by funds from the Italian Research Council.

MATERIALS AND METHODS

An experimental field was established in 1962 with 49 families of lucerne and a total of about 7.000 plants ; spacing was 90 cm between rows and 60 cm between plants on the row. In each family there were the mother clones (M.C.) and plants of three selfed generations (S_1 , S_2 , S_3) along with the corresponding OP series (OP_1 , OP_2 , OP_3), obtained from the same mother plants according to the following scheme²:



In 1964 among the 49 families were selected the 20 families with the highest number of plants and in each one of their seven generations were chosen 10 plants : i.e. the best ones, on the basis of yield, in S_1 and OP_1 and the best ones of the best strains in the second and third generations (S_2 and OP_2 , S_3 and OP_3) ; the 20 selected mother clones of each family were also represented at least by 10 plants obtained by vegetative propagation.

In the spring of 1965 we had at our disposal seven groups of plants (one for each generation), with 200 individuals (10 plants for each one of the 20 families). The seven groups of plants were used to establish seven polycross nurseries in order to obtain seven different types of polycrossed seed (poly M.C., poly S_1 , poly OP_1 , etc.). In each nursery there were ten rows ; in each row there were 20 completely randomized plants, one for each family. In 1966 the seven nurseries were isolated and in each one was placed a small nucleus hive of 5 combs of honey-bees. The aim was to obtain 140 lots of seed (7 generations x 20 families). Plants of some families died in the transplanting year, while in other families seed production was very poor ; therefore we were

forced to reduce our material, and at the end of 1966 we had at our disposal 70 lots of seed derived from 10 families ; each lot was prepared with an equal amount of seed from each plant of the family entered in the polycross nursery. The seventy lots were sown in greenhouse on 26 of September 1967 and seedlings were transplanted in the field on 4 of May 1968, following a split-plot design with four replicates. Families were put in the main plots and generations in the sub-plots made up of a single row of 10 plants. Spacing was 50 cm between rows and 30 cm between plants on the row. Plants were cut four times per year in the period 1968/70 ; at each cutting the height of the plants and their green matter yield were scored.

The value of the experimental synthetics will be given by the overall means relative to the ten polycrossed progenies from the same nursery.

The analysis of variance of the data for the 12 cuts made in 1968/70 has shown that the interaction item "generation x family" is borderline of significance for height and not significant for green matter yield, so family data will not be presented.

RESULTS

Height and green matter yield of the plants entered in 1965 in the 7 polycross nurseries are presented in table 1 ; the data were gathered in 1964 when the plants were in their third year of life.

Table 1. Height (cm) and green matter yield (g/plants) measured in 1964 on the plants entered in 1965 in the 7 polycross nurseries (4 cuts averages).

| Generation | Height (cm) | Green matter yield (g) |
|-----------------------------|-------------|------------------------|
| Mother clones (M.C.) | 56.8 | 342.1 |
| S ₁ | 49.6 | 228.3 |
| S ₂ | 49.6 | 221.7 |
| S ₃ | 53.4 | 272.2 |
| S means | 50.9 | 240.7 |
| OP ₁ | 59.3 | 363.5 |
| OP ₂ | 59.6 | 336.8 |
| OP ₃ | 60.2 | 338.6 |
| OP means | 59.7 | 346.6 |

The data of the maternal material (table 1) will permit a useful comparison with their polycross progenies (table 3).

It is worth to note (table 1) that the mean height of S plants (50.9 cm) is 14.7% below the height of OP plants (59.7 cm). The selected S_3 (53.4 cm) and OP_3 (60.2 cm) plants seem to be the best among the S and OP groups, respectively. The mother clones appear weaker than OP_1 plants, which should have the same level of heterozygosity ; this can be due to the vegetative origin of the former and/or to the effect of pollen contribution in the OP_1 .

The data of the green matter yield follow the same pattern of those relative to heights ; here differences between groups, selfed and open pollinated, are much more evident than for height. In fact the green matter yield of the S plants (240.7 g) is 30.6% below that of the OP plants (346.6 g). S_3 and OP_1 plants appear to be the best among the S and OP groups respectively. The mother clones (342.1 g) are again lower than OP_1 plants (363.5 g).

In table 2 the relative values ($OP_1=100$) of the material selected in 1964 in order to enter the seven polycross nurseries in 1965 are referred along with the relative values ($OP_1=100$) of the materials among which they were selected ; the latter value have been already reported by Panella and Lorenzetti (1966). It is evident from table 2 that the relative value of the chosen material was not different from that of all the plants of the same unselected generation, so the selective pressure should have been almost constant in all the generations.

Heights and green matter yields of the polycross progenies are reported in table 3. Due to different spacing and years the absolute values observed in these filial generations are lower than those observed for the mother plants (table 1).

The data of table 3 indicate that mean height of the progenies from S plants (43.7 cm) is lower than height of the progenies from mother clones (44.9 cm) and OP plants (45.3 cm). Differences like these can be observed for all the sister generations (S_1 vs OP_1 , S_2 vs OP_2 , S_3 vs OP_3), although the only significant difference is that for OP_2^2 - S_2 comparison.

The value of the progenies from S plants is 3.5% below that of the progenies from OP plants. Progenies from mother clones (44.9 cm) do not differ significantly from the progenies from OP_1 plants (44.1 cm).

The green matter yield follows the same pattern of the data relative to the height. In fact progenies from OP plants (114.4 g) are better than progenies from S plants (103.5 g), the latter value being 9.5% lower than the former. In all the sister generations progenies from OP plants are better than progenies from S plants, the differences being significant for the S_2 - OP_2 comparison. Progenies from mother clones (109.1 g) do not differ, as expected, from the progenies from OP_1 plants (107.4 g), but differ from the progenies given by S_1 plants (95.9 g). There is a tendency of the progenies to improve in the successive generations of selfing and selection, in fact, poly- S_3

Table 2. Relative values ($OP_1 = 100$) of heights and green matter yields for unselected and selected plants (1964).

| Generations | Height | | Green matter yield | |
|-----------------|--------------|-------------|--------------------|-------------|
| | Unselected * | Selected ** | Unselected * | Selected ** |
| S ₁ | 83 | 84 | 62 | 63 |
| S ₂ | 77 | 83 | 55 | 61 |
| S ₃ | 85 | 90 | 68 | 75 |
| OP ₁ | 100 | 100 | 100 | 100 |
| OP ₂ | 93 | 100 | 91 | 92 |
| OP ₃ | 101 | 101 | 98 | 93 |

* Data from all the 7000 plants of the original field
 ** Data from the plants entered in 1965 in the polycross nurseries

Table 3. Height (cm) and green matter yield (g/plants) of the polycrossed progenies from 10 lucerne clones and their selected S and OP progenies (12 cuts averages, 1968-1970).

| Parental generation | Height (cm) | Green matter yield (g) |
|-----------------------|-------------|------------------------|
| Mother clones (M.C.) | 44.9 | 109.1 |
| S ₁ | 43.7 | 95.9 |
| S ₂ | 42.7 | 101.6 |
| S ₃ | 44.9 | 113.1 |
| OP ₁ | 44.1 | 107.4 |
| OP ₂ | 45.8 | 117.0 |
| OP ₃ | 45.9 | 118.9 |
| L.S.D. ($P_{0.05}$) | 1.9 | 12.8 |
| S means | 43.7 | 103.5 |
| OP means | 45.3 | 114.4 |
| L.S.D. ($P_{0.05}$) | 0.7 | 4.9 |

(113.1 g) and poly-OP₃ (118.9 g) progenies are the best among the S and OP series, respectively.

DISCUSSION OF THE RESULTS

In lucerne, polycross of unselected S plants does not seem able to restore the original level of vigour but, until now, the value of progenies from genotypes selected during inbreeding has not been well explored. In the present experiment polycrosses of selected S plants have permitted a considerable restoration of vigour which, in the case of progenies from S₂ and S₃ plants, has been complete.

Height and green matter yield of the S plants entered in 1965 in the polycross nurseries were, as a whole, 85.3% and 69.4% those of the OP plants, respectively, while height and green matter yield of the poly-S progenies grown in 1968/70 have been 96.5% and 90.5% those of the poly-OP progenies, respectively. It seems therefore that a single generation of crossing has been able to improve the relative value of S progenies which, in any case, do not reach the values of OP progenies.

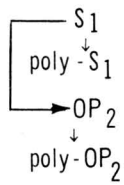
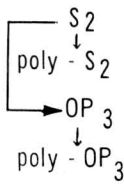
The data we are considering could have been influenced by the amount of selfed and sibbed seed produced in the polycross nurseries. On the percentage of this seed we have not data, but it seems that selfed seed should have been more frequent in the case of OP-polycrosses than in the case of S-polycrosses. In fact, it is well known that there is a drop of self-fertility from the first to the second and third selfed generation. Sibbing, on the other hand, could have had the same frequency in OP- and S-polycrosses because the number of plants of each family was always the same, but the complex, and in many respects still unknown, system of incompatibility working in lucerne should have lowered the frequency of sibbed seed in the S-polycrosses below that present in OP-polycrosses. The lower performance of polycrossed progenies from S plants seems, therefore, strictly dependent on the genetic nature of the mother plants.

The value of the progenies obtained from genotypes selected at various stages of the inbreeding process can give valuable informations for practical work. Poly-S₃ yielded 17.93% more than poly-S₁ progenies ; while poly-OP₃ yielded 10.70% more than poly-OP₁ progenies ; the corresponding increase of height from the first to the third generation was 2.74% and 4.08%. Therefore, on the basis of production data, it can be concluded that the selection work seems more efficient when carried out in advanced generations.

It is now interesting to compare poly-S₁ with poly-OP₂ and poly-S₂ with poly-OP₃ progenies. The data are reported in table 4 along with those of the parental generations (S₁, OP₂ and S₂, OP₃ respectively). In order to make possible all the comparisons the data have been expressed as values relative to OP₁ (1964) or poly-OP₁ (1968/70) ; for our purposes OP₁ and poly-OP₁ plants can be, in fact, considered equivalent.

The data refer to the green matter yield because it is well known that this character is related to vigour better than any other. The open pollination progenies, OP₂ and OP₃, gave in 1964 a green matter yield (91 and 98) slightly higher than that given in 1968-70 by the corresponding poly-S₁ and poly-S₂ progenies (89 and 95 respectively). Poly-S₁ and poly-S₂ progenies have been obtained exclusively from gametes of S plants, while in the OP₂ and OP₃ progenies half of the gametes have been contributed at random by the entire population. The observed behaviour of progenies from S and OP plants seems to indicate that the characters considered in the present work are governed by genes which, due to the autotetraploid origin of lucerne, do not act in a disomic way but, probably, in a tetrasomic one as it should be. In this case crosses among S plants involve homozygous gametes more frequently than crosses among OP plants ; as a consequence, the complex heterozygosity of trigenic and tetragenic type, that Busbice and Wilsie (1966) consider strictly related to vigour, is not frequent. Gametes from S plants are surely more homozygous than random gametes from the entire population and this can explain why OP progenies are better than the corresponding poly-S progenies. OP₂ and OP₃ do not attain the vigour of OP₁ because half of the gametes comes from S₁ and S₂ plants, respectively.

Table 4. - Relative value (OP₁ = poly OP₁ = 100) of different populations of lucerne grown at Perugia (1964-1970)

| Years | Populations * | Relative values (OP ₁ = 100) | Populations * | Relative values (OP ₁ = 100) |
|---------|---|---|--|---|
| 1964 |  | 62 |  | 55 |
| 1968-70 | | 89 | | 95 |
| 1964 | | 91 | | 98 |
| 1968-70 | | 109 | | 111 |

* OP₂ and OP₃ plants were obtained by open pollination of S₁ and S₂ plants ; see scheme at page 2.

The relative values (poly-OP₁=100) of poly-OP₂ and poly-OP₃ plants obtained from S₁ and S₂ individuals after one generation of open pollination (S₁ → OP₂ ; S₂ → OP₃) followed by one generation of polycrossing are 109 and 111 respectively.

On the basis of the previous data, it can be concluded that, at least as a tendency, OP₂ and OP₃ plants are better than the corresponding poly-S plants and that a successive generation of selection and intercrossing among such OP plants, improve the value of the progenies which attain and overcome the level of the OP₁ plants.

According to Demarly (1963), in an autotetraploid plant such as lucerne, crossing among S plants does not permit a complete restoration of vigour. Our data show that synthesis of S₁ and S₂ plants gives progenies below the level of the parental population ; only synthesis of S₃ plants (table 3) seems to permit a complete restoration of vigour, indicating that selection during inbreeding shows some positive effect.

The present data are not sufficient to demonstrate if advanced generations of synthetics obtained from S materials permit or not to overcome the parental population. Gallais (1968) has shown that the vigour of synthetics in tetraploid plants should tend to an equilibrium value in an asymptotic manner in succeeding generations of random mating. If the parents are not inbred or slightly inbred the vigour should decrease ; if the parents are sufficiently inbred the vigour should increase.

Our materials will give, in the next future, informations on the advanced generations of the synthetics here discussed, which come from inbred and not inbred genotypes ; so it should be possible to ascertain if synthetics from selected S plants can give a valuable contribution to the breeding of lucerne.

REFERENCES

- Allard, R.W., 1960. Principles of Plant Breeding. John Wiley and Sons, Inc. New York, London
- Busbice, T.H. and Wilsie, C.P., 1966. Inbreeding depression and heterosis in autotetraploids with application to Medicago sativa L. Euphytica, 15 : 52-67
- Demarly, Y., 1963. Génétique des tétraploïdes et amélioration des plantes. Ann. Amélior. Plantes, 13 (4) : 307-400
- Gallais, A., 1968. Evolution de la vigueur des variétés synthétique tétraploïdes au cours des générations de multiplication. Ann. Amélior. Plantes, 18 (1) : 5-15
- Lorenzetti, F., 1964. Contributo alla valutazione dell'attitudine alla combinazione nell'erba medica. Sementi Elette, X (6) : 452-458

- Lorenzetti, F. and Monotti, M., 1964. Campi di polincrocio e variabilità genetica per caratteri quantitativi nell'erba medica. Ann. Fac. Agraria dell'Univ. di Perugia, XIX : 201-214
- Panella, A. and Lorenzetti, F., 1966. Selfing and selection in alfalfa breeding programmes. Euphytica, 15 : 248-257
- Tysdal, H.M., Kiesselbach, T.A. and Westover, H.L., 1942. Alfalfa breeding. Research Bulletin 124, Nebr. Agr. Exp. Stat.
- Wilsie, C.P., 1958. Effect of inbreeding on fertility and vigour of alfalfa. Agronomy Journal, 50 : 182-185

USE OF HETEROSIS IN BREEDING AUTOTETRAPLOIDS

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INTRODUCTION

One of the main objectives of the different methods of plant breeding applied to allogamous and sometimes even to autogamous plants is to cumulate the vigour linked to heterozygosity with that linked to the quality of the genes and their interactions.

Regarding practical use of heterosis, it is possible to distinguish two types of varieties :

- 1) The hybrids resulting from "controlled hybridization" of lines or families genetically different. This type of varieties allows for maximum use of the variability in heterosis.
- 2) Synthetic varieties from species where "controlled hybridization" is not possible on a large scale. The genetic base of such varieties, usually wider, don't allow maximum use of heterosis.

In this paper we will show the effects of different systems of mating in autotetraploids (selfing, crossing, random mating) on the means and the variances of quantitative characters influenced by heterosis. Consequently it will be possible to precise the probability of success of a system of selection. Experimental results are included.

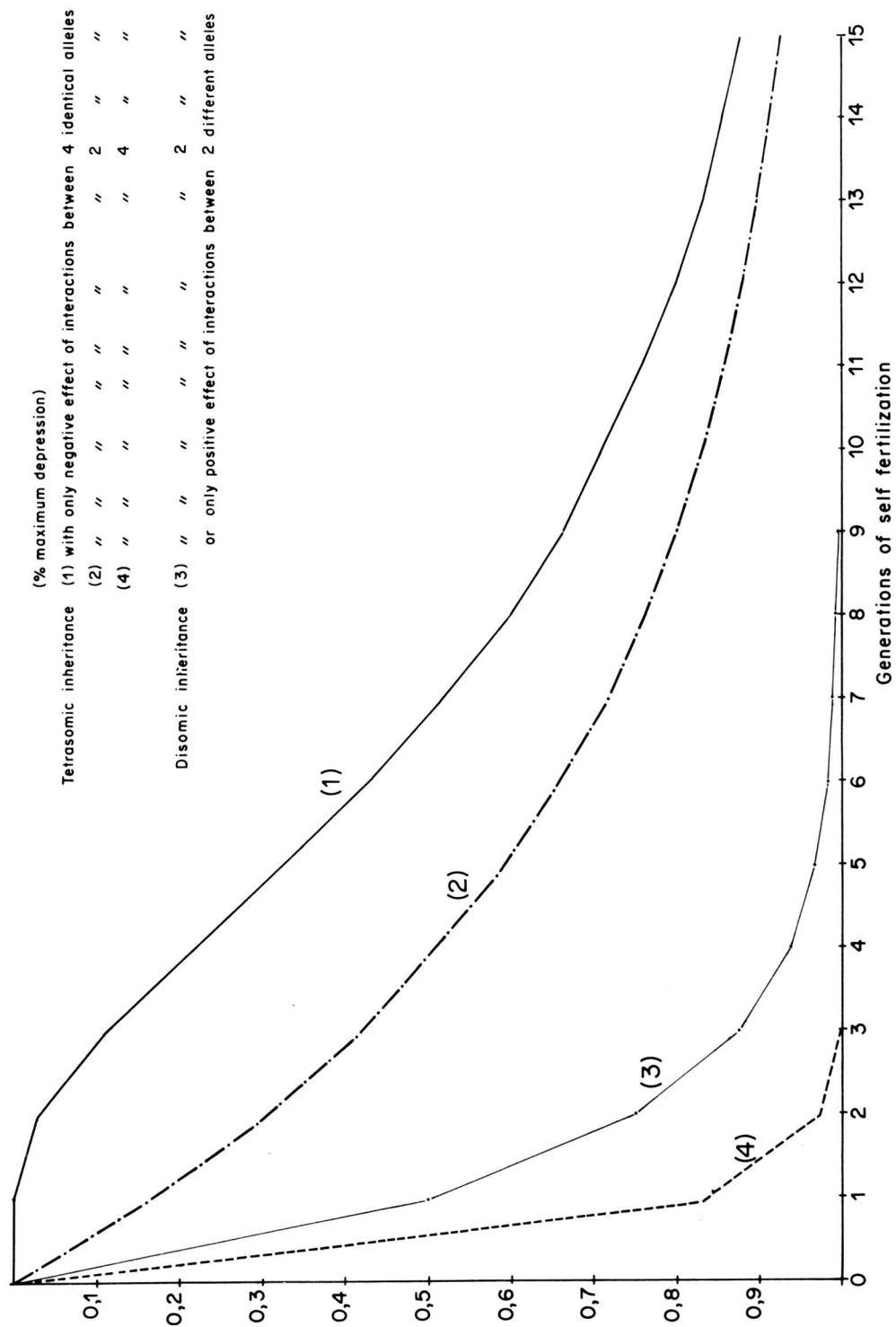
SELF-FERTILIZATION

1) Effect on the mean

Demarly (5) has shown that progress towards homozygosity is very slow with autotetraploids (around three times slower than with diploids). If vigour were only related to the proportion of homozygous loci or to the coefficient of inbreeding F , the loss of vigour should be slow.

FIGURE I

Inbreeding depression according to some extreme genetic hypotheses, without epistasis



Many authors, Berninger (1), Busbice and Wilsie (2), have shown with different autotetraploids species (Allium porum, Medicago sativa) a loss in vigour, more rapid than that expected from the coefficient of inbreeding. The results table 1, obtained by Gallais on Dactylis glomerata from two different experiments, show :

- a behaviour specific to each character (yield, which is a complex character, is most affected),
- a large variability in the reaction to inbreeding arising from different origin.

The coefficient of inbreeding is not sufficient to completely explain the results and other hypotheses giving greater weight to the heterozygous structures (tetragenic) are more satisfactory (1, 2, 9, 13, 16). When the tetragenic genotypes are very favourable, as it is readily destroyed, the loss in vigour will be important (Graph 1).

The variability in the loss of vigour is more difficult to explain. It may come from a variability in the degree of homozygosity of the different origins, but also from a simple variability in the gene effects.

2) Effect on the variance

Gallais (12) has shown that the part of variation due to the additive effects as well as that due to the interactions between two identical genes increases with the generations of selfing. The inter-family variance will thus increase, if the additivity and the interactions between two identical genes are the main sources of variation.

With regards to the variation due to other effects, it is noted that the part of the variance due to the interactions between two different alleles will be at a maximum at the second generation of selfing. These interactions may thus be selected by selfing, if they are an important source of variation.

The part of the interactions between two or more different alleles, can only diminish if they are preponderant, the total variability could even decrease. To our knowledge, this has never been observed. Indirectly, this is evidence of the importance of additive and interaction effects between identical genes as a source of variability. The experimental results by Gallais, on Dactylis glomerata and Medicago sativa, should show more precisely the evolution of the variability in selfing with regards to the importance of the various genetic effects.

3) Practical consequences

Results in table 1 show various characters as to their sensitivity and variability of responses to inbreeding.

Selection towards a tolerance to inbreeding is thus possible. But the conse-

Table 1.

a) - Effect of inbreeding on different characters with coksfoot. (Vegetative growth two years after seeding)
(7 of S₀ origins, 21 of S₁)

| Characters | Mean depression $\frac{S_1 - S_0}{S_0} \times 100$ | Variability according to origins |
|-------------------------|---|----------------------------------|
| Yield | - 24,1 | - 5,1 à - 68,2 |
| Number of tillers | - 22 | + 16 à - 43,2 |
| Tillers/Cm ² | - 0,6 | + 24 à - 22,1 |
| Weight/tillers | - 1 | + 59 à - 22,5 |

b) - Effect of inbreeding on different characters with coksfoot in the seeding year. (4 of S₀ origins, 20 of S₁, 39 of S₂).
Results from GALLAIS- BERTHOLLEAU

| Characters | Mean depression $\frac{S_2 - S_1}{S_1} \times 100$ | Variability according to origins |
|-------------------|---|----------------------------------|
| Yield | - 22 | - 19 à - 39 |
| Number of tillers | - 5 | + 10 à - 21 |
| Leaf length | - 11 | - 6 à - 20 |
| Leaf width | - 5 | 0 à - 13 |

quence of such a selection on the success of other phase are difficult to predict.

Under selfing, simple natural selection can decrease the genetic variability of the material. Furthermore we know that the production of homozygous lines in this way is illusive.

However, a limited phase of inbreeding may be of interest, e.g. for homogeneity of the material, selection of additive characteristics, preparation of the hybridization phase.

HYBRIDIZATION

We shall only consider crosses between non-related plants resulting from selfing. This scheme of producing hybrid varieties becomes more and more important with the use of male sterility.

1) Effect on the mean

As the parents are more inbred, the weaker are single crosses. The more favourable tetragenic interactions are, the greater is the superiority of double crosses over single crosses (11). These results, valid for natural or artificial autotetraploids are fundamentally different from those obtained at the diploid level. Experimental results obtained with Dactylis glomerata show the influence of the level of inbreeding on the mean of single crosses and their highest value (table 2). Results with Medicago sativa show the superiority of double crosses over single crosses at the F_1 (table 4).

2) Effect on the variance

a - General and specific combining ability variances at single cross level

If the variability is essentially due to additive effects and interaction between identical genes, the variance of the general combining ability and more so that of the specific combining ability can only increase with the degree of inbreeding of the parents (12).

If the variability is essentially due to the interactions between four different alleles the variance of the specific combining ability will be at a maximum with the second generation of selfing (12) (Graph 2).

Results obtained with Dactylis glomerata from diallel crosses at three levels of inbreeding (S_0, S_1, S_2) show always a very large general combining ability. The specific combining ability is shown to increase with the level of inbreeding (table 3). This is also apparent from the fact that the difference between the best crosses and the mean of the hybrids increases with the level of inbreeding of the parents. Thus, a better selection for hybrid vigour, may be achieved when inbreeding is used.

Table 2.

Effect of the level of inbreeding of S_0 , S_1 , S_2 parents on hybrid means and variability.

| | (1) Mean | (2) Maximum | (3) Mean of the highest | (4) Difference (3) - (1) |
|--------------------------|-------------|----------------|----------------------------|--------------------------------|
| $S_0 \times S_0$ (7 × 7) | 102 (21) | 126 | 124 (4) | 22 |
| $S_1 \times S_1$ (7 × 7) | 103 (21) | 130 | 126 (4) | 23 |
| $S_2 \times S_2$ (8 × 8) | 94 (28) | 134 | 127 (6) | 33 |

× lesser degree of selection at the S_1 , S_2 levels.

Results obtained by GALLAIS on spaced plants of cocksfoot during the year of seeding (% of the best check). Identical origins at each level concerned.

Table 3.

Significance of the general (G.C.A.) and specific combining (S.C.A.) ability in three diallel cross experiments with cocksfoot at different levels of inbreeding.

| | $S_0 \times S_0$ | $S_1 \times S_1$ | $S_2 \times S_2$ |
|----------|------------------|------------------|------------------|
| G. C. A. | ×× | ×× | ×× |
| S. C. A. | NS | × | ×× |

NS : non significant

× : significant at 5 % level

×× : significant at 1 % level

Table 4. Comparison of yields of spaced plants in per cent of the original population of *Medicago sativa* (GENIER and GUY)

| Model of variety | Generation | | | Mean of the 2 highest (1) |
|-----------------------------------|-----------------|-----------------|-----------------|---------------------------|
| | 1 st | 2 nd | 3 rd | |
| HS | 94 | 80 | <u>69</u> | <u>97</u> |
| HD (between HSF ₁) | 102 | 76 | <u>68</u> | <u>81</u> |
| HD (between HSF ₃) | <u>102</u> | 77 | 72 | <u>122</u> |
| II (HSF ₁) Syn. | 89 | 79 | <u>73</u> | <u>87</u> |
| II (HSF ₃) Syn. | <u>89</u> | 82 | 77 | <u>98</u> |
| IV (HSF ₁) Syn. | 93 | 91 | <u>84</u> | <u>94</u> |
| IV (HSF ₃) Syn. | <u>94</u> | 89 | 92 | <u>104</u> |

HS : single cross

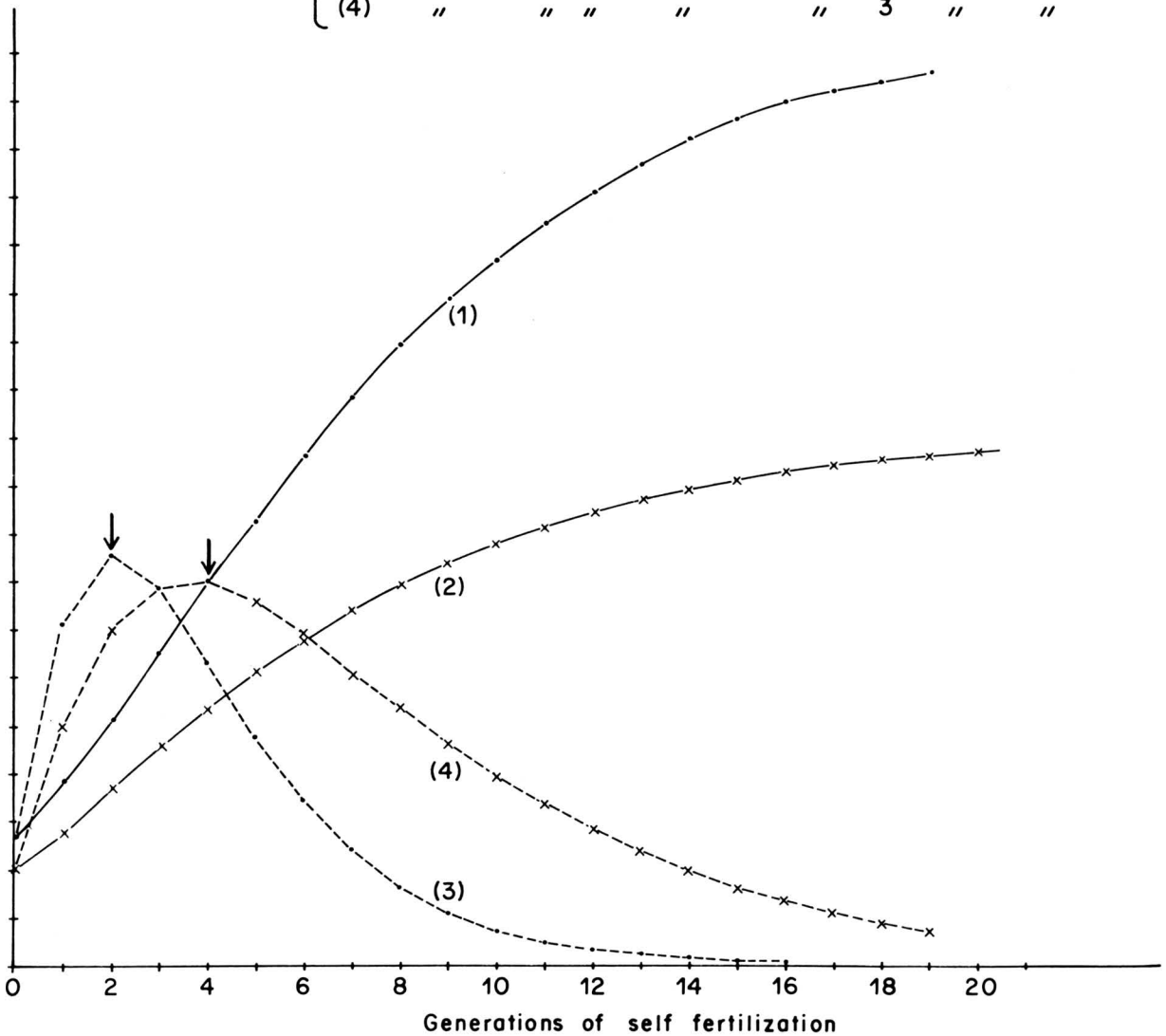
HD : double cross

(1) Means obtained from the two highest hybrids or synthetics within a generation that could be commercialized. the generation that could be commercialized are underlined.

FIGURE II

Distribution of some components of variance between crosses
according to level of inbreeding of parents

| | |
|------------------|--|
| DOUBLE - CROSSES | (1) Variability due to interactions between 4 different alleles (2) " " " " " " 3 " " |
| SINGLE - CROSSES | |



b - Choice between single cross and double cross hybrids

This choice depends on :

- the average gain due to the structure of genotypes at the double cross level,
- the loss of gain expected from interactions between two alleles due to the widening of the genetic base,
- the possible gain due to the variability of the interactions between three and four different alleles.

Experience will show for a given autotetraploid specie, it is better to take for a single cross than a double cross hybrid. In lucerne better hybrids are at the double cross level (table 5).

If double crosses are chosen, the combining ability of parents tested at the single cross level, does not necessarily reflect on the combining ability of single crosses tested at the double cross level mainly with interactions between more than two alleles.

The classical formula used with the diploids for the evaluation of double cross hybrids in relation to non related single cross hybrids does not apply strictly to tetraploids. One experiment is underway to obtain statistical prevision of the value of the double cross hybrid.

The use of heterosis in the hybrid varieties will be easier for the plant breeder if the general combining ability is the main source of variation. But experimental results, (table 6) show the importance of both, the general and specific combining ability.

MULTIPLICATION BY SUCCESSIVE GENERATIONS OF RANDOM MATING - SYNTHETIC VARIETIES -

The impossibility of controlling hybridization on a large scale leads to the multiplication, by successive generations of random mating of single and double cross hybrids (with a narrow genetic base) or of synthetic varieties (generally with a wider genetic base). The synthetic variety may result either from a mixture of the constituents followed by successive generations of random mating or from one final multiplication of a mixture of constituents multiplied separately (see annex). In this last case, partial control of pollination is possible and the value of the synthetic variety depends on the gametic and zygotic competition. The constituents may be clones, inbred or not, hybrids. Experimental results for different types of synthetic varieties are given table 4.

On the average, the narrower the genetic base (small number of parents, or and highly inbred parents) the lower the level of equilibrium. On the other hand a narrow genetic base will allow a greater intensity of selection. As we have shown, (13, 14) the vigour of a particular structure for the equilibrium is the resultant of two elements :

Table 5

Yield of single cross and double cross hybrids, in per cent of the original population, from a breeding nursery of *Medicago sativa* (GENIER and GUY).

| Year | Single-cross hybrids | | Double-cross hybrids | |
|------|----------------------|---------|----------------------|---------|
| | Minimum | Maximum | Minimum | Maximum |
| 1964 | 67 | 128 | 59 | 137 |
| 1965 | 59 | 118 | 54 | 127 |
| 1966 | 57 | 109 | 56 | 116 |
| 1967 | 59 | 116 | 62 | 136 |
| × | 60 | 118 | 58 | 129 |

Table 6

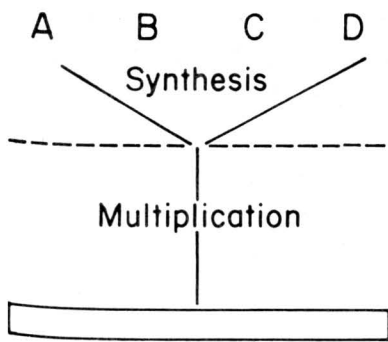
Significance of the general (G.C.A.) and specific (S.C.A.) combining ability according to different authors.

| Authors | G.C.A. | S.C.A. |
|---------------------------|---------|---------|
| DACTYLIS GLOMERATA | | |
| Kalton Leffel (1955) | SS | NS |
| Gallais 1970 | SS | NS |
| Level S_0 | | |
| Level S_1, S_2 | SS | S or SS |
| MEDICAGO SATIVA | | |
| Carnahan et al (1960) | SS | SS |
| Kehr (1961) | NS | S |
| Wileox-Wilsie (1964) | SS | S |
| Evans and al (1966) | S or SS | S or SS |
| Chaume R. (1968) | | |

S significant at 5 % level
 SS significant at 1 % level.

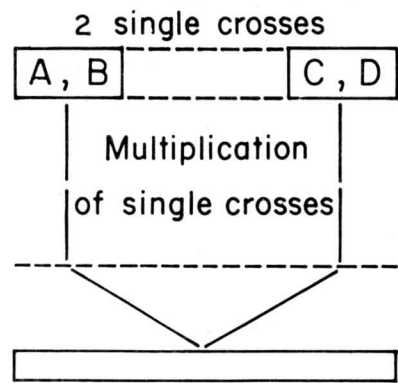
ANNEXE

SOME DIFFERENT COMBINATIONS of 4 CLONES
in a SYNTHETIC VARIETY
4 clones or lines A, B, C, D

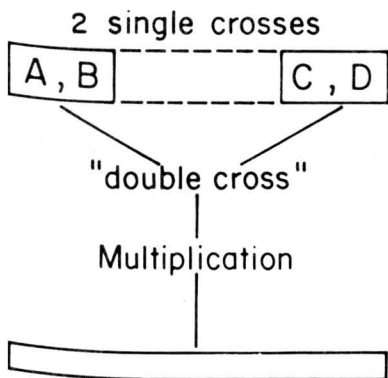


Model 1

Commercial seed

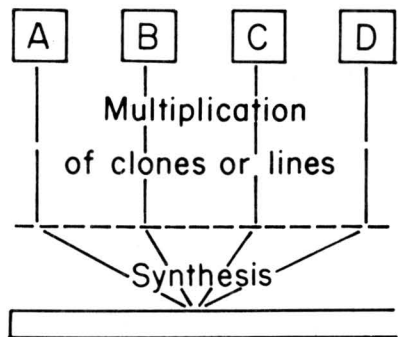


Model 2



Model 3

Commercial seed



Model 4

- 1 - the mean of any possible structure (the number and the structure of the parents being fixed),
- 2 - intensity of selection.

Therefore it is not impossible, if the selection differential is superior to this loss of vigour, to choose from a narrow genetic base. There must exist as with the diploids (13, 14) an optimum genetic base, as the factors which tend to increase the variability decrease the mean value of the structures (small number of parents, inbreeding). This implies an optimum number of constituents for a given level of inbreeding.

How to predict the value of a structure at equilibrium? the laws governing evolution and above all the relationships one can establish between parental characteristics (value under inbreeding, general and specific combining ability), and the value of the variety at equilibrium should be known. Such a study is underway with Dactylis glomerata.

Theoretically, the laws of evolution are precise. There must be a decrease or an increase of vigour with regards to the number of parents, their degree of inbreeding and the floral biology (10, 11, 13, 14, 15, 16).

Hybrids can only decrease in vigour with advanced panmictic generations (11, 13, 15, 16). If the interactions between four different alleles are important, double cross hybrids, even they are always superior to single cross hybrids, must decrease more. This seems to be confirmed by experimental results (1,13) (table 4).

The case of the synthetic varieties is more complex. With panmixia and in the absence of interactions between more than two alleles, Gallais has shown (10) that, on the average, one generation of inbreeding was sufficient for all synthetic varieties with more than two constituents to increase in vigour with advanced panmictic generations.

With self-sterility, two-clone synthetics are equivalent to single cross hybrids and their vigour can only decrease. Synthetics with non inbred clones can also only decrease in vigour whatever number of parents. Vigour can also increase, e.g. synthetics with more than 4 clones with two or more generations of selfing.

The rate of evolution is dependent on the nature of the alleles and is very variable. Practically mainly with multiplied hybrids, we must not make a very hard selection in the F_1 . It will be better to make it in the F_2 . Any subsequent evolution of vigour should be weak in the absence of epistasis. The wider is a genetic base (great number of parents, and weak inbreeding) the weaker are the risks of bad selection. A better theoretical and experimental knowledge of relationships between parental characteristics and value of the synthetic varieties at equilibrium should allow for a harder selection at the F_1 . This would limit the number of synthetic varieties to multiply.

In the development of synthetic varieties, as the general combining ability will become a more important source of variation it will be much easier to exploit the hybrid vigour.

But, experimental results of many authors (table 6) show also the importance of the specific combining ability. As it is theoretically better to develop synthetic varieties with few constituents, which allow maximum use of specific combining ability, it is necessary to undertake new studies on this subject.

CONCLUSION

The importance of heterosis upon the yield of allogamous natural or artificial tetraploid forage crops justifies and explains theoretical and experimental research towards its best use. The eventual use of male sterility gives this kind of research further interest. Furthermore many theoretical results presented in this review for tetraploid state are also valid for other autopolyploid states. We have underlined for hybrids and synthetic varieties the importance of limited inbreeding. It increases the chances to obtain a good variety. The choice of a selection scheme rests on the nature and the variability of genetic effects. Therefore it is important, to set up experiments which will give an evaluation of genetic effects and their variances on the material being selected. General laws exist, but numerous and important parameters depend on the species and the characters studied.

REFERENCES

- Berninger, E. 1967. Contribution à l'étude des modalités d'expression de l'hétérosis chez les autotétraploïdes. *Ann. amélior. Plantes*, 17, 1, 93-104. (1)
- Busbice, T.H., Wilsie, C.P. 1966. Inbreeding depression and heterosis in autotétraploïdes with application to *Medicago sativa* L. *Euphytica* 15, 1, 52-67. (2)
- Carnahan, H.L. and al. 1960. General vs specific combining ability in alfalfa for seedling vigour and fall growth habit in the year of establishment. *Agron. J.*, 52, 9, 511-516. (3)
- Chaume, Régine. 1968. Etude de la structure de la variance génétique d'une population panmictique à l'équilibre chez une espèce autotétraploïde. Thèse de 3^{ème} cycle, Fac. Sci. Paris. (4)
- Demarly, Y. 1963. Génétique des tétraploïdes et amélioration des plantes. *Ann. Amélior. des Plantes*, 13, 307-400. (5)
- Dèssureaux, L., Gallais, A. 1969. Inbreeding and heterosis in autotétraploïde alfalfa. I - Fertility. *Canad. J. Genet. and Cytol.*, 11, 3, 706-715. (6)

- Evans, K.H., Davis, R.L. and Nyquist, W.E. 1966. Interaction of plant spacing and combining ability in an eight clone diallel of *Medicago sativa* L. *Crop. Sci.*, 6,5, 451-454. (7)
- Frakes, R.V. and Al., 1961. The breeding behaviour of yield and related variables in alfalfa : I, II, III. *Crop. Sci.*, 1,3, 205-212. (8)
- Gallais, A. 1967. Moyenne des populations tétraploïdes. *Ann. Amélior. Plantes*, 17, 3, 215-228. (9)
- Gallais, A. 1968 a. Evolution de la vigueur des variétés synthétiques tétraploïdes au cours des générations de multiplication. I - En régime de panmixie. *Ann. Amélior. Plantes*, 18, 1, 5-15. (10)
- Gallais, A. 1968 b. Etude théorique et comparée de la vigueur de différentes structures variétales chez les plantes allogames autotétraploïdes. *Ann. Amélior. Plantes* 18, 2, 99-124. (11)
- Gallais, A. 1968 c. Interaction between alleles and their variability in autotetraploid cross-fertilized plants. Consequences for their selection. V Congr. Eucarpia - Milan - (Italie). (12)
- Gallais, A., Guy, P., Lenoble, M. 1970. Models for varieties in cross fertilized forage plants. *Proc. XI Int. Grassld Congr.* 254-259 (Australie). (13)
- Guy, P. 1966. Etude de l'évolution de quelques structures autotétraploïdes au cours des générations de multiplication. XI Congr. Soc. Ital. Genet. Agric. (14)
- Guy, P., Genier, G. 1967. Structure et évolution au cours des générations de multiplication en panmixie d'un hybride autotétraploïde. *Ann. Amélior. Plantes*, 17, 3, 277-290. (15)
- Guy, P. 1970. Structure génotypique d'un hybride autotétraploïde multiplié en panmixie. I. *Ann. Amélior. Plantes*. 20, 3, (in press). (16)
- Kalton, R.R. and Al. 1955. Evaluation of combining ability in *Dactylis glomerata* L. I - Clonal and outcross progeny performance. *Iowa State College J. Sci.*, 29, 4, 631-658. (17)
- Kehr, W.R. 1961. General and specific combining ability for agronomic traits in a diallel series among six alfalfa clones. *Crop. Sci.*, 1,1, 23-55. (18)
- Rotili, P. 1968. Effets de l'autofécondation dans la luzerne. V Congrès Eucarpia, 391-403 - Milan - (Italie). (19)
- Wilcox, J.B. and Wilsie, C.P. 1964. Estimated general and specific combining ability effects and reciprocal effects in crosses among nine clones of alfalfa. (*Medicago sativa* L.). *Crop. Sci.*, 4,4, 375-377. (20)

BREEDING METHODS IN FIELD BEANS (*Vicia faba* L)

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SOURCE OF VARIABILITY

Vicia faba is an isolated species in as much as it has not been possible to make crosses with related species of *Vicia*. However, a considerable range in plant characters can be found within the species, and, because of the 30 to 40% natural cross-fertilisation (shown by Fyfe and Bailey, 1951, and Picard, 1953), some considerable genetic diversity is maintained within local populations and more is probably added through contrasting varieties being cultivated in adjacent fields. At the Plant Breeding Institute, Cambridge crosses have been made between varieties of widely different origins with a view to supplementing the variation obtainable from within local populations. Such crosses are expected to be useful where improvements in hardiness, maturity dates, pod shape and disease resistance are required. In terms of yield, many local populations have been found to contain a variability which has not yet been fully exploited by breeding.

SELECTION METHODS

The following main classes of varieties are being cultivated or are being developed by breeding :

- 1) **Open-pollinated varieties**, where only populations are maintained.
- 2) **Synthetic varieties**, periodically reconstituted from :
 - a. non-inbred components or
 - b. inbred components
- 3) **Hybrid varieties**, produced from male-sterile and restorer parents
Planted in :
 - a. alternate blocks or
 - b. in mixtures

Methods which do not involve inbreeding have distinct limitations. It is true that some open-pollinated populations have become locally adapted, e. g. winter

FIGURE I

Production of synthetic varieties of field beans

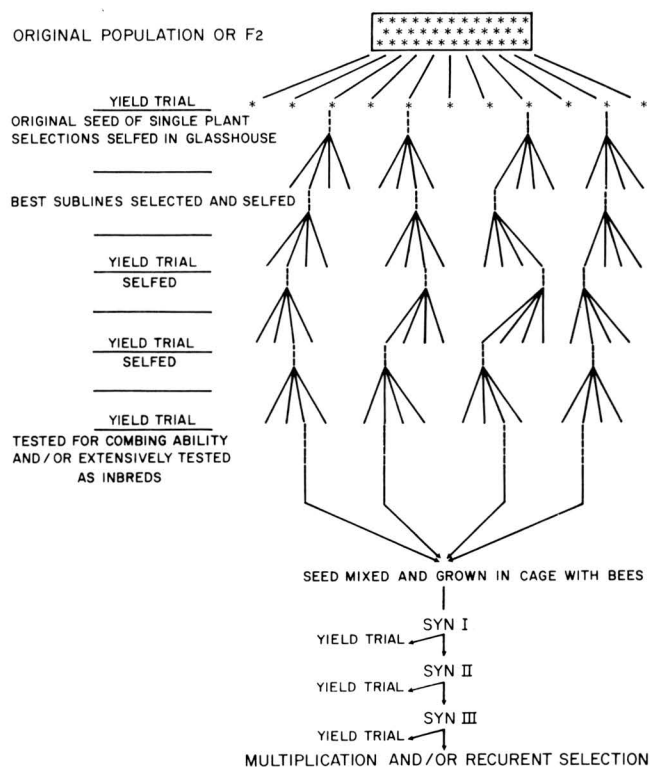
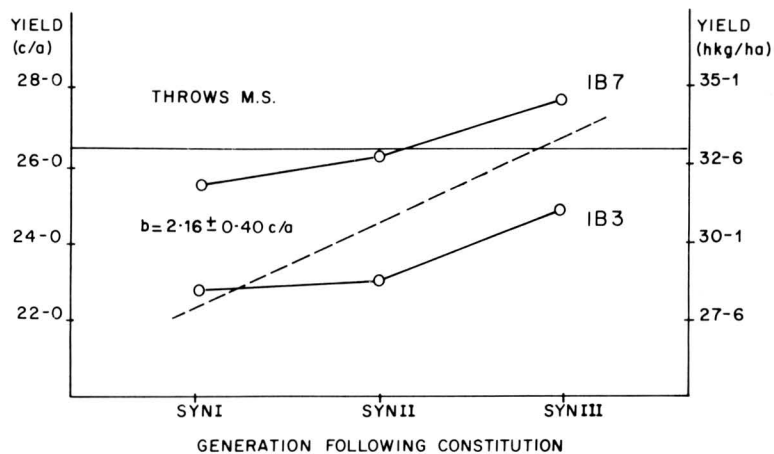


FIGURE II

Yields of synthetic varieties in the 3 generations following constitution from inbred lines



beans in central and southern England, but mass selection has given little improvement in yield and is useful only for improving the uniformity of characters which can be easily screened, such as pod shape and seed size. Single-plant progeny testing under open pollination, can give significant differences in yield between progenies but performances are not reproducible in future generations. In the case of synthetic varieties with non-inbred components, it has been found necessary to make continued selection under open pollination conditions within the populations forming the components if the performance of the synthetics is to be maintained. Thus, it is considered that the only method of evaluating the genetic potential and of arriving at stable components for synthetics or parents of hybrids, is by inbreeding.

PRODUCTION AND EVALUATION OF INBREDS

The method of inbreeding and composition of synthetic varieties used at Cambridge is shown in Fig. 1. The first selection is based on the performance of open-pollinated, single-plant progenies (about 100) but only original seeds (about 4) from each single plant are used for inbreeding. Selfing is assisted by hand-tripping of the flowers. The higher-yielding sublimes are selected from within each line until no significant differences are detected between sublimes within lines. This stage is usually reached after four generations of selfing, when sublimes may be mixed together to produce enough seed for testing at more than one location or year.

Assessment of combining ability has been mainly in diallel crosses or with three or more inbred lines as testcrosses. In a topcross, the progenies would be small and too dependent on variation within the population used as tester. Double-crosses have been used as testers where differences in only general, not specific, combining ability were sought.

In both diallel crosses and testcrosses between inbreds, general combining ability accounted for most of the variability; specific crosses tended to interact with seasons or locations or they were outyielded by crosses between lines of superior general combining ability. Some inbred testers gave a better discrimination between lines than others, but general combining ability effects could be more easily detected in some components of yield than in yield itself. Yields of crosses were correlated with those of their parents, though with significant residual variances in yields of crosses after removal of regression on parents. Thus, it should be possible to increase yields of synthetics and hybrids by selection based on the performance of the lines themselves, and a further improvement could be expected following selection for general combining ability.

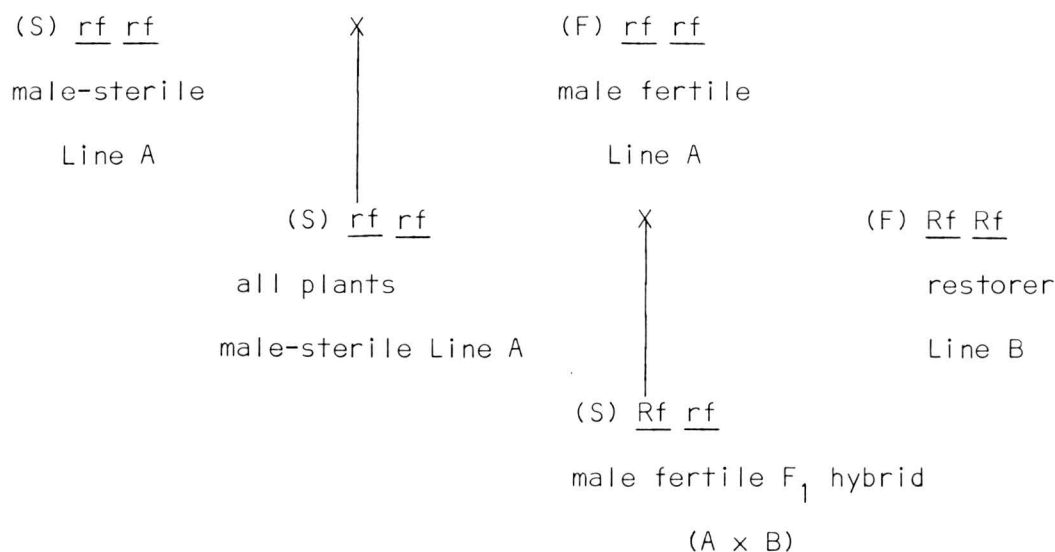
All selfed lines suffered from inbreeding depression, the yield after S_4 varying from 40% to 90% of the open-pollinated control variety. Random inbreeding also resulted in some loss of ability to self without tripping, and in susceptibility to disease, though the latter was mainly eliminated by selection during inbreeding and testing of lines.

SYNTHETIC VARIETIES

At Cambridge, the components of synthetics are selected inbreds, and usually four or five in number. Yields of the Syn_1 generation in recent trials were greater than the mean of the components and there were slight increases in yield from the Syn_1 to Syn_3 generation (See Fig. 2.), but none of the synthetics averaged more than 5% higher yield than the control variety. The low yield of the Syn_1 relative to the known combining ability of the components, was attributed to the high degree of selfing and sibbing in the generation of composition. Seed markers in one synthetic showed only 16% crossing between lines and in another 36% crossing. This was despite the observation of Drayner (1959) that in mixed plantings of inbreds and hybrids, inbreds undergo more outcrossing than hybrids. Liberal supplies of bees did not improve the degree of outcrossing. There may have been some unintended selection for automatic self-fertility during inbreeding.

HYBRID VARIETIES

The level of heterosis in Vicia faba was established as at least 20% (Bond 1966) and the discovery of male sterility allowed an investigation of the possibility of commercial hybrids. A genetic male-sterile ms was used to avoid hand emasculation in the production of crosses to test combining ability, but the cytoplasmic male-sterile (S), with nuclear gene rf rf, opened up the possibility of large-scale hybrids. The following is the basis of the method used in the production of hybrids :



Evidence of the mode of inheritance of the restorer gene (Rf) was given by Bond, Fyfe and Toynbee-Clarke (1966). Inbreds have been sterilized by back-crossing male-sterile plants to non-restorer lines as recurrent parent of the type, (F) rf rf. There is also the need for the absence of factors controlling partial restoring ability, or modifiers, the mode of inheritance of which has not yet been determined. Many inbreds restore the fertility in a small proportion (between 1 and 5%) of the progeny of male-steriles ; and those inbreds with less than 1% restoring ability need to be multiplied without contamination so that a high degree of male-sterility is maintained during multiplication and a high degree of purity obtained in the F₁.

The fertility-restoring pollinator line gives complete restoration of fertility provided it is homozygous Rf Rf and is maintained in good isolation. The Rf allele, which was detected by testcross data, has been transferred from the inbred in which it was discovered, to other lines. All hybrids now being officially tested in England however, have the original restorer line as male parent. Parents of hybrids are grown in alternative blocks of convenient widths for sowing and harvesting machinery. The ratio of male to female parent has been 1 : 1 but a 1 : 2 ratio will soon be tested. Mixed plantings of male-sterile and restorer plants are being investigated. Pod-setting on male-steriles in mixtures was 20 per cent greater than that in alternate blocks and equal to that on male-fertiles ; but whereas in one hybrid it was shown that a one-eighth admixture with its inbred parent could be tolerated without loss of yield, in another hybrid a mean of 1.99 cwt/acre (2.5 qx/ha) was lost for every one-eighth of parent added. Where the two parent lines have contrasting seed size or colour, it is possible to separate the seed after harvesting, with sieves or a photoelectric colour sorter.

Pollination of male-sterile beans in England is brought about mainly by long-tongued bumble-bees, e.g. Bombus hortorum but honey-bees are also effective when collecting pollen. Seed setting may be better in countries with good numbers of pollinating insects.

PERFORMANCE OF F₁ HYBRIDS

In Fig. 3 are summarized yields of winter bean F₁ hybrids, inbred lines and synthetic varieties in relation to the control in trials at the Plant Breeding Institute, Cambridge, from 1964 to 1969. The hybrid 349 x S45 averaged 20% higher than the control, Throws M.S., in all Plant Breeding Institute trials and 17% in all official (N.I.A.B.) trials. I acknowledge the collaboration of the Station d'Amélioration des Plantes at Rennes, and can report that in trials there, 349 x S45 again yielded 20% more than Throws M.S. Differences at each station were significant at P = 0.05 and were the equivalent of an increase of 6 cwt/acre (7.5 qx/ha) on 30 cwt/acre (38 qx/ha). Trials in cages which excluded insects showed a greater advantage of hybrids over controls than in open-field trials. This results is consistent with the hypothesis of Drayner (1959) that automatic self-fertility is closely related to heterozygosity, and suggests that F₁ hybrids would be especially advantageous in regions with few wild bees. Honey-bees are sometimes provided by farmers to supplement wild bees, but they are not always effective and as is shown in Fig. 4., the

FIGURE III

Yields of hybrids, synthetics and inbreds in relation to throws M.S.

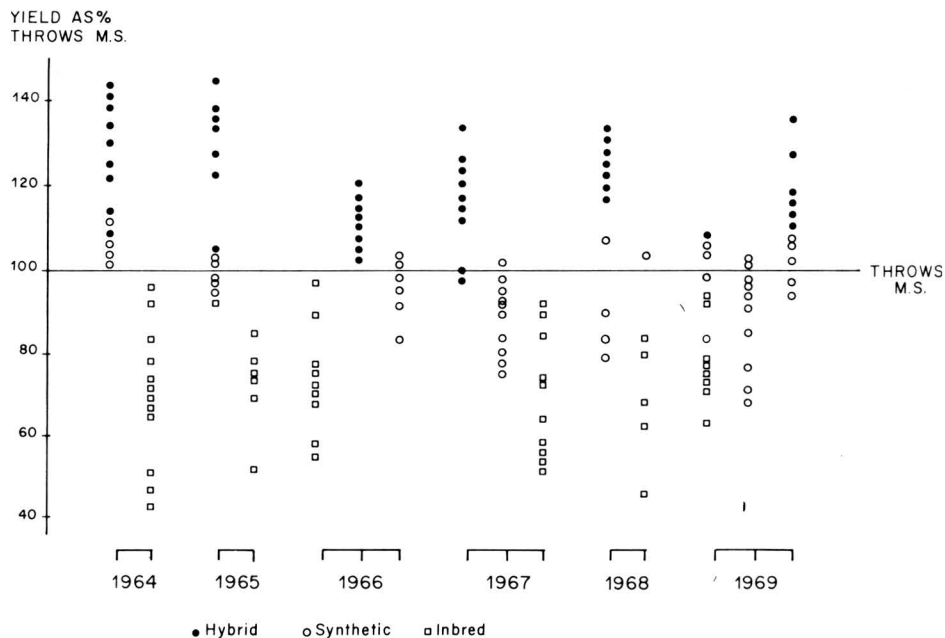
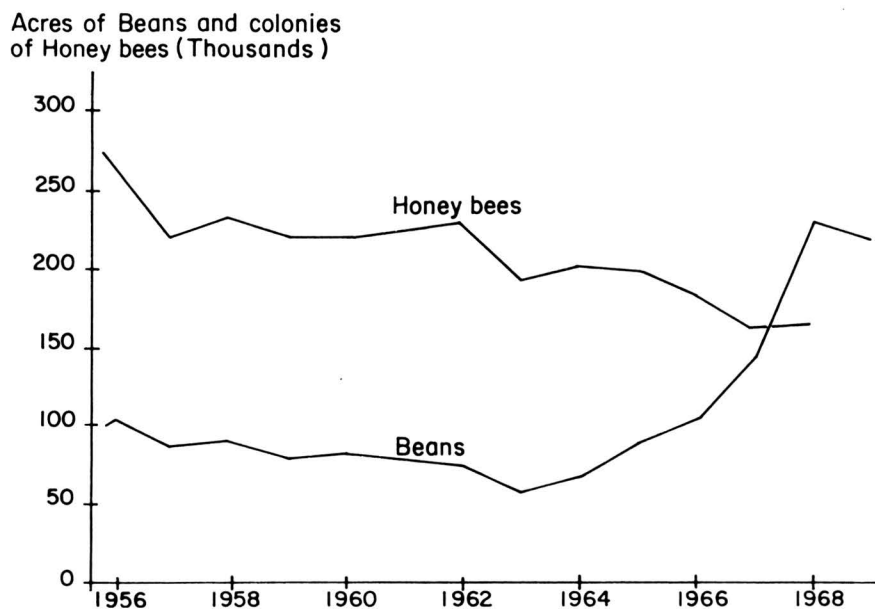


FIGURE IV

Acreage of field beans and numbers of honey bee colonies (England and Wales)



number of colonies in England has now fallen below the number of acres of field beans grown. Even one hive per hectare would not be possible because most of the honey-bees are in fruit-growing areas not bean-growing regions. Thus, automatic self-fertility is highly desirable in bean varieties, and this attribute may be realised in F_1 hybrids provided their parents are pure so that fertility is restored to a high degree.

F_1 hybrids based on inbred parents are more uniform and easier to identify than open-pollinated or synthetic varieties. These and their other characteristics apply only to the F_1 generation however, yields of the F_2 in trials at Cambridge fell to the level of or below that of control varieties.

The cost of producing hybrids (i.e. from parents in alternate blocks) is estimated to be double that of standard varieties. At present English prices, this means, for the farmer changing from open-pollinated to hybrid varieties, an increase of £6/acre (195 francs/ha.) expenditure on seed, which, to equal the profit margin of open-pollinated varieties, must bring an extra yield of at least 4 cwt/acre (5 qx/ha) or 13% increase on 30 cwt/acre. Where open-pollinated varieties yield only 20 cwt/acre (25 qx/ha) a 20% increase is needed before hybrids bring more profit ; therefore hybrids may be suited to fertile soils and situations where yield levels are high. Winter bean hybrids are vigorous in tillering and may be sown at a slightly lower seed rate than open-pollinated varieties. Costs of producing hybrids in mixtures are expected to be less than from alternate blocks but the method has not yet been fully developed.

CONCLUSIONS

The hybrid method of breeding shows greater promise of a substantial improvement in yield than other methods, but it is as important to devise the most economical method of producing hybrid seed as it is to breed new higher-yielding hybrids. Synthetic varieties have shown only a slow advance in yield, in relation to that achieved by hybrids. This could be partly due to the small scale on which the selection of inbreds can be justified in a crop of minor economic importance. On the other hand, the evidence of overdominance presented by Bond (1966) suggests that part of the heterosis in yield of the F_1 is unfixable in inbreds or synthetics no matter how large the scale of testing. A programme of breeding synthetics should proceed alongside hybrid breeding however, so that the relative advantage of hybrids can be continually assessed. It so happens that concomitant synthetics are the only way in which immediate use can be made of the inbreds which are difficult to sterilize with the male-sterility factors available at Cambridge. Synthetics may be more useful in the improvement of characters other than yield, for example, hardiness and earliness. Although there may be some response to selection under open-pollination, inbreeding appears to be the best way of evaluating, stabilizing and exploiting the genetic potential ; and resultant hybrid and synthetic varieties should be more suited than open-pollinated varieties to the requirements of schemes for seed certification and plant breeders' rights.

REFERENCES

- Bond D. A. (1966). Yield and components of yield in diallel crosses between inbred lines of winter beans (Vicia faba) J. agric. Sci., Camb. 66 325-36.
- Bond D.A., Fyfe J.L. and Toynbee-Clarke G. (1966). Male-sterility in field beans (Vicia faba L.) III Male-sterility with a cytoplasmic type of inheritance. J. agric. Sci., Camb. 66 359-67.
- Drayner J.M. (1959). Self and cross-fertility in field beans (Vicia faba Linn.). J. agric. Sci., Camb. 53 387-403.
- Fyfe J.L. and Bailey N.T.J. (1951). Plant breeding studies in leguminous forage crops. I Natural crossbreeding in winter beans. J. agric. Sci., Camb. 45 141-147.
- Picard J. (1953). Recherches sur la féverole Ann. Amel. Plantes 3 57-106.

USE OF MENDELIAN MALE-STERILITY IN RECURRENT SELECTION
AND HYBRID BREEDING IN BEETS

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Inbred, monogerm, cytoplasmic male-sterility maintainers (O-types) in sugar beet mostly carry also a gene (S^f) for self-fertility. This gene was present already in the genetic monogerm plant found by V.F. Savitsky in the American sugar beet variety Michigan Hybrid 18, the increase of which was later made available to sugar beet breeders throughout the world under the accession SLC 101 (V.F. Savitsky, 1950 ; H. Savitsky, 1952, 1954).

Although detrimental in conventional breeding programmes aiming at synthetic or multistrain varieties without the use of male-sterility, the S^f gene has greatly facilitated the development and maintenance of inbred monogerm O-types, especially in areas with hot summers, where under isolation normal self-sterile beets set very little or no seed.

However, since the S^f gene results in a high degree of selective fertilization favouring the plants own pollen (Savitsky, 1952), it severely hampers the development and continuous improvement of monogerm source populations from which superior lines can be selected. Owing to the rapid change over to genetic monogerm seed, the amount of self-sterile multigerm material subject to intensive breeding has decreased and many lines of less immediate interest have no doubt already been lost. With the bulk of genetic variability still to be found in self-sterile, multigerm populations, efficient and inexpensive methods of gene introduction and population improvement at the monogerm level are required, or the combination self-fertility-hybrid breeding will in the long run result in an impoverishment of the gene pool of cultivated beet. The usefulness of Mendelian or genic male-sterility in genetic improvement programmes with self-fertile beets, such as convergent improvement of inbred lines or incorporation of desirable genes into established inbreds, was emphasized by Owen as early as 1952 in connection with his description of the recessive gene a_1 for pollen abortion in sugar beet (Owen, 1952). Later Doggett and Jowett (1963, 1964) and Gilmore (1964) have suggested the use of male-sterility to facilitate recombination in recurrent selection programmes with predominantly self-fertile crops. A system employing the Coes genetic male-sterile (ms_3) in Sorghum for this purpose has recently been outlined by Doggett and Eberhart (1967). Two divergent populations are formed one homozygous

for the genetic restorer factors for cytoplasmic male-sterility and the other homozygous for the maintainer factors, but in the fertile cytoplasm. Both populations should segregate for the ms_3 gene. Elite lines selected from the maintainer population are used to develop their cytoplasmic male-sterile counterparts. Recurrent selection is practised in both populations. Reciprocal test-crossing of lines developed from the populations is simplified by the use of genetic male-sterility in the restorer population and the cytoplasmic-sterile equivalent of the maintainer population. The commercial products can be single-cross hybrids, variety-cross hybrids or pure line varieties.

The present report describes an adaptation of this system to beets. It permits the use of various kinds of recurrent selection in the development of self-fertile, monogerm, 0-type lines and their male-sterile counterparts, and greatly simplifies the incorporation of new genetic variability into the population from self-sterile, multigerm populations. As in Sorghum pollinator populations can be developed along the same lines. Since there is no advantage in having monogerm pollinator lines for hybrid seed production and the degree of fertility of the commercial hybrid is unimportant in a root crop, the handling of pollinator populations for single-cross hybrids or three-way hybrids presents no difficulties.

In a diploid hybrid programme, the development of inbred lines from the best roots in each cycle of selection appears to be most efficient, at least in the long run. If practised in both the 0-type and the pollinator populations, four-way hybrids can be produced utilizing cytoplasmic male-sterility in one of the single crosses and the final cross and Mendelian male-sterility in the other single cross, as proposed by Owen (1954). However, a serious disadvantage of this method is that at least 50% of the plants in the line segregating for Mendelian male-sterility will have to be rogued out since they are pollen producers. If instead, a pollen restorer population is formed and subjected to the same system of selection as the type 0 population, it should be possible to develop double-cross restored hybrid varieties based on four inbred lines : two of which are cytoplasmic male-sterile, one an 0-type and one a pollen fertility restorer (Theurer and Ryser, 1969).

However, the author's interest in the present method is primarily the possibilities it seems to offer to develop triploid hybrid sugar or fodder beet varieties. A selected, broad-base cytoplasmic male-sterile population is probably a better female parent of triploid hybrids than inbreds or F_1 -hybrids, especially if the cytoplasmic male-sterile equivalent of the 0-type population is used as a tester in the development of the tetraploid pollinator population (Bosemark, 1969). In the following emphasis is placed on this alternative, and the development of diploid pollinator populations or restorer populations is not discussed.

The recessive gene a_1 for Mendelian male-sterility usually gives a very clear-cut 3:1 segregation with only slight environmental modification (Owen, 1952). Lines incorporating the a_1 gene have been widely distributed to sugar beet breeders through the U.S. Department of Agriculture. A self-fertile, monogerm

0-type line segregating for \underline{a}_1 ($\underline{S}^f \underline{S}^f \underline{m} \underline{m} \underline{A}_1 \underline{a}_1 \underline{x} \underline{x} \underline{z} \underline{z}$) is used to initiate the maintainer population. Its development from the appropriate crosses should not present any difficulties. Male-sterile segregants from this line may be crossed directly with self-sterile, multigerm populations. However, since most breeders already carry a number of acceptable monogerm, self-fertile type 0 inbreds, the use of these as initial pollinators will expedite the formation of a maintainer population (see Fig. 1(1)). All such F_1 -crosses may be bulk propagated (2). Stecklings from the following F_2 -generation, which will segregate for the \underline{a}_1 gene, are planted out in a crossing plot (3-4). At flowering time, sterile F_2 -plants are tagged, and seed is harvested only from these plants. The off-spring from these sterile plants (5) will give a 2:1 segregation (2 fertile : 1 sterile). To start the cytoplasmic-sterile counterpart of the population, a number of rows of a good, monogerm, cytoplasmic male-sterile (CMS) line may be planted out already in this first F_2 -plot and harvested separately. To achieve a certain degree of linkage equilibrium in the population, it may be advisable not to select in the initial population, but in the next cycle.

Unless the number of inbreds included in the starting population is high, the introduction of new material from multigerm, self-sterile populations should start as soon as possible. Again self-fertile, Mendelian male-sterile, type 0 plants will be required as females for the crossings (6). However, these plants should not belong to the line used to initiate the population but be taken from the F_2 - or F_3 - generation. If the male-sterile plants used for introductions are always taken from the most recent population, the risk of introducing unwanted characteristics through these steriles is minimized ; moreover, a single genotype is not allowed to put its stamp on the population. If, for the sake of expediency, we accept the hypothesis for the genetic background of cytoplasmic male-sterility put forward by Owen (1945), the F_1 -plants from introductions will consist of four genotypes ; $\underline{X} \underline{x} \underline{Z} \underline{z}$, $\underline{X} \underline{x} \underline{z} \underline{z}$, $\underline{x} \underline{x} \underline{Z} \underline{z}$ and $\underline{x} \underline{x} \underline{z} \underline{z}$, all of which will be heterozygous for the gene for monogermity (\underline{m}) and for \underline{a}_1 (7). The proportions of the four genotypes affecting fertility in sterile cytoplasm will, of course, depend on the constitution of the multigerm population. To find the $\underline{x} \underline{x} \underline{z} \underline{z}$ -plants, all F_1 -plants are test-crossed with a cytoplasmic sterile line carrying the dominant gene \underline{B} for annual habit (Owen, 1950). F_1 -progenies with this line (F_1 AMS) having 100% sterile plants (8) arise from crosses with plants that are 0-types ($\underline{x} \underline{x} \underline{z} \underline{z}$). At the same time as the crossings with the AMS, all plants are also back-crossed to new $\underline{S}^f \underline{S}^f \underline{m} \underline{m} \underline{A}_1 \underline{a}_1 \underline{x} \underline{x} \underline{z} \underline{z}$ -plants (7). The off-springs from crosses with $\underline{x} \underline{x} \underline{z} \underline{z}$ -plants, identified through the AMS test cross, will segregate 1:1 for each of the factors \underline{m} and \underline{a}_1 while being homozygous for the sterility genes. Stecklings from all such crosses are planted in the crossing and recombination plot together with stecklings from the segregating maintainer population and the CMS back-cross population as outlined in the diagram (9). If the initial unselected maintainer population is used, it will segregate for fertile (F) and sterile (S) plants in the ratio 2:1 as illustrated. In later cycles when the population is formed through intercrossing of selected S_1 -lines the segregation will be 3:1. At flowering, male-sterile segregants from the maintainer population are tagged for individual seed harvest. The fertiles may be selected for vigour and morphological characters. The CMS plants are checked for male-sterility and off-types. The introductions are selected for both monogermity and male-sterility, which means that on an

FIGURE I

Diagram illustrating the use of mendelian male-sterility in recurrent selection with a self-fertile, monogerm, type O sugar beet population

(In the text reference is made to the figures 1-13 in the left hand margin)

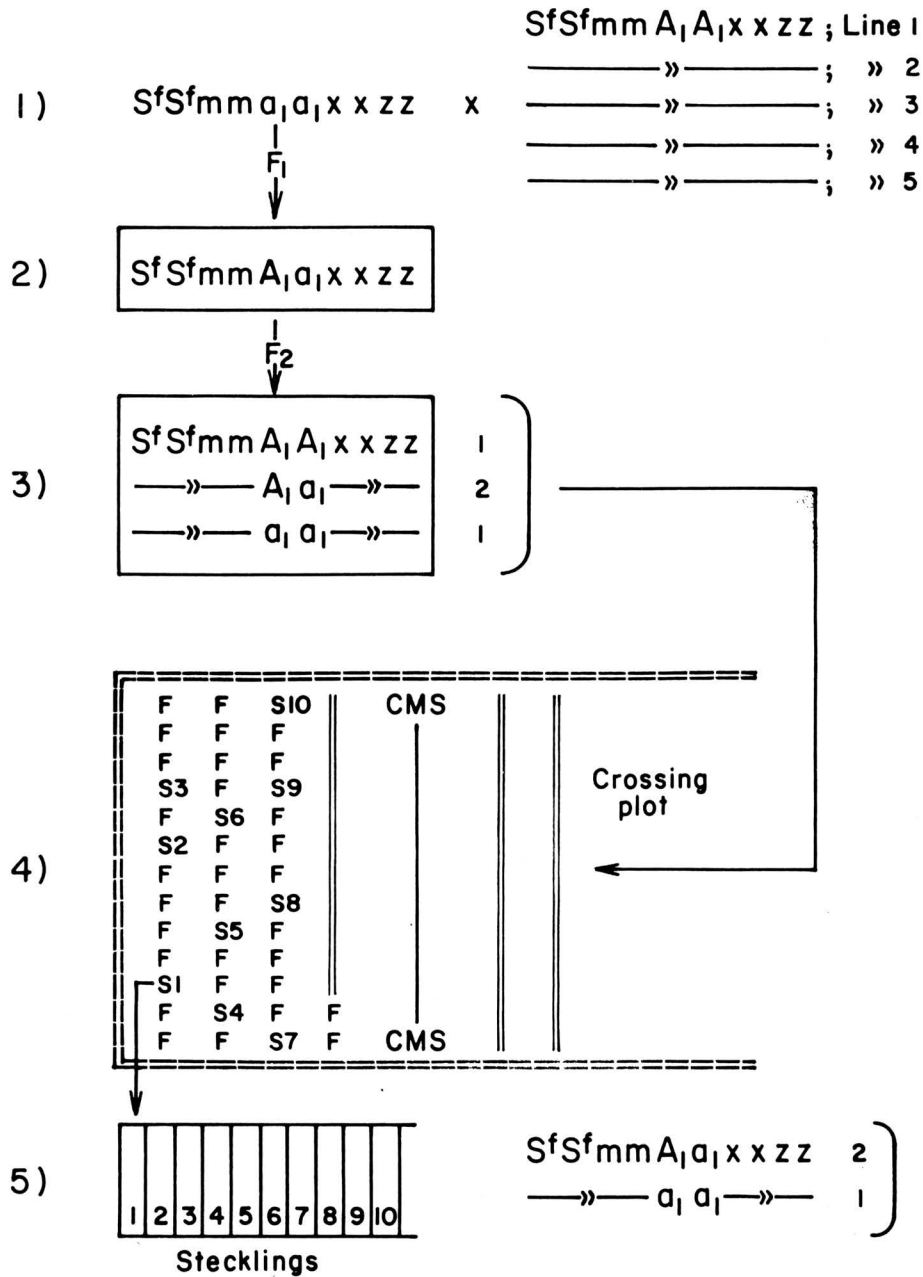
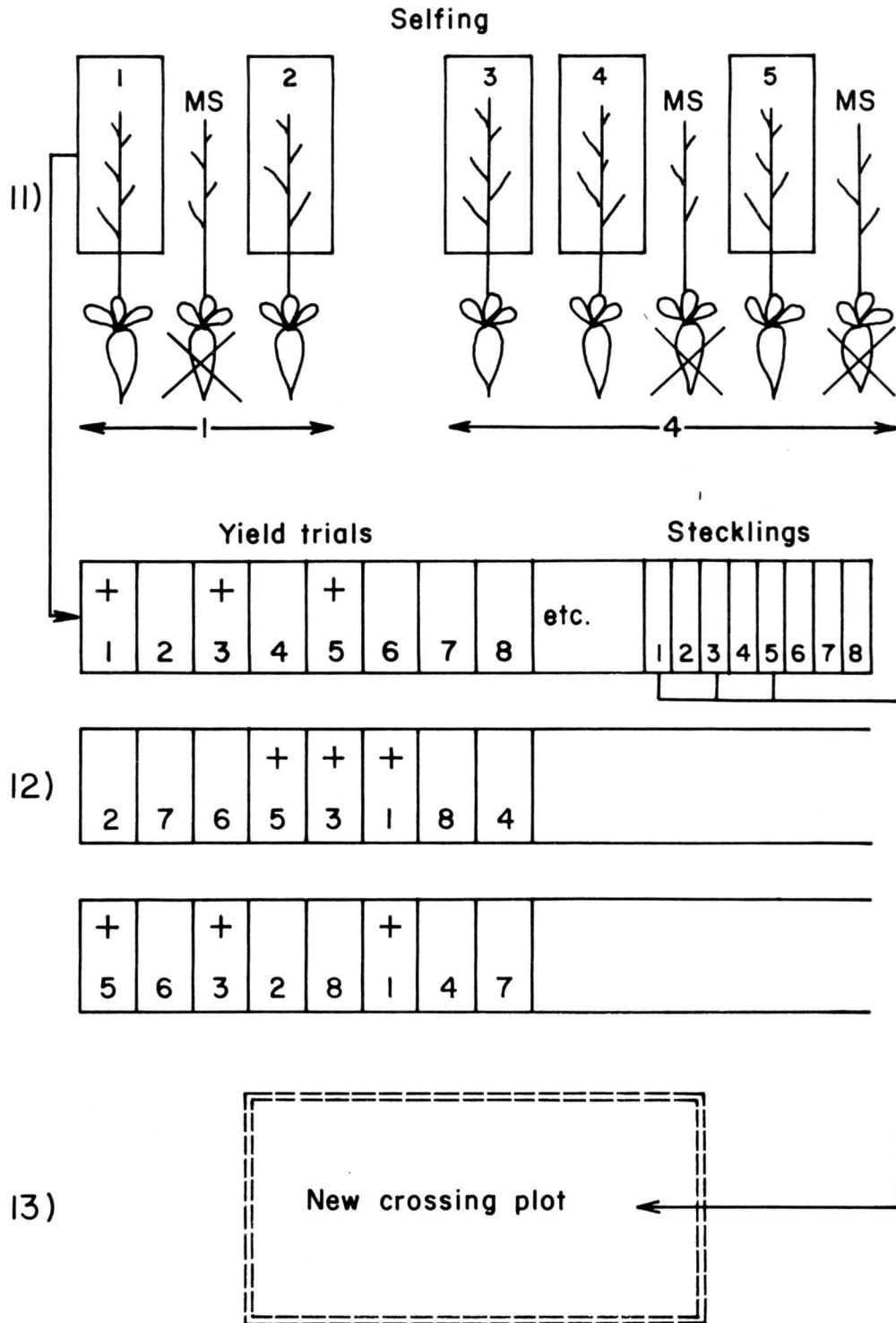


FIGURE I (continued)



average 1 plant in 4 will be saved. The reason for removing all fertiles is that the newly introduced genotypes have not been previously tested. By allowing only male-sterile plants to remain in the crossing plot, the introductions cannot contaminate the population. After evaluation of the hybrids formed on the male-sterile plants, it can be decided if the introductions should remain in the population or not.

In the following year, the seed harvested on the male-steriles -those from the population, as well as the introductions- are subjected to a screening trial (10). The seed harvested from the CMS plants is sown out for steckling production. Since the number of entries in the yield trial is large, and the screening is of a preliminary nature, 2-row plots replicated twice will suffice. One of the replications is harvested and analysed in the usual way ; the other is used as a selection plot and harvested only when the trial results are known. From each of the selection plots belonging to the best 25% of the entries 4 or 5 desirable roots are selected. Next year all of these are planted out in a seed plot well isolated by distance, hemp, mustard or other means (11). One half of the plants will be male-sterile and are removed leaving at least one good fertile plant from each F_1 to produce selfed seed. (When the maintainer population is based on S_1 -lines only one third of the plants will be male-sterile). To ensure complete self-pollination it is necessary to isolate each plant. However, plants carrying the \underline{S}^f gene will usually produce about 75-95% selfed seed even without isolation, and it is very likely that for the present purpose we can dispense with isolation and still look upon the seed as selfed seed. Another possibility is to use isolators that are open at the top. Such isolators can be made from bamboo canes and plastic sheets and should have the advantage over cotton tents of not diminishing the seed yield.

In this connection, it should be pointed out that all fertile plants selected will not be self-fertile. The inheritance of the self-fertility conditioned by the \underline{S}^f gene is explained by the oppositional factor hypothesis. Thus, hybrids between self-sterile and self-fertile plants are all highly self-fertile in F_1 , F_2 and successive selfed generations. A line in which the \underline{S}^f gene has been introduced consequently rapidly approaches homozygosity for this factor. However, if male-sterile plants heterozygous for the \underline{S}^f gene are pollinated by fertile heterozygotes, self-sterile plants will arise in the offspring. Therefore, it is important that among the plants entering the maintainer population the percentage of plants heterozygous for the \underline{S}^f gene is as low as possible. Hence, the progenitors of the male-sterile monogerm O-types used for the first cross, as well as the back-cross with the introductions, should be homozygous for the \underline{S}^f gene. This can be ascertained by crossing some such plants with self-sterile, Mendelian male-sterile tester plants. If the tester plants also carry the gene \underline{B} for annual growth habit, the F_1 will flower without photothermal induction and the genotype of the test-plants can be rapidly determined by bagging a few of the fertile F_1 -plants. All the same 1 out of 16 (maximally) of the plants selected for selfing will be self-sterile. If pollen-proof isolators are used for production of the S_1 -generation, such plants will of course be readily spotted by their low seed set. However, if the selective fertilization of plants carrying the \underline{S}^f gene

is relied upon to produce selfed seed, self-sterile plants will have to be identified by bagging a small branch on each plant with a kraft paper bag. Two to three weeks after flowering, self-sterile plants can be identified and removed (Savitsky, 1952). All S_1 seed lots are tested in yield-trials over as many replications as the seed quantities permit. Preferably, replications should be spread over as many locations as possible (12). Stecklings are also sown from each S_1 -line. It is important to carry over some seed should the material in the field be lost for one reason or other. From the trial results, 10 to 20% of the best S_1 -lines are selected. A number of stecklings from each of these selected lines are mixed together and planted out the following spring to form the improved population (13). At flowering time the most desirable male-sterile plants in the population are again tagged. Unwanted fertiles should be removed as soon as they can be identified. As in the previous cycle, CMS plants are planted out in strips in the plot to produce the next CMS back-cross. If new materials are introduced, their male-steriles will also be allowed to form seed in the plot. The seed harvested on the male-sterile plants belonging to the population is bulked, except the seed from the tagged plants, which is harvested separately. This completes the cycle.

For the benefits of the present system to be fully realized, it is important to maintain a large number of plants in each cycle so that selection can be carried out on root characteristics as well as seed plant characters. The last is not the least important since seed-production capacity, degree of monogermity, lack of fasciation, shape and size of the seed, percentage of false twins, as well as germination capacity will have to be subjected to a continuous selection pressure.

Going back to the CMS back-cross seed, this can be tried out as the female partner in various hybrid combinations with already approved diploid or tetraploid pollinators. If and how soon it will be of interest in commercial seed production, of course, depends on the materials that have gone into the population. However, irrespective of this it should be used as tester in the development of new and improved pollinator populations. If the goal is a commercial triploid hybrid, we thus have to consider the system for improving the tetraploid population. In principle recurrent selection utilizing self-fertility and the a_1 gene should be possible also at the tetraploid level. However, there are still many questions to be answered before such a system can be put into practice. At present, conventional family line breeding is nearest at hand, and is likely still to result in considerable improvement.

A bulk cross of the best available self-sterile, tetraploid, multigerm strains, would be a suitable starting material. Single plant progenies (or two-plant families) based on individually selected roots are tested in a preliminary trial and selected families separately increased. The lines thus developed should be allowed to cross with the latest CMS back-cross population. The lines producing the best 25% of these $3x$ -crosses may then intercrossed to form the new improved tetraploid population and the whole procedure is again repeated. To maintain genetic variability in the improved population it should not be based on too few families. The average of the selected $3x$ -crosses can be used to predict when commercial seed with satisfactory yielding capacity

can be expected from crossing the CMS population and the 4x population. Another advantage of this method is that selection for flowering time can be made by eliminating those tetraploid families in which flowering does not coincide with that of the CMS-population.

Summing up, the proposed system retains the advantages of the \underline{S}^f gene is development of O-types, especially inbred type O lines. At the same time it greatly facilitates gene introduction and continuous improvement of mono-germ populations by the use of recurrent selection based on S_1 -testing. Whether the final goal is varieties based on inbreds or populations, the possibilities for mutual test-crossing in the evaluation of the result of each cycle of selection should also be useful. This applies not the least to the development of triploid hybrids.

REFERENCES

- Bosemark, N.O. 1969. Polyploidy and hybrid breeding in sugar beet. Sveriges Utsädesförenings tidskrift. Suppl, 1969 : 107-115.
- Doggett, H. and Jowett, D. 1963. Sorghum breeding research. Record of Research, Ann. Rep. E. Afr. Agric. For. Res. Org. : 33-36.
- Doggett, H. and Jowett, D. 1964. Sorghum breeding research. Record of Research, Ann. Rep. E. Afr. Agric. For. Res. Org. : 89-91.
- Doggett, H. and Eberhart, S.A. 1967. Recurrent selection in Sorghum. African Soils (2/3) : 119-122.
- Gilmore, E.C. Jr. 1964. Suggested method of using reciprocal recurrent selection in some naturally self-pollinated species. Crop Science 4 : 323-325.
- Owen, F.V. 1945. Cytoplasmically inherited male-sterility in sugar beets. Jour. Agr. Res. 71 : 423-440.
- Owen, F.V. 1950. The sugar beet breeder's problem of establishing male-sterile populations for hybridization purposes. Proc. Amer. Soc. Sugar Beet Tech. : 191-194.
- Owen, F.V. 1952. Mendelian male sterility in sugar beets. Proc. Amer. Soc. Sugar Beet Tech. : 371-376.
- Owen, F.V. 1954. Hybrid sugar beets made by utilizing both cytoplasmic and Mendelian male sterility. Proc. Amer. Soc. Sugar Beet Tech. VIII(2) : 64.
- Savitsky, H. 1952. Selective fertilization studies and recovery of self-sterile from self-fertile races of monogerm sugar beets. Proc. Amer. Soc. Sugar Beet Tech. : 339-343.

Savitsky, H. 1954. Self-sterility and self-fertility in monogerm beets.
Proc. Amer. Soc. Sugar Beet Tech. VIII(2) : 29-33.

Savitsky, V.F. 1950. Monogerm sugar beets in the United States. Proc.
Amer. Soc. Sugar Beet Tech. : 156-159.

Theurer, J.C. and Ryser, G.K. 1969. Double-cross sugar beet hybrids
utilizing pollen restorers. Crop Science 9 : 610-612.

GENERAL AND SPECIFIC COMBINING ABILITIES IN
WHITE CLOVER (*Trifolium repens*).

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SUMMARY

The heritable and non-heritable variation between single crosses for three white clover (*Trifolium repens*) characters, (a) legume yield, (b) mixed herbage yield and (c) magnesium content of the legume component, was investigated. The ten parents of the diallel set of crosses were selected from evolutionary distinct populations of white clover.

Genetic variation was predominantly additive for all characters. Legume yield and yield of mixed herbage showed positive genetic correlation. There was evidence that the progeny of some parents formed a more efficient association with the grass component of the sward than others.

The implications of the results regarding the breeding of this species and improvement of sward productivity is discussed.

1 - INTRODUCTION

Increase in yield, particularly in early Spring and late Autumn, and improved persistency under high levels of nitrogen are the major objectives in the selection of white clover (*Trifolium repens*) in Ireland. Breeding programmes with similar objectives are in progress in U.K. (Davies and Evans, 1968 ; Davies, 1970) and New Zealand (Barclay, 1960, 1964). Ecotypes and varieties introduced from areas where adaptation to long periods of winter dormancy does not occur have provided desirable genetic variation, in respect of some of the characters concerned. Hybridization of adapted varieties with these ecotypes and selection in subsequent generations is the general pattern adopted in these breeding programmes. Response to selection will depend on the extent to which the variation expressed by the characters concerned is of the additive genetic type.

The purpose of the work described in this paper was to estimate the degree to which the observed variation was genetic and to partition this component into

additive and non-additive fractions.

The problem of evaluation of single plants or families (where seed supply is a limiting factor) of white clover and of pasture species in general has been the subject of much research (Atwood and Garber, 1942 ; Myers and Garber, 1942 ; Ahlgren et al, 1945 ; Wilsie, 1949 ; Wright, 1960 ; Davies, 1961, 1970 ; Lazenby and Rogers, 1962 and others). These experiments showed that for such characters as yield, the performance of widely spaced plants is an unreliable guide to productivity under normal pasture conditions. In the investigation of the genetic control of productivity and in selection for this character it is, therefore, desirable that measurements are made on grass/clover swards. Methods of evaluating individual plants and families in simulated pasture conditions have been described by Atwood and Garber (1942), Gibson (1964), Davies (1958, 1969). These techniques were employed in the present study.

2 - MATERIAL AND METHODS

During the period 1961 - 63 approximately 180 ecotypes and varieties introduced from a range of climatic regions were evaluated as spaced plants. Variation between populations was observed for such characters as seasonal growth pattern, leaf size, petiole length and density. Within populations the plants showed a high degree of uniformity for these characters.

One genotype was selected from each of the following ten populations.

| <u>Parent</u> | <u>Population</u> | <u>Major Characteristics</u> |
|---------------|-----------------------------|--|
| 1 | Ecotype France | Vigorous spring and mid-summer, long petiole, medium-large leaf. |
| 2 | Ecotype France | Similar in most respects to parent 1. |
| 3 | Ecotype France | Poor growth, short petiole, small leaf, prostrate. |
| 4 | Ecotype Belgium | Dense, erect growth habit, medium leaf size. |
| 5 | Variety "Pilgrim" Canada | Ladino clover, large leaf, good establishment, poor persistency. |

| <u>Parent</u> | <u>Population</u> | <u>Major Characteristics</u> |
|---------------|---------------------------------------|---|
| 6 | Variety "S100" U.K. | Vigorous adapted variety medium leaf size. |
| 7 | Variety "Crau" France | Good early spring growth medium-large leaf size. |
| 8 | Variety "Kentish Wild White". U.K. | Prostrate growth habit, small leaf size. |
| 9 | Ecotype Israel | Very good Autumn growth, poor winter hardiness, medium-large leaf size. |
| 10 | Ecotype Ireland | Good spring growth, medium-leaf size. |

These 10 parents were diallel crossed by hand pollination. The plants were not emasculated, tests of self fertility showed that all parents were highly self sterile. In April 1964, seeds from each of the 45 single crosses (equal amounts of seed from reciprocal crosses were bulked to provide sufficient plants for field plot establishment) were sown individually in 2.5 cm² peat pots in the glasshouse. At the same time the experimental area in the field was sown with perennial ryegrass. Parent 4 had died during winter, the remaining nine parents were clonally propagated to give 160 propagules per parent. Field plots were established by transplanting 5 - week old clover seedlings and parent clones into the newly sown ryegrass sward. A randomized block design with four replications was used.

Each plot representing a single family or parent consisted of 39 seedlings (or propagules) in three rows spaced 30 cm apart within and between rows. Within a short period (5 - 6 weeks) these plants had spread and formed a good grass/clover sward. This technique is similar to that described by Davies (1958) for the evaluation of families under simulated sward conditions.

Yield was estimated from an area 3.6m x 0.9m cut through the centre of each plot (overall plot size was 4.6m x 1.8m). This herbage was sub-sampled for dry matter determination and for separation into grass and clover components. During the period 1964 - 67 eighteen cuts were taken, two in the establishment year 1964, six in 1965 and 1966, and four in 1967.

3 - ANALYSIS OF DATA

From field observation and examination of treatment means it was apparent that single cross families grown from seed were in general more vigorous than

the parent plots which were established from clones of the parent genotypes. Similar difficulties in comparing vegetatively propagated parents with progeny established from seed have been recorded in other experiments (Fejer, 1969 ; Cooper and Wilson, 1969 ; Connolly, 1970). The diallel analysis was, therefore, based on the set of 45 F₁ family means following Griffing (1956) Method 4 Model 1. Of the characters measured only the results for dry matter yield of (a) legume yield (b) mixed herbage yield (i.e. grass + legume) and (c) magnesium content of the legume dry matter will be presented here.

4 - RESULTS

1) Estimates of combining abilities for characters (a) and (b) :

Preliminary analyses showed that significant differences between crosses were present for each cut in all years. Partitioning of this between family variation showed that in all 36 diallel analyses (both characters) the general combining ability effect (g.c.a.) was highly significant. The specific combining ability (s.c.a.) mean square was significant only in 11 of these analyses. Estimates of the parameter, g_i , measuring the accumulative additive gene effects of the i^{th} parent are shown graphically in figs. 1 and 2 for each of the 18 cuts. (Note, the number given to each cut indicates the month in which the yields were measured as follows : 1 April, 2 May, 3 June, 4 July 5 August - September, 6 November). The estimates of g_i for both characters, legume and mixed herbage dry matter yield are shown together for each parent. The corresponding values for accumulated total yield over all cuts in each year are given in table 1.

The main features of these results are summarized as follows :

- Average performance : Parent 6, (derived from the variety S100) although outranked at certain periods of the year, showed very high average level of general combining ability. Parent 3, 5 and 8 were poor at all harvests.
- Seasonal differences : Parent 10 showed the highest level of g.c.a. for early growth particularly in the first 2 harvest years. Parent 1 and 2 were rather disappointed in this respect. The populations from which these two parents were selected had shown good early growth. The most striking seasonal effects were shown by parent 9 derived from the Israeli population. This parent had the highest values of g.c.a. for the two cuts in the September-November period. At other cuts the \hat{g}_i values for this parent were mainly negative. This oscillation from positive to negative values with changing season of growth is clearly shown in fig. 5. The superiority of this parent in late Autumn growth decreased as the experiment progressed. This was probably due to poor persistency.
- Repeatibility : Repeatibility over years, although not analysed statistically, appeared to be very good.
- Relationship between legume and mixed herbage yield : The correlation coefficient between the 18 pairs of \hat{g}_i values for each parent is given in

figs. 1 and 2. The correlations ranging from 0.46 - 0.79 are all positive and differ significantly from zero. Comparison of these r values indicates that they do not differ significantly from each other. Nevertheless, there is evidence that the rank order of parents based on \hat{g}_i values is not the same for both characters. For legume yield the progeny of parents 1 and 2 were similar to those of parent 6 in the three years 1964 - 66 but much poorer in terms of yield of mixed herbage (Table 1, figs. 1 and 2).

- Genetic Control : The genetic differences between the parents are mainly additive in nature. The s.c.a. effects, where these were significant, were relatively small and estimates of s_{ij} are not presented here.

2) Components

Estimation of g.c.a. and s.c.a. variance components is not relevant in the strict statistical sense where, under Model 1 assumptions (Eisenhart, 1947), the g_i and s_{ij} effects are regarded as constants. It is, however, possible to calculate an average of "squared fixed effects" (Wearden, 1964) which estimates the relative contributions of additive and non-additive gene effects to the total phenotypic variation. Estimates of these components and of error variance were expressed as a percentage of the total phenotypic variation. This data is summarized in tables 2 and 3 where :

$$\hat{\sigma}_g^2 = \frac{1}{9} \sum g_i^2 ; \hat{\sigma}_s^2 = \frac{1}{35} \sum_{i < j} s_{ij}^2 ;$$

$$\hat{\sigma}_e^2 = \text{error variance (see Griffing 1956)}$$

$$\text{and } \hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \hat{\sigma}_e^2 = \text{total phenotypic variance}$$

With regard to legume yield these results show that for most cuts the additive genetic component accounted for more than half the total variation. In the case of mixed herbage yield the additive contribution was almost invariably less than for legume yield. Nine of the eleven analyses (considering both characters) in which non-additive effects were significant occurred in the first three cuts of 1965 and 1966. With the exception of the second cut in 1965 and the last cut in 1967, the results were in good agreement over years.

3) Magnesium content of legume (g/100g dry matter)

This character was measured for ten cuts. In all analyses highly significant g.c.a. effects were found but only in one cut (first cut 1966) were the s.c.a.

FIGURE I

Estimates of $g_i \times 1000$ for parents 1 - 5 for each cut 1964-67

- Legume dry matter yield.
- - -■ Mixed herbage dry matter yield.

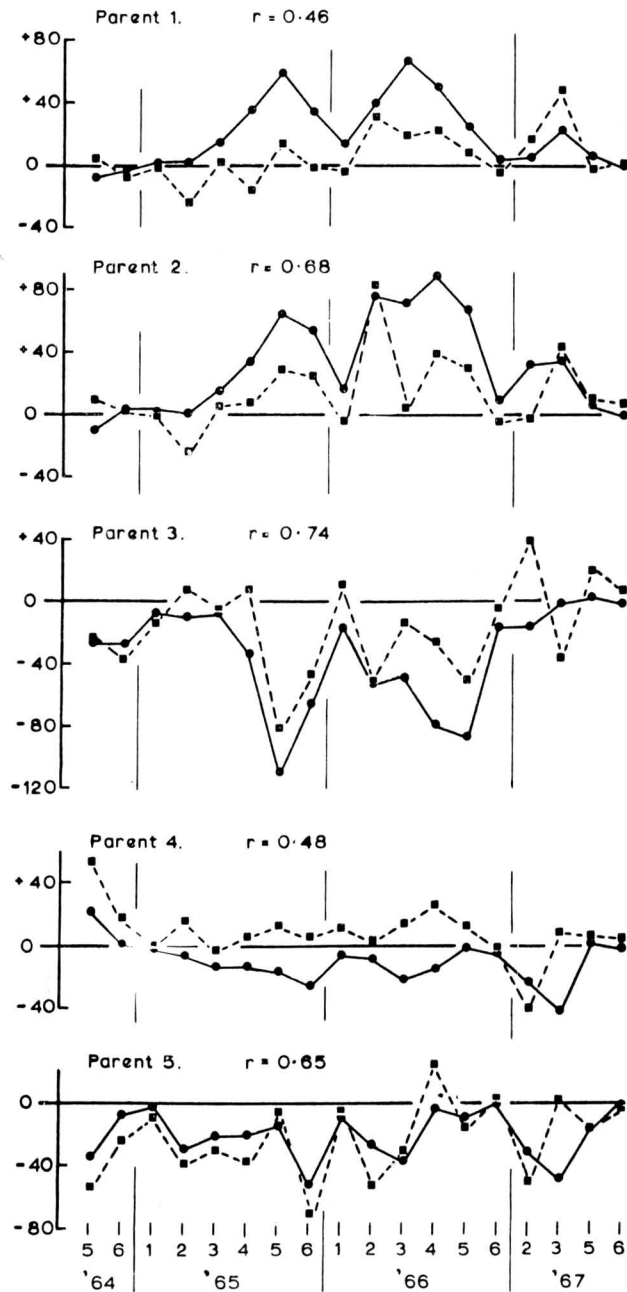


FIGURE II

Estimates of $g_i \times 1000$ for parents 6 - 10 for each cut 1964-67.

- Legume dry matter yield.
- - -■- - Mixed herbage dry matter yield.

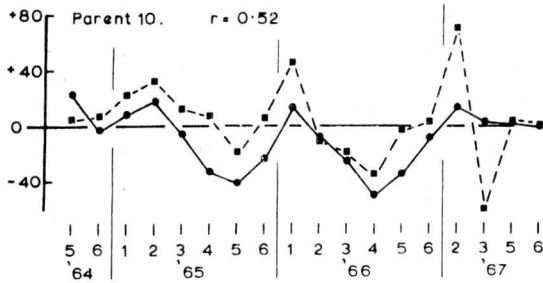
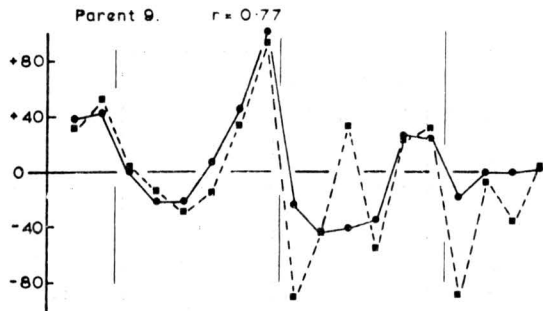
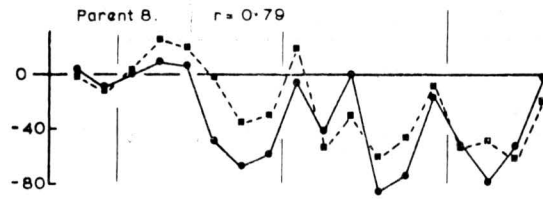
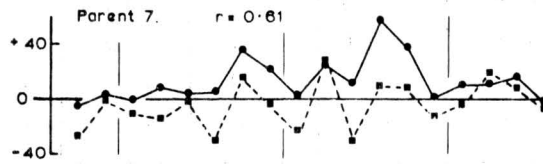
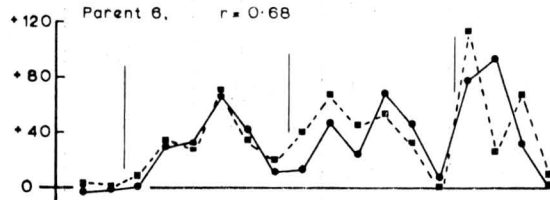


Table 1. Estimates of $g_i \times 1000$ for accumulated dry matter yield of legume and mixed herbage in each year - g_i (L) and g_i (M) respectively.

| Parent No. | 1964 | | 1965 | | 1966 | | 1967 | |
|------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | g_i (L) | g_i (M) | g_i (L) | g_i (M) | g_i (L) | g_i (M) | g_i (L) | g_i (M) |
| 1 | - 12 | - 4 | 144 | - 28 | 203 | 73 | 35 | 65 |
| 2 | - 5 | 14 | 168 | 43 | 334 | 149 | 75 | 59 |
| 3 | - 53 | - 62 | - 231 | - 129 | - 305 | - 133 | - 12 | 29 |
| 4 | 24 | 69 | - 75 | 39 | - 53 | 65 | - 63 | - 19 |
| 5 | - 41 | - 69 | - 138 | - 190 | - 85 | - 78 | - 95 | - 68 |
| 6 | - 5 | 0 | 182 | 196 | 231 | 239 | 206 | 219 |
| 7 | - 2 | - 29 | 76 | - 44 | 147 | - 13 | 40 | 17 |
| 8 | - 7 | - 15 | - 156 | - 17 | - 313 | - 178 | - 187 | - 182 |
| 9 | 81 | 83 | 112 | 74 | - 52 | - 102 | - 16 | - 132 |
| 10 | 20 | 14 | - 83 | 56 | - 107 | - 21 | 17 | 13 |
| S.E. \hat{g}_i | 8.6 | 16.8 | 30.7 | 35.9 | 41.2 | 41.0 | 26.8 | 36.3 |

S.C.A. effects not significant.

Table 2. Estimates of components σ_g^2 , σ_s^2 , and σ_e^2 expressed as percent of the total phenotypic variance.

| Date of cutting | Legume yield | | | Mixed herbage yield | | |
|-----------------|--------------------|--------------------|--------------------|---------------------|--------------------|--------------------|
| | $\hat{\sigma}_g^2$ | $\hat{\sigma}_s^2$ | $\hat{\sigma}_e^2$ | $\hat{\sigma}_g^2$ | $\hat{\sigma}_s^2$ | $\hat{\sigma}_e^2$ |
| 16/9/64 | 45.9 | - | 54.1 | 30.1 | - | 69.9 |
| 3/11/64 | 75.6 | 14.1 | 10.3 | 71.7 | - | 28.3 |
| Total 1964 | 63.9 | - | 36.1 | 45.7 | - | 54.3 |
| 8/4/65 | 62.3 | - | 37.7 | 37.3 | 23.5 | 39.2 |
| 12/5/65 | 28.9 | 34.7 | 36.4 | 37.2 | - | 62.8 |
| 9/6/65 | 19.8 | 29.7 | 50.5 | 15.7 | 29.1 | 55.2 |
| 15/7/65 | 48.4 | - | 51.6 | 32.8 | - | 67.2 |
| 25/8/65 | 81.8 | - | 18.2 | 55.7 | - | 44.3 |
| 1/11/65 | 80.8 | - | 19.2 | 81.4 | - | 18.6 |
| Total 1965 | 72.8 | - | 27.2 | 47.8 | - | 52.2 |

Table 3. Estimates of components, σ_g^2 , σ_s^2 , σ_e^2 expressed as percent of total phenotypic variance

| Date of cutting | Legume yield | | | Mixed herbage yield | | |
|-----------------|--------------------|--------------------|--------------------|---------------------|--------------------|--------------------|
| | $\hat{\sigma}_g^2$ | $\hat{\sigma}_s^2$ | $\hat{\sigma}_e^2$ | $\hat{\sigma}_g^2$ | $\hat{\sigma}_s^2$ | $\hat{\sigma}_e^2$ |
| 20/4/66 | 66.2 | 17.4 | 16.4 | 57.8 | 16.3 | 26.0 |
| 16/5/66 | 71.8 | 10.6 | 17.6 | 73.9 | 7.8 | 18.3 |
| 4/7/66 | 25.3 | 44.3 | 30.4 | 18.0 | - | 82.0 |
| 17/8/66 | 67.8 | - | 32.2 | 47.2 | - | 52.8 |
| 20/9/66 | 75.9 | - | 24.1 | 49.0 | - | 51.0 |
| 2/11/66 | 61.9 | - | 38.1 | 41.9 | - | 58.1 |
| Total 1966 | 68.3 | 10.0 | 21.7 | 50.7 | - | 49.3 |
| 2/5/67 | 67.7 | - | 32.3 | 43.8 | - | 56.2 |
| 20/6/67 | 60.8 | - | 39.2 | 35.9 | - | 64.1 |
| 27/8/67 | 33.1 | - | 66.9 | 43.7 | - | 56.3 |
| 23/11/67 | 16.0 | - | 84.0 | 14.9 | 29.5 | 55.6 |
| Total 1967 | 61.6 | - | 38.4 | 48.5 | - | 51.5 |

Table 4. Estimates of $g_i \times 1000$ for Magnesium content of legume dry matter. Cut numbers as in figs. 1 and 2.

| Parent No. | Year and cut number | | | | | | | | | |
|------------------|---------------------|------|------|------|------|------|------|------|------|------|
| | 1964 | | 1965 | | | | | | 1966 | |
| | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 6 |
| 1 | 9 | 14 | - 5 | - 1 | - 15 | - 7 | 13 | - 6 | - 2 | 5 |
| 2 | 2 | - 10 | 1 | - 10 | 18 | 2 | - 9 | - 8 | 9 | - 5 |
| 3 | - 21 | - 6 | - 6 | - 21 | - 12 | - 15 | - 29 | - 14 | - 18 | - 21 |
| 4 | 7 | - 3 | 7 | 7 | 2 | 2 | - 7 | 2 | 4 | 2 |
| 5 | 7 | 0 | 16 | 8 | 15 | 22 | 15 | 13 | 28 | 34 |
| 6 | - 1 | 9 | 4 | 14 | 6 | 27 | 19 | 18 | 23 | 27 |
| 7 | 5 | 15 | 10 | 9 | - 1 | - 2 | - 4 | - 1 | - 8 | - 8 |
| 8 | 9 | 20 | 7 | 17 | 18 | 11 | 21 | 16 | 17 | 22 |
| 9 | - 6 | - 16 | - 22 | - 19 | - 13 | - 27 | - 11 | - 13 | - 38 | - 42 |
| 10 | - 9 | - 22 | - 12 | - 4 | - 18 | - 13 | - 8 | - 7 | - 16 | - 14 |
| S.E. \hat{g}_i | 4.3 | 5.8 | 4.0 | 4.6 | 7.0 | 5.2 | 6.7 | 3.8 | 3.2 | 4.5 |

effects significant. Estimates of the \hat{g}_i values are listed in table 4. Parents 3, 9 and 10 had consistently negative estimates of g_i for all periods of the year while parents 5, 6 and 8 had positive values. The values for parent 4 did not differ significantly from zero, i.e. this parent was average ($\hat{g}_i = 0$) for the group. The sign of the estimates for parents 1, 2 and 7 varied with season and repeatability over years for corresponding cuts was much poorer than for the other parents.

5 - DISCUSSION

Yield : It should be recalled that the parents of the diallel were selected from widely contrasting populations, the results and their interpretation have, therefore, a limited application. Genetic differences between the parents were predominantly additive. Non-additive genetic effects were relatively small and confined almost entirely to spring and early summer growth. Barclay (1960) also found s.c.a. effects present for summer growth but absent for winter production while g.c.a. effects were significant for both periods. Wilson and Cooper (1969), working with ryegrass, found that heritable variation for a number of characters related to growth rate was mainly due to additive gene effects.

Variation in productivity characters of populations of Lolium perenne derived from old pasture have been studied by Fejer (1958) and by Hayward and Breese (1966, 1968). They concluded that reciprocal differences and additive nuclear gene effects were present but found no evidence of dominance or epistasis.

Hayward and Breese (loc. cit.) have argued that, under conditions where sexual reproduction is absent or very infrequent (as occurs for persistent ryegrass populations such as those used in their study), adaptive response would be conditioned mainly by extra-nuclear variability. This would account for the cytoplasmic variation which they found and also for the absence of non-additive effects which might reasonably be expected to occur in crosses between differentiated populations where sexual reproduction is the rule rather than the exception (Breese and Mather, 1960). No information on reciprocal differences is available from the present study and comparisons with the results obtained by Hayward and Breese (loc. cit.) are limited in this respect.

Assuming that the additive genetic variation measured was due to nuclear gene effects and allowing for the fact that realised response to selection is frequently less than the predicted value, the results indicate that substantial improvement in productivity should be achieved by selection among the progeny of these crosses. In particular, it should be possible to introduce the desirable characteristics of Israeli ecotypes into existing adapted varieties.

With regard to pasture productivity the desired objective is to improve the yielding ability of the grass/clover sward rather than change one particular

component of it. Because the additive genetic effects governing yield of the clover component are correlated with those controlling mixed herbage yield, improvement of the desired character (i.e. yield of mixed herbage = character b) can be achieved by direct selection or, indirectly, by correlated response to selection for legume yield (= character a).

The conditions under which correlated response is expected to exceed direct response have been discussed by Lerner and Cruden (1948) and Falconer (1960 a, b,). Taking as an example, the accumulated yield of mixed herbage over all cuts in each year as the desired trait (b) and assuming that the intensity of selection is the same for both characters then the ratio of correlated to direct response for character (b) is :

$$\frac{h_a r_g}{h_b} = \frac{0.82 \cdot 0.65}{0.69} = 0.77$$

where h_a and h_b is the square root of the heritability for characters (a) and (b) ; (the average values of $h^2 =$

$$\frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \hat{\sigma}_e^2}$$

are taken from tables 2 and 3 ; $h^2 = 0.67$ and $h_b^2 = 0.48$). r_g is the combined estimate over all parents of the correlation between the additive genetic effects for both characters (see figs. 1 and 2 for individual values). This ratio is less than 1.0. In the absence of other compensating factors (e.g. different selection intensities) direct selection should be more efficient.

As pointed out earlier there was strong evidence (table 1) that the progeny of some parents (e.g. no. 6) formed a more efficient association with the companion grass than others, e.g. parents 1 and 2. The array means for legume yield of these three parents were similar for most cuts but they differed considerably for the amount of grass present. A comparable result was reported by Myers and Garber (1942) for their evaluation of individual grass plants in association with white clover. This situation would also favour direct selection for yield of mixed herbage.

Magnesium : Genetic differences between the parents for this character were additive, accounting for 40 - 50 % of the observed variation. Differences between natural populations of white clover for a number of minerals including Mg have been recorded by Snaydon and Bradshaw (1969), the present results bear out their findings.

The high level of additive gene effects indicates that selection among the progeny of these single crosses should be effective in changing the Mg content.

The low level of Mg content in the progeny of the Israeli parent highlights a danger that the use of such genotypes as a source of some desired agronomic character (e.g. late Autumn growth) might inadvertently result in a variety with reduced Mg levels if counter selective measures were not applied. In view of the relationship between the Mg content of the herbage and the occurrence of hypomagnesaemia in the grazing animal, any decrease would be undesirable.

REFERENCES

- Ahlgren, H.L., Smith, D.C., and Nielsen, E.L. 1945. Behaviour of various selections of Kentucky bluegrass, Poa pratensis L., when grown as spaced plants and in mass seedings. *J. Am. Soc. Agron.*, **37**, 268 - 281.
- Atwood, S.S., and Garber, J.R. 1942. The evaluation of individual plants of white clover for yielding ability in association with bluegrass. *J. Am. Soc. Agron.*, **34**, 1 - 6.
- Barclay, P.C. 1964. Extending the season of pasture growth. *N.Z. J.L. Agric.*, **108**, 437.
- Barclay, P.C. 1960. Breeding for improved winter pasture production in New Zealand. *Proc. 8th Int. Grassld. Congr.*, Reading, 326-330.
- Breese, E.L., and Mather, K. 1960. The organisation of polygenic activity within a chromosome in *Drosophila*. II. Viability. *Heredity*, **14**, 375-401.
- Connolly, V. 1970. Variation in seasonal productivity of populations of white clover (Trifolium repens) and their potential in breeding improved varieties. "White Clover Research". *Occ. Symp. No. 6*, 133-138. *Brit. Grassld. Soc.*
- Davies, W.E. 1958. *Rep. Welsh Pl. Breed. Stn. for 1950 - 56.* 53 - 54.
- Davies, W.E. 1961. *Rep. Welsh Pl. Breed. Stn. for 1960.* 31.
- Davies, W.E. 1969. Herbage legumes - special considerations. "Grass and forage breeding". *Occ. Symp. No. 5.* 21-27. *Brit. Grassld. Soc.*
- Davies, W.E. 1970. "White Clover Research" *Occ. Symp. No. 6*, 99 - 122. *Brit. Grassld. Soc.*
- Davies, W.E., and Evans, D.R. 1968. *Rep. Welsh Pl. Breed. Stn. for 1967*, 43 - 44.

- Eisenhart, C. 1947. The assumptions underlying the analysis of variance. *Biometrics* 3, 1 - 21.
- Falconer, D.S. 1952. The problem of environment and selection. *Am. Nat.*, 86, 293 - 298.
- Falconer, D.S. 1960a. Selection of mice for growth on high and low planes of nutrition. *Genet. Res.*, 1, 91 - 113.
- Falconer, D.S. 1960b. *Introduction to Quantitative Genetics*. Oliver and Boyd, Edinburgh and London.
- Fejer, S.O. 1958. Genetic and environmental components of the productivity of perennial ryegrass (*Lolium perenne* L.). *N.Z. J.L. Agric. Res.*, 1, 86 - 103.
- Gibson, P.B. 1964. A technique requiring few seed for evaluating white clover strains. *Crop Sci.* 4, 344 - 345.
- Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. biol. Sci.*, 9, 363 - 393.
- Hayward, M.D., and Breese, E.L. 1966. The genetic organisation of natural populations of *Lolium perenne*. I. Seed and seedling characters. *Heredity*, 21, 287 - 304.
- Hayward, M.D., and Breese, E.L. 1968. The genetic organisation of natural populations of *Lolium perenne* L. III. Productivity. *Heredity*, 23, 357 - 368.
- Lazenby, A., and Rogers, H.H. 1962. Selection criteria in grass breeding. I. *J. agric. Sci., Camb.* 51, 51 - 62.
- Lerner, I.M., and Cruden, D.M. 1948. The heritability of accumulative monthly and annual egg production. *Poultry Sci.*, 27, 67 - 77.
- Myers, W.M., and Garber, R.J. 1942. The evaluation of individual plants of pasture grasses in association with white clover. *J. Am. Soc. Agron.*, 34, 7 - 15.
- Snaydon, R.W., and Bradshaw, A.D. 1969. Differences between natural populations of *Trifolium repens* L. in response to mineral nutrients. II. Calcium, magnesium and potassium. *J. Appl. Ecol.*, 6, 185 - 202.
- Wearden, S. 1964. Alternative analysis of the diallel cross. *Heredity*, 19, 669 - 680.

- Wilsie, C.P. 1949. Evaluation of grass-legume associations with emphasis on the yield of brome grass varieties. *Agron. J.*, 41, 412 - 420.
- Wilson, D., and Cooper, J.P. 1969. Diallel analysis of photosynthetic rate and related leaf characters among contrasting genotypes of Lolium perenne. *Heredity*, 24, 633 - 649.
- Wright, C.E. 1960. The introduction of an associated legume at the progeny testing stage in perennial ryegrass breeding. *Proc. 8th Int. Grassland Congr.*, Reading, 313-317.

THE METHODS OF ALFALFA BREEDING IN THE AREA OF SLAVONIJA

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In the area of Slavonija several ecotypes of Panonia alfalfa have been formed by the natural selection and by the growing manners. These ecotypes do not differ very much in consideration of the morphological and physiological properties.

As the result of the manner of exploitation and seed production there has been formed an alfalfa of robust growth, with tall stalks, resistant to low temperatures and with good longevity, because the seed was produced in three, four or five years old alfalfa stands, when there remained only by the natural selection preferred types. Such alfalfa in comparison to the foreign varieties which have more leaves and a lower content of crude fiber and a higher protein content, could not satisfy the demand of practice for a high quality forage. Therefore the selection in this area has been orientated to the improvement of the qualitative properties of existing well adapted ecotypes of Panonia alfalfa.

The primary task of alfalfa breeding on the Agricultural Institute Osijek, has been the quality improvement of Panonia alfalfa, and in the later work the utilization of heterotic effect of the hybrid combinations of alfalfa.

I IMPROVEMENT OF QUALITATIVE PROPERTIES OF ALFALFA

In 1956, we started sampling the ecotypes and provenances of Panonia alfalfa. On that occasion we gathered about 50 different ecotypes originating from 10 different sources in the area of Slavonija, Vojvodina, Northern Bosnia and Serbia. These ecotypes were examined on the experimental field of Agricultural Institute Osijek. On the basis of visual observations and evaluations of the following characters : growth habit, general appearance, plant height, the leaf - stalk ratio, branching, recovery rate and resistance to diseases, there were selected 22 ecotypes and the next year they were put into the comparative trials for the green and dry matter yields examinations in a dense stand. After the three years' examination the 10 highest yielding ecotypes were chosen

In 1960 a nursery was established with 10 selected ecotypes of Panonia alfalfa together with 19 foreign varieties which had to serve as a source of germ

plasm for leafiness and a good quality. Those were the following varieties Flamande, Provence, Du Puits, Poitou, Gamma, Omega, FD 100, Emeraude, Cardinal, Orchesienne, Chalans, Marais de Luçon of French origin, Saint Luis, N 27, Rancher and Barsen of Minneapolis of American origin, Polesana and Romagnolo from Italy and Gebrüder Herrnfeld from Austria.

The 29 domestic and foreign ecotypes and varieties were sown in a common nursery. Each of them was represented by about 1.500 - 1.800 plants. The plants were spaced at 50 x 30 cm without repetition. In that way we obtained a large population of each ecotype or variety, enclosing the whole existing variability. During the three years' examination the following characters were analysed : Growth habit and general appearance, the rate of recovery, leafiness, height of plants and state of health. On the basis of these observations we selected 25 plants which were superior in the most of examined properties. Those plants were vegetative propagated in 1962 in a polycross nursery 25 x 25, with a distance of 80 cm between the plants. During the three years of polycross pollination we evaluated and measured the above mentioned characters. The seed, harvested at the clones in polycross nursery in 1962, was sown in 1963 in the comparative trials for progeny testing during the three years. Each offspring was sown in dense stand on the plots of 3 m² in three randomized blocks. On the basis of the obtained yields, leafiness and chemical composition in comparison to the Panonia alfalfa, 15 superior clonal offsprings were chosen. Eight of them originated from ecotypes, of Panonia alfalfa from Vojvodina and Slavonija, four clones were derived from the Italian variety Polesana, two of them originated from the French variety Omega, and one from the other French variety Du Puits.

The seed from those 15 clones obtained by polycross pollination was sown separately in a new nursery in proper isolation, destined for breeder seed production of the new synthetic variety. This new synthetic variety was registered in the Yugoslav official catalogue of species and varieties, after three years of examination at 4 locations, under the name : Osječka 66.

This new synthetic variety was superior to the others, in green and dry matter yields, quantity of leaves and the high content of carotene.

On the plants of those 15 offsprings which were sown in 15 repetitions in further three years we accomplished the additional phenological observation which was a basis for selection of 10 best plants, that vegetative propagated into three different polycross nurseries. Those were the following clones :

3/7 - originating from the Panonia alfalfa from Vojvodina

6/1 - originating from a Slavonian ecotype of Panonia alfalfa

6/10 - of the same origin

10/2 - derived from the Italian variety Polesana

10/13 - derived from the Italian variety Polesana

12/11 - derived from the Italian variety Polesana

13/13 - originating from the French variety Omega

14/11 - originating from the French variety Omega

15/14 - originating from the French variety Du Puits

65/6 - originating from the French variety Du Puits.

Polycross nursery I - contained the following clones : 3/7, 6/1, 10/2, 13/13, 15/14.

Polycross nursery II - composed of the clones : 3/7, 6/10, 10/13, 14/11 and 15/14.

Polycross nursery III - composed of the clones : 6/7, 6/1, 12/11, 14/11 and 65/6.

At the same time those clones were planted in the rows spaced at 50 cm between each other in 3 repetitions destined for open pollination.

In 1968 the offsprings of each clone from every polycross nursery and row were sown into a comparative trial together with the best domestic and foreign varieties. On the basis of the results obtained during the two years of examination it seems that we obtained polycross offsprings, which according to the growth habit, green matter yield and quantity of leaves were superior to the check varieties, for example : the progeny of clone No 3/7 from all three polycross nurseries, gave significantly greater yields of green matter than the most of check varieties. The offspring of clone No 14/11 from the polycross nursery III, and offspring of clone No 13/13 from polycross nursery I had a better leaf - stalk ratio than the other varieties with yield on the level of standard varieties.

Each year in addition to this ordinary programme of selection there were carrying out comparative examinations of domestic and foreign populations and varieties, we had obtained during the preceding year, as well as our own selection material. For that purpose the variety trials were carried out on the plots of 10 m² in dense stand. The same populations and the additional ones were tested separately in a nursery with the isolated plants spaced at 50 cm between and 30 cm within the rows in 3 repetitions. Each population or variety was represented by 120-150 plants. In that nursery we analysed the above mentioned characters on the individual plants, which have served us as a base for the further selection.

In addition to yield and quality improvement in a separate selection programme we are working on seed yield increasing.

In 1966 when the seed production was very bad and no pollination occurred, because of the unfavorable weather conditions, we selected 15 plants out of a stand of our new synthetic variety, which were normally fertilized with good seed setting. The seed from those plants was sown the next year in rows with a distance of 50 cm between them in 3 repetitions together with several standard varieties.

After three years of examination we noticed that several offspring of the selected plants were superior in green matter yield and especially in longevity to the check varieties, so that in the fourth year of vegetation they had 80-90% of the initial stand density in comparison to the check varieties in which the stand was reduced to only 40-50%. The seed yield varied largely,

depending on the climatic conditions in different years and it was higher on the selected plants.

The additional programme of alfalfa breeding encloses the selection of resistance to the acid soils. For that purpose we established our best selection material and the foreign resistant varieties in a nursery on acid soil in order to examine the plant reaction to the new soil condition. The first year we found very large variability between and within the populations and varieties. The further examinations and selection of more resistant genotypes are in the course.

II GENETIC BASE OF SELECTION

The material gathered at the beginning of our selection programme originates from different regions of the large area which has different edaphic and climatic conditions. Thus among the ecotypes adapted to these regions existed an important variability of all the characters. On the basis of phenological observation by means of individual selection we tried to enclose the positive variation of most of the examined properties. In such a way we moved the means of selected plants in the direction of selection. However in those ecotypes the existing variability of several characters was not big enough, so we had to use some foreign varieties. In that period the aim of selection was to accumulate in a population as much as possible of gene plasm for the characters which we wanted to improve in the Panonia alfalfa. That was the reason, why we took 15 parental clones originating from 7 different populations and varieties. Such material could also serve as a new initial population for the second cycle of selection in which we reduced the number of parental components, with intention to use only those ones with the best combining ability for a particular character. In the above described selection programme we haven't utilized the heterotic effects of hybrid populations, but we have used on the general combining ability basis of the parents the additive action of genes.

III THE HETEROSIS UTILIZING IN THE SELECTION PROGRAMME

In our breeding programme we began in 1967 with preparations for selfing and hand crossing of alfalfa. Those preparations consisted in finding out the best methods of selfing and crossing. The selfing was carried out in two ways. The first way was consisted in eliminating already tripped flowers of isolating the remain flowers of raceme by a paper bag, and squashing the flowers in the bag between the fingers. Using the second method we eliminated the already tripped flowers, and the remaining ones were tripped by a sharp wooden object (toothpick). The second method was more efficient in podsetting.

The crossing was also carried out on two ways : with emasculation and without emasculation. The emasculation was made by eliminating the standard petal with scissors, tripping the flowers and plunging them into a 57% alcohol for 10 seconds. After that the flowers were rinsed with water and left to

dry up for an hour. The pollen from the male plants was transported by a sharp wooden object (toothpick) into the stigma of the emasculated female flower. After that the whole raceme was isolated with a paper bag. The hand crossing without emasculation was accomplished with a toothpick, by tripping of several male flowers and transporting the pollen to the female flowers by tripping them with the same toothpick. Comparing the results we've noticed that there were no significant differences between these two methods of crossing neither with regard to pod setting, nor to the percentage of selfing.

In 1969 our co-worker was in Lusignan, France on specialism where he learned more thoroughly about genetics of tetraploids and got acquainted with problems of use of heterosis of alfalfa. This year in the Agricultural Institute in Osijek we've continued and enlarged the researching of the use of heterosis of alfalfa applying the new knowledge and experiences.

A nursery of alfalfa with 110 various populations and hybrid combinations domestic and foreign origin has been established. Alfalfa has been sown separately spaced at 60 x 60 cm with 20 plants in a plot in four randomized blocks. All the characters are being analysed separately on each plant. In the breeding programme the following is included :

- analysing the phenotypic variability of some characters between and within the populations,
- establishing and analysing genetic variability of analysed characters of Panonia alfalfa using the analysis of covariance of relatives,
- finding out genetic correlations with particular characters,
- finding out the adequate genetic structure with the highest heterotic effects in commercial generation,
- studying the combining ability of selective material,
- using the self fertilization for expanding genetic variability.

Hand crossing pollination in polycross nursery will be used in our selection Programme until the breeding of insect pollinators for alfalfa fertilization will be perfected. It's the third year we have been examining the possibility of use the Megachile rotundata being obtained from the USA.

The purpose of the new improving programme is to get the high yielding variety resistant to frequent cutting using the heterotic effect of hybrid combination in order to improve the quality in an indirect way.

This short report was an essay to explain the methodic of our breeding programme which is specific in a way but which has given an improved variety on the basis of Panonia alfalfa. We've also described here our new programme of selection with the methodology which isn't worked out to its details but which will be improved using both ours and the knowledge of the others in theoretical and practical work.

BREEDING FOR INCREASED YIELD OF DRY MATTER
AND OF SEED IN TETRAPLOID RED CLOVER

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INTRODUCTION

Already in 1939 the first tetraploid plants of red clover were produced at Svalöf through colchicin treatment. During the following years the morphological differences between diploids and tetraploids were studied and it was found that certain gigas characters, such as higher plants, larger leaves and flowers, thicker stems etc. were typical for tetraploid red clover in the same way as for many other species. In 1941 a material of diploid and tetraploid red clover was planted as spaced plants in the field. It was found that the weight of green bulk of the tetraploid plants was about 60 per cent higher than that of corresponding diploid material and the best tetraploid families had a weight which surpassed the corresponding diploids with not less than 128 per cent. One therefore had reason to believe that breeding of polyploids considerably should increase the productivity of red clover.

BREEDING FOR INCREASED FORAGE PRODUCTION

In 1942 tetraploid red clover material was for the first time included in sown comparative variety trials. The results were very disappointing. Out of ten different tetraploid varieties only one gave a dry matter yield as high as the best diploid variety and most of the tetraploids gave much lower yield. The same poor results were repeated in trials during the following years. The tetraploids demonstrated, however, in many cases better regrowth than the diploids and also a better persistancy in the second harvest year. In order to improve the material, selections were made inside the existing populations but without any remarkable improvement with regard to yield. In 1947 a mass crossing was made among three varieties of different origin. This resulted in a new population clearly superior to the three basic varieties and for the first time a tetraploid variety was obtained, which was definitely higher yielding than the existing diploid ones. Also the seed setting, which in all previous material had been rather poor, was considerably improved but was still much lower than in diploid clover.

Table 1. Yield of green bulk from different 4 × red clover types in relation to 2 × Merkur (= 100)

| Basic 2x material | No of varieties | 1 st harvest year | | | | 2 nd harvest year | | | |
|--|-----------------|-------------------|----------|----------|-------|-------------------|----------|----------|-------|
| | | No of trials | 1 st cut | 2 nd cut | Total | No of trials | 1 st cut | 2 nd cut | Total |
| 1) Few plants from one variety | 5 | 20 | 91 | 96 | 92 | 18 | 96 | 108 | 97 |
| 2) Few plants from 2 or 3 varieties | 14 | 47 | 93 | 106 | 97 | 38 | 106 | 138 | 113 |
| 3) Selections from 1 and 2 | 5 | 36 | 98 | 105 | 100 | 32 | 103 | 145 | 111 |
| 4) Masscrosses among 1, 2 and 3 | 3 | 25 | 98 | 109 | 101 | 23 | 116 | 170 | 126 |
| 5) Many plants from one variety | 1 | 9 | 106 | 114 | 108 | 7 | 119 | 167 | 129 |
| 6) Many plants from several varieties | 5 | 38 | 113 | 122 | 115 | 34 | 122 | 169 | 131 |
| 7) Selections from 5 and 6 | 6 | 30 | 111 | 116 | 112 | 23 | 132 | 180 | 138 |
| 8) Masscrosses among varieties from 1 to 6 | 2 | 7 | 107 | 113 | 109 | 5 | 119 | 133 | 123 |

Table 2. Dry matter yield from the best varieties from different groups. Relative values.

| Variety group | Variety | Merkur = 100 | | | | | | | |
|---------------|---------|-------------------|----------|----------|-------|-------------------|----------|----------|-------|
| | | 1 st harvest year | | | | 2 nd harvest year | | | |
| | | No of trials | 1 st cut | 2 nd cut | Total | No of trials | 1 st cut | 2 nd cut | Total |
| Group 2 | Sv 034 | 35 | 95.9 | 104.3 | 99 | 24 | 95.9 | 120.3 | 101 |
| Group 4 | Sv 059 | 64 | 98.8 | 110.4 | 103 | 46 | 116.0 | 128.8 | 120 |
| Group 5 | Sv 0106 | 32 | 100.5 | 108.8 | 104 | 21 | 109.7 | 119.2 | 113 |
| Group 6 | Sv 099 | 31 | 108.3 | 114.3 | 111 | 22 | 115.3 | 123.5 | 118 |
| Group 7 | Sv 095 | 41 | 105.0 | 112.0 | 108 | 30 | 114.6 | 136.4 | 121 |
| Group 8 | Sv 0164 | 13 | 105.5 | 117.2 | 110 | 9 | 109.7 | 139.2 | 117 |

All tetraploid varieties which up to that time had been investigated were based on a rather limited number of plants, and each variety was based on diploid material originating from only one or two varieties. The immediate result of this mass crossing indicated that the genetical basis of the previously tested material had been too narrow. In producing new tetraploid material we, therefore, tried to base this on as many diploid plants as possible and selected for this purpose a large number of diploid varieties. In the material obtained in this way mass selection for certain characters was made and the best varieties of the same type but of different origin were again mixed.

During the 30 years since the work with tetraploid red clover started a large number of varieties have been tested for their yield capacity in variety trials. The varieties which have been included in these trials can be divided into following eight groups with regard to their origin :

1. Varieties based on a few diploid plants from one variety.
2. Varieties based on few diploid plants from two of three varieties.
3. Selections from the varieties in the groups 1 and 2.
4. Mass crosses between varieties from the groups 1, 2, 3.
5. Varieties based on a large number of diploid plants from one variety.
6. Varieties based on a large number of diploid plants from several varieties.
7. Selections inside varieties from the groups 5 and 6.
8. Mass crosses between varieties from the groups 1 - 6.

Results with 41 varieties of medium late type from these eight groups have been compiled in Table 1. As the results have been collected over a period of 25 years and the number of trials in which the different varieties have been included varies considerably there are no possibilities for direct comparisons among all varieties. The diploid standard variety Merkur have, however, been included in every trial and the figures given in Table 1. are the relative values of the yield of green bulk of each variety in comparison with Merkur. The results show very clearly that a widening of the genetic base has resulted in increased yield of the tetraploid varieties. While in the first varieties with very narrow genetic base the transfer from the diploid to the tetraploid state hardly gave any yield increase at all, this transfer in the later varieties based on a very wide diploid material has resulted in a yield increase varying between 10 and 15 per cent in the first harvest year and 30 to 40 per cent in the second one.

The results presented in Table 1 are in each group the average for a number of varieties. Further the results are all obtained from experiments carried out at Svalöf and thus only from one place. Finally they are based on the green bulk production.

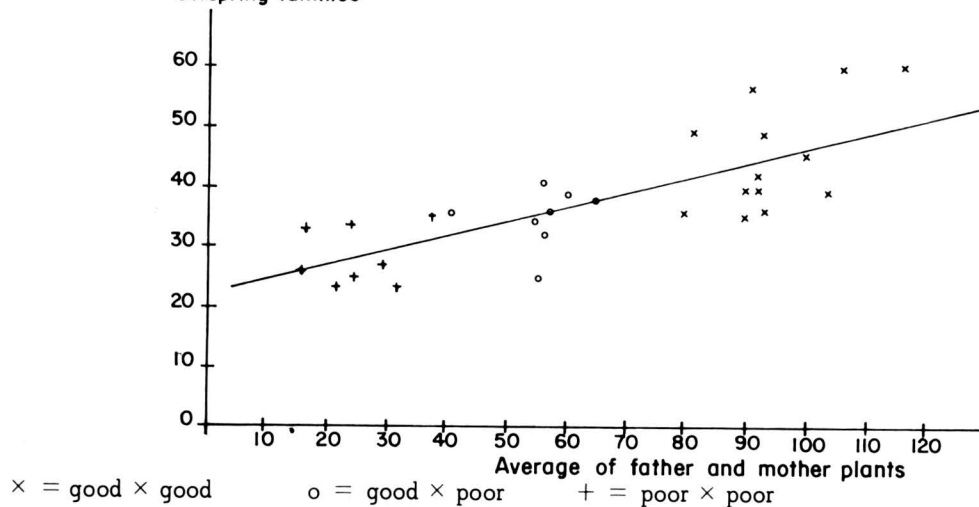
In Table 2. the results obtained with the best variety in each of the main groups are presented. In this case the figures refer to dry matter yield. As there is a considerable difference between diploid and tetraploid clover with regard to the dry matter content this is a more correct comparison. The figures given in the table are the average values for all experiments carried

Table 3. Seed yield trials with tetraploid red clover

| Variety group | Variety | Svalöf 1965 | | Svalöf 1966 | | Ultuna 1965 | | Ultuna 1966 | |
|---------------|---------|-------------|------|-------------|------|----------------------------------|------|-------------|------|
| | | kg/ha | Rel. | kg/ha | Rel. | kg/ha | Rel. | kg/ha | Rel. |
| Dipl. | Merkur | 289 | 100 | 348 | 100 | Dipl. not included in this trial | | 398 | 100 |
| | Disa | | | | | | | | |
| Tetr. | Sv 065 | 219 | 76 | | | | | | |
| 3 | Ulva | | | | | 431 | 100 | 296 | 74 |
| 4 | Sv 0259 | 208 | 72 | 269 | 77 | 549 | 127 | 303 | 76 |
| | Sv 064 | 212 | 73 | 310 | 89 | 506 | 117 | 272 | 68 |
| 6 | Sv 093 | 284 | 98 | 441 | 127 | 632 | 147 | 336 | 84 |
| | Sv 094 | 263 | 91 | | | 604 | 140 | | |
| | Sv 099 | 274 | 95 | 395 | 114 | 623 | 145 | | |
| | Sv 0100 | 283 | 98 | | | 652 | 151 | | |
| | Sv 086 | 313 | 108 | | | | | | |
| 7 | Sv 095 | | | | | 642 | 149 | | |
| | Sv 098 | 283 | 98 | | | | | | |

FIGURE I

Relation between seeds per head in parent plants and offspring families after crossing
Offspring families



out in the whole of Southern Sweden and are the relative values in relation to Merkur. Also in this case the varieties have not all been included in the same trials and therefore direct comparisons among the different varieties is not possible. The picture is, however, exactly the same as in Table 1. with a successively increasing yield from group 2 up to group 6, that is with the widening of the genetic base. Sv 095 representing group 7 is a selection for resistance to nematode from material in group 6 which has resulted in a better persistancy but without any influence on the yield capacity as such. Sv 0164 is obtained through mass crossing among a number of varieties from the groups 5, 6 and 7. The yield capacity of this variety is not higher than the varieties obtained inside the groups 5, 6 and 7 and it seems as if an uncontrolled further mixing of these types of populations has no influence on the yield capacity.

IMPROVED SEED PRODUCTION CAPACITY NECESSARY

One of the weaknesses of tetraploid red clover has always been the seed setting capacity. In the first tetraploid material the seed setting was very low. Thanks to natural selection the seed setting increased during the first generations but in all the varieties obtained during the first period the seed setting remained very low. In the varieties produced later the seed setting has considerably increased without any specific artificial selection. Results from seed yield trials carried out with varieties with good dry matter yield have been compiled in Table 3. The varieties have here been arranged in the various groups according to their type of origin. There is a clear tendency to higher seed yield from varieties belonging to the groups 6 and 7 indicating that the widening of the genetic base has had a favourable influence not only on the dry matter yield but also on the seed setting capacity. In spite of this the seed setting of all these varieties is still considerable lower than that of the diploids. If it should be possible fully to utilize the high yield capacity in the best tetraploid varieties, it is necessary to increase the seed setting capacity of these varieties. During the last 15 years great emphasis has been given to this problem in the breeding program at Svalöf.

Up to the middle of the fifties the work was mainly concentrated on studies on different characters and their influence on the seed setting. On basis of the results obtained in these studies selections were made with regard to specific characters inside the different varieties available at that time. The results were, however, not very promising and no results of practical importance were obtained. In the works carried out during the last 15 years much better results have been achieved. In order to illustrate various aspects on connected problems I will utilize the results obtained in one breeding project, started in 1957 and still going on. The scheme of this project is illustrated in Diagramm 1. As basic material well balanced tetraploid varieties of different origin in the 4th or 5th generation after polyploidization were used. In order to get as wide genetic base as possible random crosses among the different varieties were made on as large a scale as possible.

BREEDING FOR SHORTER COROLLA TUBES

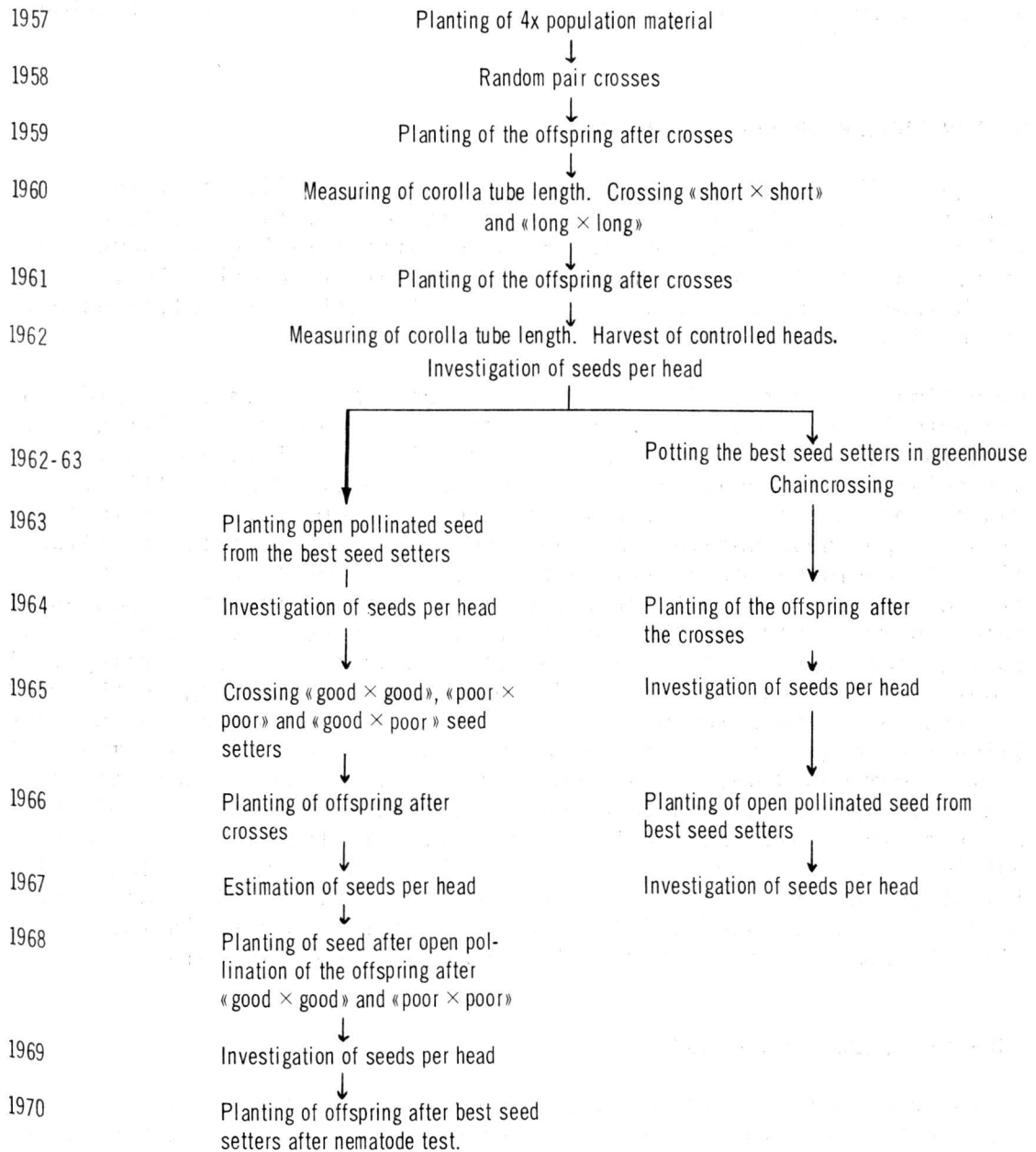
The offspring after these crosses were planted in the field as spaced plants. In 1960 the plants were studied individually for different characters among others the corolla tube length. On basis of the results obtained crosses were made among plants with short corolla tube, as it was believed that plants with short corolla tube should easier be pollinated and thus give higher seed yield. For control purposes also some crosses among plants with long corolla tube was carried out. The offspring material was planted out and investigated in 1962. The corolla tube length was measured and at the same time the number of seeds per head was investigated on each plant. In order to make sure that the seed development had taken place under similar ecological conditions on each plant, ten heads were labelled at the same time during the main flowering period. The labelled head were harvested and threshed separately and the number of seeds in each head was counted. The following results were obtained :

In the offspring

| Crossing | No of plants | Corolla tube length | No of seeds per head |
|---------------|--------------|-------------------------|-------------------------|
| Long x long | 166 | 10.63 | 32.20 |
| Short x short | 342 | 10.12 | 27.53 |
| Diff | | 0.51 [±] 0.069 | 4.67 [±] 1.357 |
| † | | 7.39 ⁺⁺⁺ | 3.44 ⁺⁺⁺ |

On an average the corolla tube of the offspring after the cross "short x short" was definitely shorter than that of the offspring after "long x long". The difference was about half a millimeter and the difference is fully significant. Thus it is evident that it is possible to reduce the corolla tube length through selection. On the other hand it is also quite clear in this material that the number of seeds per head in the offspring after "short x short" is definitely lower than in the cross "long x long". In this case the difference is almost 5 seeds per head or more than 15 per cent and also this difference is fully significant. In most families the correlation coefficient between corolla tube length and number of seeds per head is positive and in some families the correlation coefficient is fairly high up to 0.5 or more. Only in very few cases negative correlation coefficients have been obtained and in all these cases the coefficient is very low and insignificant. The correlation coefficient for the whole material is +0.23. The results indicate that a shorter corolla tube to a high degree is due to certain weaknesses in

Diagram 1. Scheme for a breeding program for improved seed setting



the plant resulting in low fertility and that the positive influence of a short corolla tube on the pollination cannot compensate for the reduced seed setting caused by these other factors. In any case in this material it, therefore, seemed not possible to achieve an improved seed setting by selection for short corolla tube.

SELECTION FOR NUMBER OF SEEDS PER HEAD

At that stage of the investigation it was, therefore, decided that in the future selection should be made not for specific characters influencing the seed setting but for the total complex of seed setting taking the number of seeds per head obtained under field condition as selection criterium. For that purpose seed from the best seed setters in the plantation was collected and the offspring from these plants was planted in 1963.

At the same time the best seed setting plants were taken from the field and planted in pots in the greenhouse. The plants were arranged in falling order with regard to the number of seeds per head obtained in field and chain crossing was carried out by hand. The number of seeds obtained per pollinated flower was estimated for each plant and the results were compared with the number of seeds per head obtained on the same plants under field conditions. No relationship could be found between the two series of figures and the correlation coefficient was only 0.1. Seedlings obtained from the crosses were planted in the field and the number of seeds per head was investigated the following year. Correlations were computed between the seed setting in the mother plants under field condition and the average seed setting in the offspring families and also between the seed setting of the mother plants after hand pollination in greenhouse and the offspring. In the first case the correlation coefficient was 0.48, in the second case 0.37. Thus it seems as if there were a stronger relationship between the seed setting of the mother plants under field conditions and the seed setting in the offspring under the same conditions than between the seed setting between the mother plants after hand pollination and the seed setting of the offspring. This indicates that when crosses are made by hand under greenhouse conditions certain factors influencing the seed setting under field conditions are eliminated.

INHERITANCE OF "SEEDS PER HEAD"

The plant material obtained from the open pollinated seed from the best seed setters in the 1961 plantation was investigated with regard to number of seeds per head in 1964. The variation in this material was very large indeed. While the best plants had an average seed setting of more than 100 seeds per head one plant had an average as low as 2 seeds per head. The average for the whole material was 54.1 seeds. Also between the families there were considerable differences varying from 75 seeds per head in the best family down to 26 seeds in the weakest one. The plants were left in the field and during the next summer crosses were made among the best seed setters, among the poor seed setters and between good and poor seed setters. The offspring after these

crosses were planted out and the number of seeds per head was investigated. Following results were obtained :

In the offspring

| Crossing | No of families | No of plants | No of heads | Average seeds/head | Variation among families |
|-------------|----------------|--------------|-------------|--------------------|--------------------------|
| good x good | 12 | 113 | 1000 | 44.5 | 60.4 - 35.5 |
| good x poor | 8 | 68 | 549 | 35.2 | 41.1 - 31.8 |
| poor x poor | 8 | 55 | 406 | 28.4 | 36.3 - 23.5 |

It is quite evident that the offspring after the crossing "good x good" is definitely better than after the crossing "poor x poor" and that the offspring after "good x poor" is closed to the average of the two extreme groups. The correlation between the seed setting of the parents and the offspring is also very strong with a correlation coefficient of 0.75. The regression coefficient is 0.23 and as can be seen in fig. 1 there seems to be a straight line correlation.

The seed from the best seed setters in the three groups was planted in four replicates and a new investigation of the number of seeds per head was carried in the following year. In this plantation also a tetraploid standard variety which has never been selected for seed setting was included. The results were :

| From offspring after | No of investig. plants | Seeds per head replicate | | | | Average |
|--------------------------------|------------------------|--------------------------|------|------|------|---------|
| | | I | II | III | IV | |
| good x good | 282 | 59.7 | 59.3 | 53.3 | 60.8 | 58.7 |
| good x poor | 20 | 55.5 | 48.4 | 63.7 | 50.2 | 55.7 |
| poor x poor | 119 | 36.9 | 36.9 | 35.9 | 35.2 | 36.3 |
| Standard material (unselected) | 45 | 46.9 | 52.6 | 43.5 | 44.6 | 47.12 |

In spite of the fact that the best seed setters in each group has been selected as parents material there is a considerable difference between the offspring from the three groups. It is also quite clear that the material selected for good seed setting is definitely better than the unselected standard material. The correlation coefficient between the seed setting in the parent plants and the offspring families was this time 0,78.

APPLICATION IN PRACTICAL BREEDING

From the results obtained in this series of experiments it seems as if there would be great possibilities to increase the number of seed set per head under field conditions through mass selection. To what extent this will influence the seed yield under normal field conditions is not yet tested. Material selected in this way has for the first time been included in a seed production trial which will be harvested in 1971. There is, however, no reason to believe that not the increased seed setting ability in the spaced plants should increase the seed production capacity of the population also when it is sown in close sward. In other experiments it has been shown that the same variation exists in the close sward as has here been demonstrated among spaced plants and that also that variation is genetically controlled. The question is not so much if the character "number of seeds per head" influences the seed yield but more to what extent. Another question is how many generations of selection will be needed before we reach a seed production level in the tetraploids, equal to that of diploids.

The basic material used in the investigation described here was an ordinary tetraploid population the general agriculture value of which was not established. It might be questioned if it is advisable to start such a laborous and long lasting selection program unless it is known that the dry matter production is high enough to justify its release as a new variety. Because of the good results obtained in this series of investigations two new tetraploid varieties, one early and one medium late which both are very high in dry matter production have been subject to similar selection during the last five years. The work is here combined with the elit seed production in that way that about 10.000 plants are planted and of these at least 1.000 are tested for the seed setting capacity. Unselected seed from the total population is harvested and used as elit seed. The next generation elit plantation is, however, based on seed from the 200 best seed setters. It is necessary to work with very large material so that a sufficiently large number of plants are selected in order to maintain the general characteristics of the variety and sufficient variability and heterozygosity to ensure high dry matter productivity.

CONCLUSION

In order to obtain high yielding capacity from tetraploid red clover it seems essential that the material has a very wide genetic base. For production of tetraploid material a number of well adapted diploid variety with good

agronomic characters should be used and from each variety a large number of tetraploid plants should be raised.

The same wide genetic basis is of importance also for a good seed production. Through a large scale mass selection for number of seeds per head this character can be improved and seems to be an efficient way to increase seed production ability.

SELFING EFFECTS ON S_2 AND S_3 GENERATIONS IN MEDICAGO SATIVA (*)

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INTRODUCTION

Our improvement programme is based on an initial selfing period, at the end of which we obtain S_4 seeds. The results of the first three experiments, dealing with parental populations and S_1 and S_2 generations (7,8) have already been published.

All tests were carried out in a state of competition : 150 plants per square metre (4 x 17 cms.), transplanted in field after 120 days of growing in greenhouse (4 x 4 cms. between the plants). The growing technique employed shows practical advantages of fundamental importance : the study of 20,000 individual plants, as in trial 1969-70, becomes possible without too large requirements of space and time. Furthermore, by the technique of spaced plants, besides the difficulty of finding the suitable extension of field, other negative consequences could be expected, concerning the evaluation of individuals and lines. Taking into account the total number of plants examined (about 45,000 over the whole of four trials since 1966 up today), the registration of all the characters listed in the program would be impossible for spaced plants.

In this paper, we report the results of the fourth experiment, concerning the performance of the lines S_2 and S_3 .

MATERIAL AND METHOD

The method was the same as in previous experiments, except for a change in the time of the first cutting, which was now earlier, and took place at the budding stage. As in trial n. 3, when necessary, the second cutting of the S_1 , S_2 , and S_3 was delayed in comparison with the corresponding S_0 , so that

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

the characters of each line could be measured at about 50% flowering. This tends to avoid the selfing effect on earliness being confused with the real effect on vigour. The following material was used :

| | | | FRIULANA Nr. | | FLORIDA Nr. | |
|----------------------|-----|-------|-----------------|--------|----------------|--------|
| | | | lines | plants | lines | plants |
| Parental Populations | | | - | 311 | - | 341 |
| S ₀ | A | lines | 8 | 695 | 6 | 493 |
| S ₂ | AA | " | 11 | 584 | 4 | 184 |
| | AB | " | 8 | 673 | 5 | 339 |
| S ₃ | AAA | " | - | - | - | - |
| | AAB | " | - | - | - | - |
| | ABA | " | 7 | 224 | 3 | 126 |
| | ABB | " | 5 | 366 | 4 | 255 |
| S ₀ | B | " | 18 | 1568 | 21 | 1801 |
| S ₂ | BA | " | 14 | 1049 | 21 | 1301 |
| | BB | " | 21 | 1816 | 23 | 1859 |
| S ₃ | BAA | " | 4 | 92 | 5 | 115 |
| | BAB | " | 4 | 265 | 5 | 279 |
| | BBA | " | 18 | 861 | 16 | 645 |
| | BBB | " | 24 | 1813 | 25 | 1606 |
| Totals | | | 143 | 10328 | 138 | 9349 |

We also grew four S_1 lines as test of the previous experiments. The inbreeding depression values of these S_1 lines in 1969-70 were the same than in 1968-69. The same must be said as for the inbreeding depression values of the S_2 lines. For this reason, it was possible to make a reliable estimate of the S_1 mean value over all the S_1 lines grown in 1968-69. This estimated S_1 average was used for the calculation of the selfing effects.

RESULTS AND DISCUSSION

Effects of selfing on the averages.

1 - As in test 3, $B S_0$ is slightly higher than $A S_0$ in both populations (tables 1 and 2) as concerning the dry matter weight. $A S_0$ and $B S_0$ have always higher mean values than in parental populations, though no significantly.

In order to understand these results better, it must be born in mind that both the two groups and the parental populations have the same precocity value ; in fact the second cutting was carried out at the same time for all these plants. The decision to effect the first cutting at the budding stage was necessitated by the need to find out the effect of selfing when the influence of precocity on the dry matter weight and the plant height is less than at the moment of flowering. In fact, if we compare the inbreeding depression values, calculated at the first and second cutting (table 3), it is clear that if we want to find out the selfing effect as nearly as possible, and measure the dry matter weight at the same time for all the material, then we must cut at the budding stage. The values for the two cuttings are, in fact, fairly similar, especially with Florida. Vice versa if we want to measure the characters at the flowering stage, the delayed cut is absolutely necessary. In fact, the AA of Florida, cut a good five days after the respective S_0 , show only 38% of plants in flower against 59% for S_0 . So, if the S_0 and selfed lines are cut on the same day, the effect of selfing on weight and height would be overestimated. And even though we ourselves used delayed cuttings, our results still showed an overevaluation of the effects of selfing on the dry matter weight as, at the moment of cutting, the S_0 had a higher percentage of plants in flower. On the contrary, the effect of selfing on precocity, measured as the number of days necessary for the selfed lines to bring a similar percentage of plants to flower as S_0 , is undervalued. However, the phenomenon is clear : lateness in flowering

- a) is greater in group A than in group B, especially in Florida ;
- b) increases with each generation of selfing ;
- c) is more noticeable in the weak lines within one generation, especially inside group A.

2 - From examination of inbreeding depression values (table 4) taken for the two cuttings and the two populations together, the results were as follows :

Table 1. FRIULANA - Mean values of plant characters

| Parental population | 1rst cutting | | | 2nd cutting | | |
|---------------------|-------------------|--------|--------------|-------------------|--------|--------------|
| | Dry matter weight | Height | Nr. of stems | Dry matter weight | Height | Nr. of stems |
| Group A : | | | | | | |
| S0 | 2.78 | 61 | 3.2 | 3.38 | 81 | 5.2 |
| S2 | 2.89 | 59 | 3.3 | 3.51 | 78 | 5.7 |
| AA | 1.56 | 45 | 2.7 | 1.87 | 61 | 3.8 |
| AB | 2.32 | 53 | 3.3 | 2.63 | 71 | 4.9 |
| S3 | - | - | - | - | - | - |
| AAA | - | - | - | - | - | - |
| AAB | 1.65 | 44 | 2.8 | 1.81 | 57 | 4.2 |
| ABA | 2.20 | 52 | 3.3 | 2.40 | 69 | 4.9 |
| ABB | | | | | | |
| Group B : | | | | | | |
| S0 | 2.97 | 61 | 3.2 | 3.58 | 81 | 5.6 |
| S2 | 1.85 | 51 | 3.0 | 2.17 | 70 | 4.7 |
| BA | 2.38 | 56 | 3.1 | 2.75 | 75 | 4.9 |
| BB | 1.05 | 37 | 2.4 | 1.26 | 41 | 2.9 |
| S3 | 1.93 | 48 | 2.8 | 2.42 | 73 | 4.4 |
| BAA | 1.50 | 45 | 2.7 | 1.77 | 64 | 4.2 |
| BAB | 2.27 | 53 | 3.0 | 2.43 | 70 | 4.8 |
| BBA | | | | | | |
| BBB | | | | | | |

Table 2. FLORIDA - Mean values of plant characters

| Parental population | 1 st cutting | | | 2 nd cutting | | |
|---------------------|-------------------------|--------|--------------|-------------------------|--------|--------------|
| | Dry matter weight | Height | Nr. of stems | Dry matter weight | Height | Nr. of stems |
| | | | | | | |
| Group A : | | | | | | |
| S0 | 3.02 | 70 | 3.0 | 3.59 | 83 | 5.0 |
| S2 | 3.07 | 64 | 3.1 | 3.68 | 77 | 5.6 |
| AA | 1.57 | 43 | 3.0 | 1.98 | 58 | 4.7 |
| AB | 2.26 | 55 | 3.1 | 2.48 | 67 | 4.9 |
| S3 | - | - | - | - | - | - |
| AAA | - | - | - | - | - | - |
| AAB | 1.61 | 44 | 2.7 | 1.51 | 50 | 3.6 |
| ABA | 2.15 | 53 | 2.8 | 2.61 | 66 | 5.0 |
| ABB | | | | | | |
| Group B : | | | | | | |
| S0 | 3.20 | 65 | 3.1 | 3.98 | 83 | 5.7 |
| S2 | 1.91 | 52 | 2.7 | 2.33 | 68 | 4.4 |
| BB | 2.37 | 57 | 2.9 | 3.03 | 77 | 5.0 |
| S3 | 1.44 | 46 | 2.5 | 1.77 | 59 | 4.7 |
| BAA | 2.00 | 50 | 2.9 | 2.48 | 67 | 5.2 |
| BAB | 1.56 | 47 | 2.6 | 1.83 | 63 | 4.3 |
| BBA | 2.00 | 51 | 2.8 | 2.47 | 69 | 4.6 |
| BBB | | | | | | |

Table 3. Selfing effects on dry matter weight ($S_0 = 100$), budding and blooming percentage, and blooming delay *

| | FRIULANA | | | | | | FLORIDA | | | | | | | | |
|-----------|-------------------|-----------------|-------------------|------------------|---------------|-------------------|-----------------|-------------------|------------------|---------------|-------------------|-----------------|-------------------|------------------|---------------|
| | 1st cut | | | 2nd cut | | | 1st cut | | | 2nd cut | | | | | |
| | Dry matter weight | Budding percent | Dry matter weight | Blooming percent | Cutting delay | Dry matter weight | Budding percent | Dry matter weight | Blooming percent | Cutting delay | Dry matter weight | Budding percent | Dry matter weight | Blooming percent | Cutting delay |
| Group A : | | | | | | | | | | | | | | | |
| S0 | 100 | 63 | 100 | 66 | - | 100 | 69 | 100 | 59 | - | 100 | 69 | 100 | 59 | - |
| S2 AA | 54 | 38 | 53 | 37 | 3.2 | 51 | 30 | 54 | 38 | 5.0 | 54 | 30 | 54 | 38 | 5.0 |
| AB | 80 | 65 | 74 | 58 | 2.0 | 74 | 62 | 67 | 58 | 2.0 | 67 | 62 | 67 | 58 | 2.0 |
| S3 AAA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| AAB | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ABA | 57 | 42 | 52 | 40 | 3.3 | 52 | 42 | 41 | 24 | 6.3 | 41 | 42 | 41 | 24 | 6.3 |
| ABB | 76 | 52 | 68 | 49 | 3.0 | 70 | 42 | 71 | 58 | 5.8 | 71 | 42 | 71 | 58 | 5.8 |
| Group B : | | | | | | | | | | | | | | | |
| S0 | 100 | 63 | 100 | 64 | - | 100 | 71 | 100 | 74 | - | 100 | 71 | 100 | 74 | - |
| S2 BA | 62 | 35 | 61 | 47 | 3.9 | 60 | 42 | 59 | 56 | 2.7 | 59 | 42 | 59 | 56 | 2.7 |
| BB | 80 | 50 | 77 | 58 | 1.9 | 74 | 51 | 76 | 65 | 1.7 | 76 | 51 | 76 | 65 | 1.7 |
| S3 BAA | 35 | 32 | 36 | 44 | 3.8 | 45 | 40 | 44 | 53 | 3.6 | 44 | 40 | 44 | 53 | 3.6 |
| BAB | 65 | 45 | 68 | 65 | 3.7 | 63 | 43 | 62 | 50 | 3.4 | 62 | 43 | 62 | 50 | 3.4 |
| BBA | 51 | 29 | 49 | 45 | 4.4 | 49 | 31 | 46 | 39 | 3.1 | 46 | 31 | 46 | 39 | 3.1 |
| BBB | 76 | 52 | 68 | 54 | 3.6 | 63 | 36 | 62 | 51 | 2.4 | 62 | 36 | 62 | 51 | 2.4 |

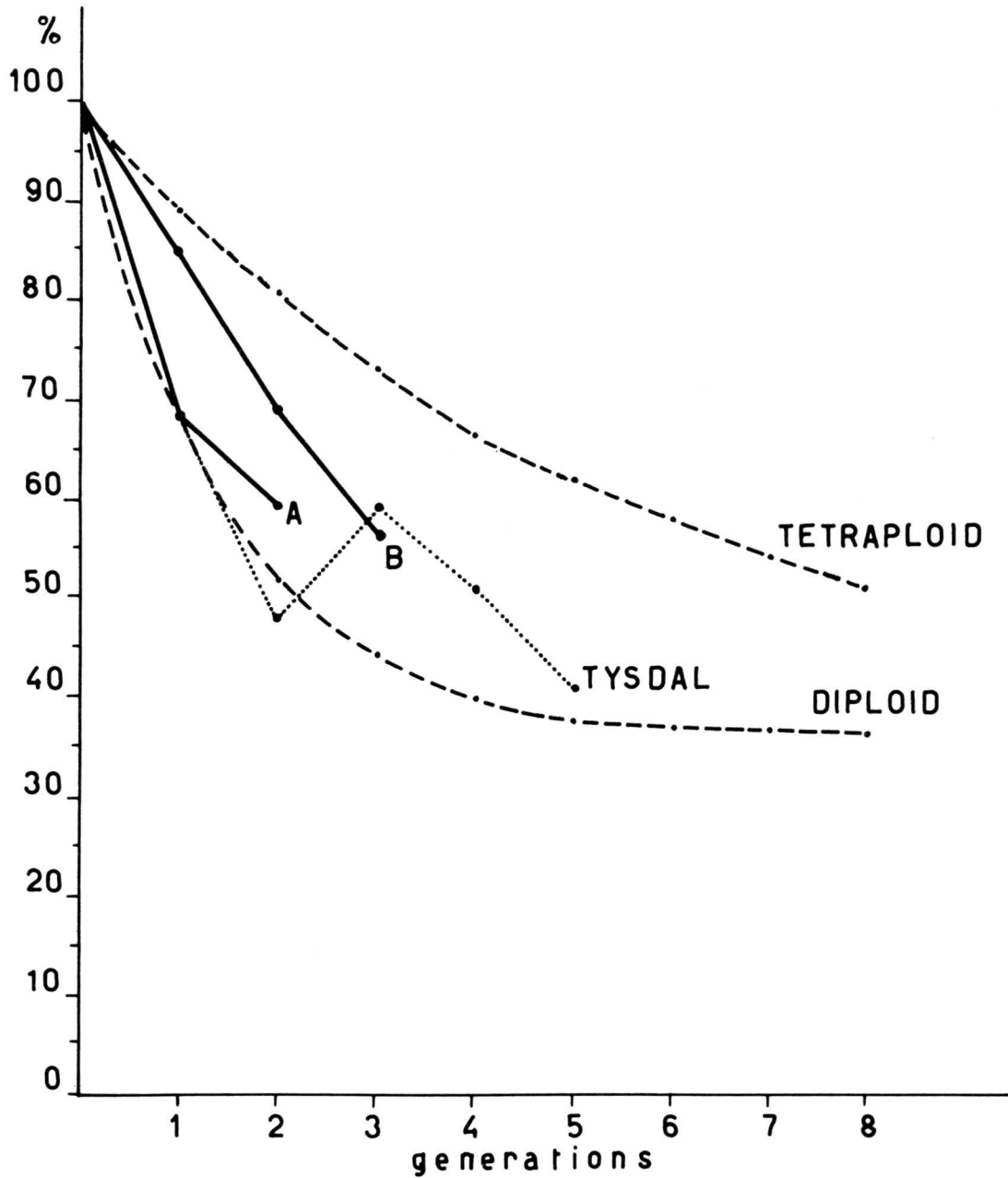
* Average number of days after cutting of S_0

Table 4. Selfing effects over 2 cuttings in Friulana and Florida

| Comparison at different levels of inbreeding | Dry matter weight | Plant height | Number of stems |
|--|-------------------|--------------|-----------------|
| $\frac{AS_1 - AS_0}{AS_0} \cdot 100$ | - 32 | - 10 | - 12 |
| $\frac{(AA, AB) S_2 - AS_1}{AS_1} \cdot 100$ | - 8 | - 10 | 0 |
| $\frac{(ABA, ABB) S_3 - ABS_2}{ABS_2} \cdot 100$ | - 17 | - 4 | - 3 |
| $\frac{BS_1 - BS_0}{BS_0} \cdot 100$ | - 17 | - 5 | - 7 |
| $\frac{(BA, BB) S_2 - BS_1}{BS_1} \cdot 100$ | - 16 | - 9 | - 6 |
| $\frac{(BAA, BAB) S_3 - BAS_2}{BAS_2} \cdot 100$ | - 12 | - 12 | - 6 |
| $\frac{(BBA, BBB) S_3 - BBS_2}{BBS_2} \cdot 100$ | - 25 | - 12 | - 8 |

FIGURE I

Inbreeding effect on vigour



Note - The S_1 value is the average of the 1968 and 1969 experiments. The S_2 value the average of the 1969 and 1970 one. The S_0 lines were the same all in the three experiments 1968 - 1969 - 1970.

- a) Dry matter weight is the character most susceptible to selfing and the number of stems is the least susceptible. This would lead us to suppose that amongst the characters directly connected with the yield (height, thickness and number of stems) the thickness of the stems is perhaps, on average, the characters affected most by the inbreeding effect.
- b) the trend of inbreeding depression in the subgroups can be resumed thus :
- except for A S_1 which will be discussed later, the subgroups belonging to group B show a greater loss in vigour than the corresponding ones in group A : -16 compared to -8 and -25 to -17 ;
 - within group B, selfing of the vigorous S_2 lines (BB) causes S_3 to show a greater inbreeding depression than S_3 derived from the weak S_2 lines (BA):

Up to now we have not been able to obtain a sufficient number of AAA and AAB lines to provide valid information for group A. In fact, the plants of the AA lines are almost always autosterile or produce vestigial flowers, or else they are unable to produce flowers in the first year owing to their greatly reduced vigour after the second cutting. However, it seems probable that the results obtained for the S_3 of the B group, would be even more valid for those of group A. This is because inbreeding depression seems to follow this pattern : the selfing of plants belonging to a weak line causes a lower inbreeding depression than when plants of a vigorous line are selfed.

It is not easy to explain these differences of reaction to selfing. We feel that the series of facts observed in our experiments is much more likely to be explained by the hypothesis of heterozygotic structure than by the hypothesis of gene or interaction effects. What is more, gene effects are probably conditioned by structural effects, so that their importance is modified with inbreeding. The explanation for the great S_1 depression (-32) of the A lines might be the following : considering the very low value registered for S_2 (-8), the great depression in S_1 is to be ascribed to the fact that plants of the A group, open-pollinated with pollen from both the A and the B groups, produced S_1 lines with a higher average level of heterozygosis than the plants of group A possessed.

Figure 1 shows that the general loss in vigour was more rapid than the expected from the inbreeding coefficient, but was still slower than that so far reported by other authors.

In a previous paper we held that the differences between our results and those of other research-workers were probably mainly the result of growing methods (competition). We now add that the precocity effect is of the greatest importance from the first generation of selfing. We calculated the general inbreeding depression by the values of the separate sub-groups, that is, two in the second generation and four in the third generation. The same number of plants for each of the separate sub-groups was used.

FIGURE II

Dry matter C.V. distribution (a group)

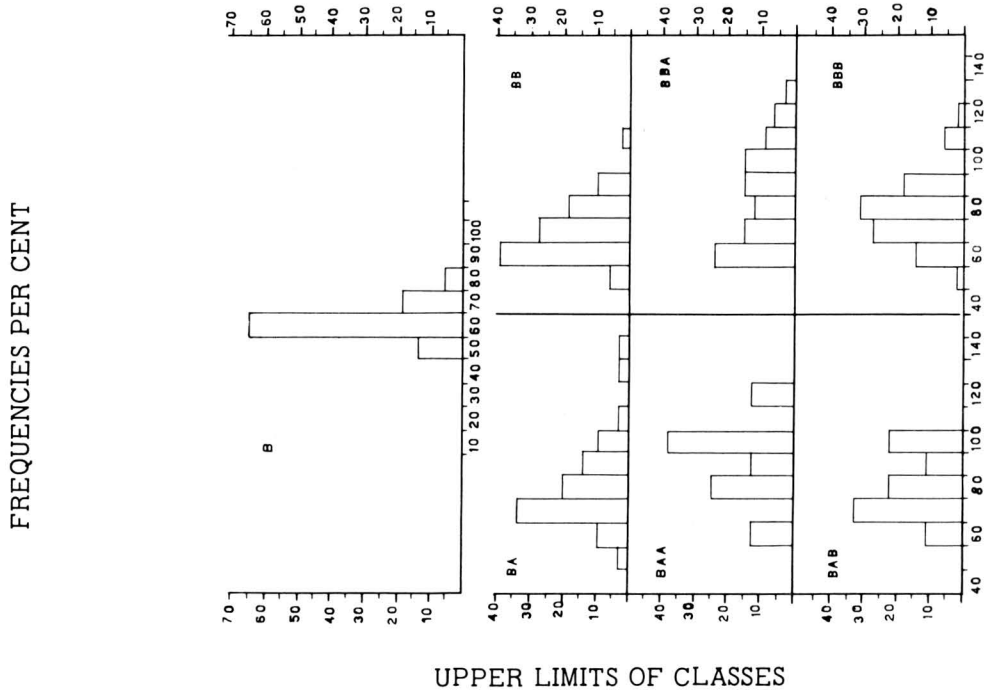
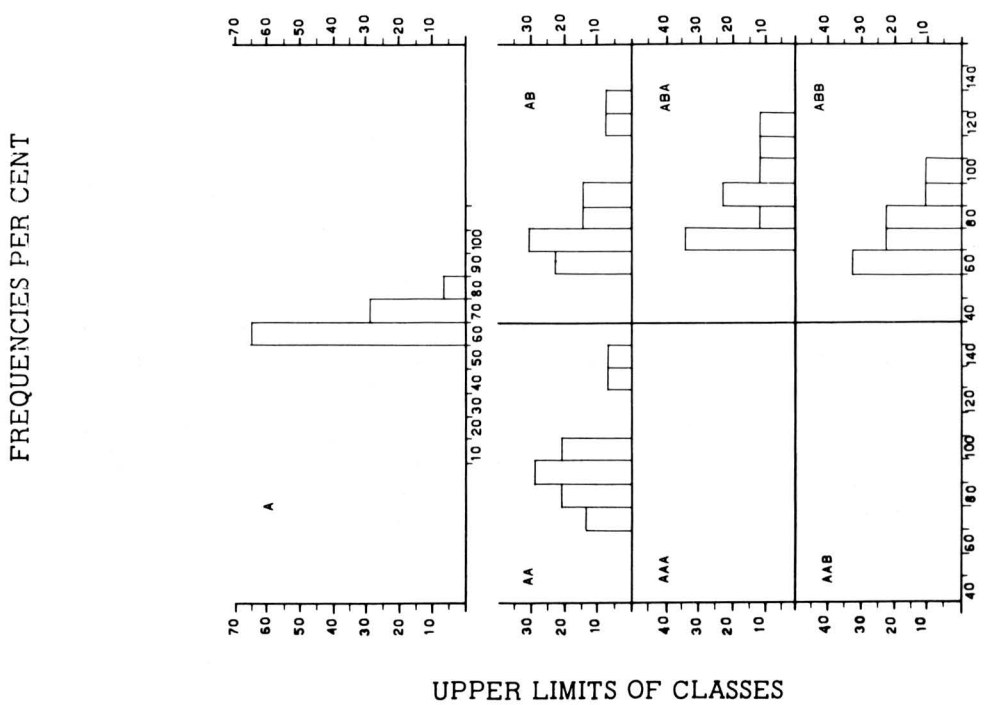


FIGURE III

Dry matter C.V. distribution (b group)



Effect of inbreeding on the variability

For dry matter weight, the variance decreases from S_0 to S_2 to S_3 , following the drop in the averages, though not proportionally; in fact, the coefficients of variability increase with the selfed generations and are greater for the weak lines (table 5).

As there exists no correlation between average and variance for plant height (table 6), the increase in variability directly appears in the variance. In this case too, the increase is in relation to selfing and is, on the whole, greater in the weak lines than in the vigorous ones.

We offer the following explanation: it is well known that in autopolyploids the progress towards homozygosity is very slow; between maximum heterozygosity and homozygosity there exist several intermediate levels of heterozygosity, with a corresponding number of vigour levels. Four or five generations of selfing produce this type of material, which, from a structural point of view, is more heterogeneous than the S_0 lines. Interplant competition (intra-genotype) heightens this heterogeneity and causes a higher phenotypic variance (competition variance) in the selfed lines than in S_0 .

This hypothesis is confirmed (8) by observation of S_0 , S_2 and S_3 material at the seedling stage, 35 days after sowing (before competition begins).

In fact:

- a) the coefficients of variability for height show the same trend as those of the adult plant;
- b) in the selfed lines, especially in the weak ones, there are pale green plants besides plants morphologically normal but growing very slowly etc..

These plants, whether in greenhouse or in field, are not killed by competition, but are merely depressed in favour of the more vigorous plants, so that the dry matter weight of adult plants in these groups may vary from 0.05 gm. to 9-10 gm.

The distribution curves of the variability coefficients are more dispersed, yet, at the same time tend towards maximum values in the weak selfed lines, so confirming our hypothesis (Figures 2 and 3).

Degree of response to selfing in vigorous plants (B)

As can be seen in the figure 4 all the lines in S_3 which derive from S_2 plants type B (ABB-BAB-BBB lines) have more or less the same dry matter weight.

Therefore, in selecting vigorous plants in S_2 we must bear in mind that:

- the difference between the parental populations is of little importance;
- it does not matter whether vigorous or weak plants are selfed at the start.

Table 5. Variances and coefficients of variability of dry matter weight

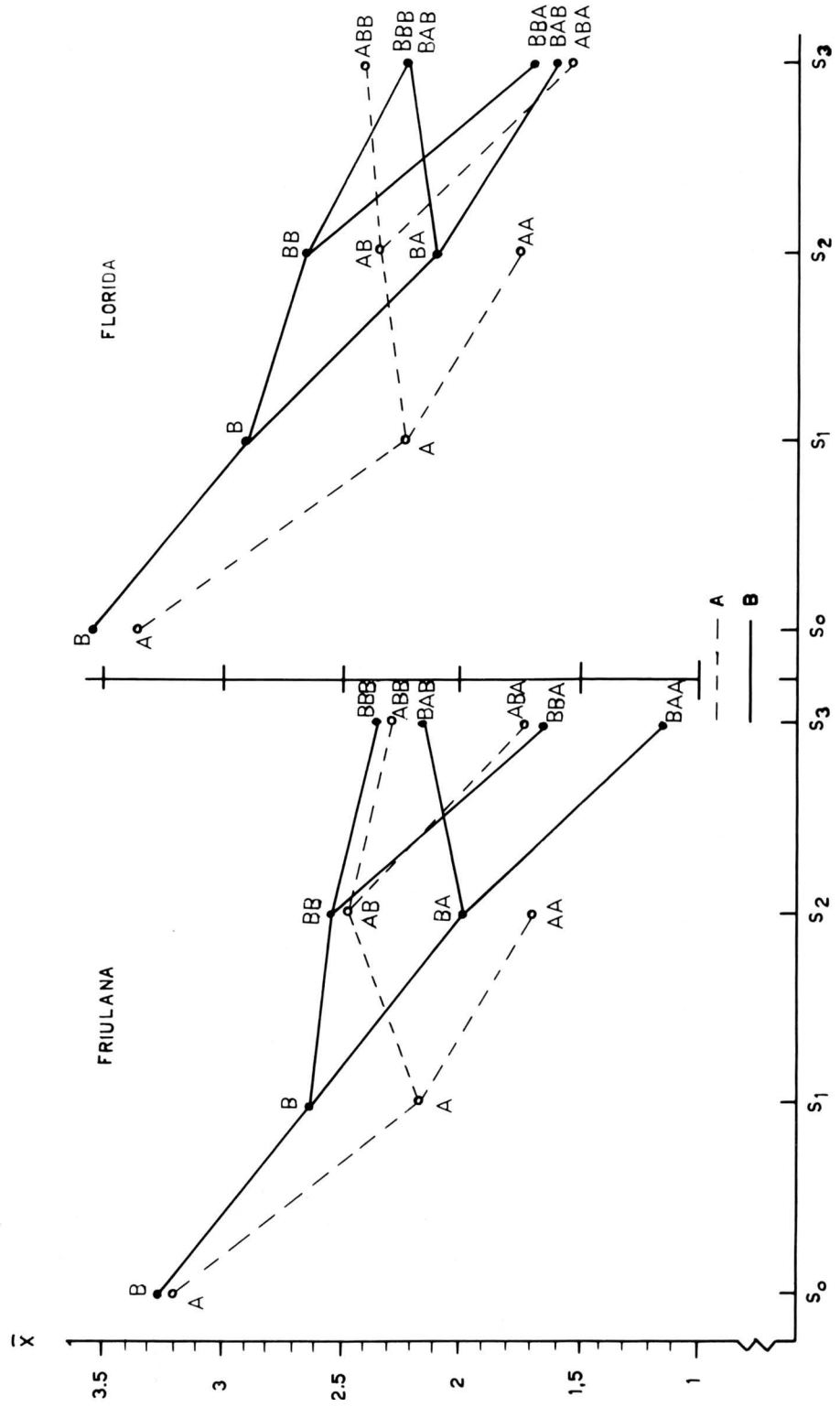
| | FRIULANA | | | | FLORIDA | | | |
|-----------|---------------------|------|---------------------|------|---------------------|------|---------------------|------|
| | 1 st cut | | 2 nd cut | | 1 st cut | | 2 nd cut | |
| | S 2 | C.V. | S 2 | C.V. | S 2 | C.V. | S 2 | C.V. |
| GROUP A : | | | | | | | | |
| S 0 | 2.17 | 50 | 4.34 | 59 | 2.82 | 54 | 4.84 | 59 |
| S 2 | 1.14 | 69 | 2.85 | 84 | 1.22 | 75 | 2.11 | 78 |
| AB | 1.68 | 58 | 3.40 | 65 | 2.43 | 68 | 4.98 | 83 |
| S 3 | - | - | - | - | - | - | - | - |
| AAA | - | - | - | - | - | - | - | - |
| AAB | - | - | - | - | - | - | - | - |
| ABA | 1.58 | 72 | 1.87 | 85 | 1.28 | 88 | 1.05 | 85 |
| ABB | 1.77 | 59 | 2.97 | 72 | 1.59 | 59 | 2.64 | 63 |
| GROUP B : | | | | | | | | |
| S 0 | 2.05 | 48 | 4.38 | 57 | 2.57 | 50 | 2.29 | 57 |
| S 2 | 1.49 | 65 | 3.50 | 75 | 1.60 | 67 | 3.63 | 77 |
| BB | 1.35 | 48 | 3.13 | 62 | 1.81 | 57 | 4.26 | 67 |
| S 3 | 0.71 | 80 | 1.00 | 79 | 0.98 | 65 | 2.10 | 76 |
| BAB | 1.38 | 59 | 3.41 | 73 | 1.60 | 65 | 3.07 | 74 |
| BBA | 0.94 | 64 | 1.96 | 78 | 1.45 | 71 | 2.49 | 82 |
| BBB | 1.54 | 55 | 3.08 | 70 | 1.71 | 68 | 3.39 | 77 |

Table 6. Variances and coefficients of variability of plant height

| | FRIULANA | | | FLORIDA | | |
|--------------------|----------------|------|----------------|----------------|------|---------|
| | 1st cut | | 2nd cut | 1st cut | | 2nd cut |
| | S ² | C.V. | S ² | S ² | C.V. | C.V. |
| GROUP A : | | | | | | |
| S ₀ | 63 | 13 | 91 | 67 | 14 | 11 |
| S ₂ AA | 92 | 21 | 191 | 136 | 26 | 25 |
| AB | 56 | 18 | 168 | 121 | 20 | 18 |
| S ₃ AAA | - | - | - | - | - | - |
| AAB | - | - | - | - | - | - |
| ABA | 136 | 27 | 225 | 112 | 26 | 33 |
| ABB | 116 | 20 | 236 | 99 | 22 | 22 |
| GROUP B : | | | | | | |
| S ₀ | 68 | 13 | 92 | 98 | 15 | 11 |
| S ₂ BA | 94 | 19 | 194 | 139 | 24 | 24 |
| BB | 67 | 14 | 138 | 125 | 19 | 16 |
| S ₃ BAA | 132 | 32 | 230 | 167 | 30 | 27 |
| BAB | 106 | 21 | 172 | 142 | 23 | 25 |
| BBA | 97 | 22 | 216 | 184 | 29 | 27 |
| BBB | 95 | 18 | 204 | 153 | 22 | 23 |

FIGURE IV

Dry matter weight - Averages of two cuttings (gm)



More exact indications can be gained by comparing the average weight of the lines with that of their plants of origin (table 7). The ratio of the lines to the plants averages gives the "degree of response to selfing" of the same plants. By examining the results in table 8, we can see that : the B plants chosen from the weak S_1 lines have a "degree of response" 8-9 points higher than B plants chosen from vigorous S_1 lines. This is true both of Friulana and of Florida. The same trend has the ratio of the S_3 lines to the S_2 lines but on the whole the "degree of response" does not increase, except for vigorous plants chosen from BA lines of Florida.

These results would seem to imply that if inbreeding is to be limited to the first generation, then non-vigorous plants should be selfed. The ideal solution would be to select plants from the S_2 AA lines which were as vigorous as those in BB. The probability of finding such plants is slight but is not to be excluded. Lastly, we must stress that the "degree of response" to selfing is the same in Florida and Friulana at the mother plants level but begins greater in Florida than in Friulana, in the following selfed generations. This perhaps because the Florida variety has a narrower genetic base than the ecotype Friulana.

Correlation between generations

In order to estimate the general combining ability of mother plants to be employed in a synthetic variety, in addition to the better known methods such as polycross and top-cross, also progenies derived from selfing and open-pollination are employed in lucerne. The correlation coefficients between these progenies and their plants of origin give a measure of the general combining ability of the latters.

The coefficients of correlation between plants selected within S_1 and S_2 lines and their self-progenies are reported in table 9.

The results can be resumed as follows :

- a) the correlation coefficients concerning the dry matter weight increase with the selfed generations, whereas those concerning the plant height remain unchanged ;
- b) within a given generation, the correlation is higher for the plants selected in weak lines ;
- c) the S_2 - S_3 correlation coefficients concerning the dry matter weight are higher than those concerning the plant height in both the populations.

This latter is the more interesting feature of these results. We have to ask ourselves if the plant height was not a character which had receive too much importance in the lucerne breeding. We have to point out that in the experiments on competition in lucerne we have found that the plant height is not a "leader character", as it is generally defined but it is a character subordinate to the vigour (9). This is sound as concerning the high density stand, while as for the spaced plants both the plant height and the number of stems

Table 7. Plants selected in S₁ and S₂ lines - Mean values (2nd cutting)

| | FRIULANA | | | FLORIDA | | | | |
|-----------------------------------|-------------------|--------------|-----------------|-------------------|--------------|-----------------|----|-----|
| | Dry matter weight | Plant height | Number of stems | Dry matter weight | Plant height | Number of stems | | |
| Plants selected in S ₁ | A { | A | 1.35 | 66 | 3.6 | 1.57 | 65 | 3.9 |
| | | B | 7.24 | 84 | 6.4 | 5.17 | 77 | 5.5 |
| | B { | A | 1.32 | 68 | 3.9 | 1.69 | 68 | 3.2 |
| | | B | 9.89 | 90 | 9.0 | 7.85 | 90 | 6.4 |
| Plants selected in S ₂ | AA { | A | - | - | - | - | - | - |
| | | B | - | - | - | - | - | - |
| | AB { | A | 1.56 | 67 | 4.0 | 1.03 | 57 | 3.3 |
| | | B | 6.78 | 91 | 7.2 | 6.71 | 83 | 7.3 |
| | BA { | A | 0.84 | 65 | 2.0 | 1.34 | 65 | 3.2 |
| | | B | 6.50 | 86 | 7.3 | 4.03 | 78 | 6.0 |
| | BB { | A | 1.86 | 68 | 3.9 | 1.45 | 64 | 3.1 |
| | | B | 8.01 | 88 | 8.5 | 7.02 | 85 | 7.2 |

Table 8 . Degree of response to selfing in vigorous plants (B)

| | FRIULANA | FLORIDA |
|--|----------|---------|
| BS ₁ lines vs B mother plants | 0.28 | 0.29 |
| AB S ₂ lines vs B plants selected within A S ₁ | 0.36 | 0.48 |
| BB S ₂ " " " " B S ₁ | 0.28 | 0.39 |
| ABB S ₃ lines vs B plants selected within AB S ₂ | 0.35 | 0.39 |
| BAB S ₃ " " " " BA S ₂ | 0.37 | 0.62 |
| BBB S ₃ " " " " BB S ₂ | 0.30 | 0.35 |

Table 9. Correlation coefficients between generations (2nd cut)

| | FRIULANA | | | FLORIDA | | |
|---|--------------|-------------------|--------------|--------------|-------------------|--------------|
| | Nr. of pairs | Dry matter weight | Plant height | Nr. of pairs | Dry matter weight | Plant height |
| S ₂ lines vs S ₁ plants selected within the A group (AA, AB/A) within the B group (BA, BB/B) | 20 | 0.64* | 0.58** | 8 | 0.65 | 0.67 |
| | 35 | 0.50** | 0.51** | 44 | 0.53** | 0.53** |
| S ₃ lines vs S ₂ plants, selected within the A group within AA lines within AB lines (ABA, ABB/AB) within the B group within BA lines (BAA, BAB/BA) within BB lines (BBA, BBB/BB) | - | - | - | - | - | - |
| | 12 | 0.77** | 0.59* | 7 | 0.77** | 0.68 |
| | 8 | 0.82** | 0.68* | 10 | 0.75** | 0.76** |
| | 42 | 0.68** | 0.40** | 41 | 0.49** | 0.41** |

are autonomous vis-à-vis the vigour (dry matter weight).

CONCLUSION

From the material we have studied since 1966 up today - two parental populations and S_1 - S_2 - S_3 generations - we obtained the following general informations.

- 1) it is advantageous from every point of view (as scientific, as technical and economical) to study the parental populations and their progenies in state of competition.
- 2) in state of competition the most important character is the vigour, measured as dry matter weight.
- 3) in order to evaluate as correctly as possible the inbreeding depression, it is necessary to cut if we want to measure the characters at the budding stage ; to the flowering stage, the cut of selfed lines has to be delayed, in order to keep for each line the stage of about fifty per cent of flowering. In this way it is possible to exclude from the estimation of vigour the delaying effect of selfing on the precocity.
- 4) the selfing of weak mother plants would perhaps hasten the obtention of lines in which the greatest number of favourable genes would accumulate.

SUMMARY

We give results for S_2 and S_3 generations. As in previous experiments, the material was studied in competition at a density of 150 plants per square metre, transplanted after 120 days of growing in greenhouse.

We summarize the results as follows :

- 1) the average loss in vigour was the same for the two populations ; in the Florida variety, which has a narrower genetic base than the Friulana ecotype, it was more advisable to choose vigorous plants.
- 2) loss of vigour was more rapid than the expected from the inbreeding coefficient, but still lower than that obtained so far by other research workers.
- 3) the trend of vigour depression in plants of the A group is very different from that in plants of the B group.
- 4) the vigorous lines in S_3 (ABB-BAB-BBB) show the same average weight in both populations. It does not seem to make a difference to self the vigorous plants or the weak ones ; indeed, weak plants seem to give better results.

- 5) in order to evaluate the loss in vigour as accurately as possible, it is necessary to effect the cutting at the budding stage ; if we want to measure the characters at the flowering stage, the delayed cut is absolutely necessary, so that the effect of selfing on precocity will not affect the evaluation of vigour.

REFERENCES

- Busbice, T.H., Wilsie, C.P., 1966. Inbreeding depression and heterosis in tetraploid with application to *Medicago sativa*. *Euphytica*, 15, 1. (1)
- Demarly, Y., 1963. Génétique des tétraploïdes et amélioration des plantes. *Ann. Amél. Plantes*, 13, (4). (2)
- Demarly, Y., 1968. La sélection des variétés synthétiques. Cinquième Congrès Eucarpia, Milano. (3)
- Dessureaux, L., 1960. Analysis of continuous variation in autotetraploid. Report of the seventeenth improvement conference. (4)
- Gallais, A., 1968. Evolution de la vigueur des variétés synthétiques tétraploïdes au cours de générations de multiplication. *Ann. Amél. Plantes*, 18, (1). (5)
- Guy, P., Genier, G., 1967. Structure et évolution au cours des générations de multiplication en panmixie d'un hybride autotétraploïde. *Ann. Amél. Plantes*, 17, (3). (6)
- Rotili, P., 1968. Effets de l'autofécondations dans la luzerne. Cinquième Congrès Eucarpia, Milano. (7)
- Rotili, P., 1970. L'autofecondazione nel miglioramento genetica dell'erba medica (self-pollination in alfalfa breeding). *Quad. Sper. Ist. Foraggiere. Lodi n° 1* (English summary pag. 50-69). (8)
- Rotili, P., Zannone, L., 1970. Intra and intergenotypic competition in "*Medicago sativa*" Eucarpia Fodder Crops Section. Lusignan. (9)
- Tysdal, H.M., Kiesselbach, T.A. and Westover, H.L., 1942. Alfalfa breeding *Univ. of Nebraska Agr. Exp. Sta. Bull.*, 124. (10)

METHODS OF YIELD ASSESSMENT DURING INDIVIDUAL
PERFORMANCE TEST AND SUBSEQUENT REALIZATION OF YIELD
POTENTIALS IN SYNTHETICS

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INTRODUCTION

Increased annual forage production has always been high on the list of priorities of grass breeders. Yet, the success in this field falls far short of the considerable achievements in other breeding objectives, like persistency, winter hardiness, seasonal yield etc. Recognised varieties often produce no greater annual D.M. yield than their commercial counterparts or when they exceed them, the differences are usually rather modest. No doubt the wide range of management, practiced on grasslands, accounts for a great deal of the shortcomings in realizing the full potentials of bred grass varieties. It is also possible however that the usual procedures adopted by the breeder for yield assessment in the various phases of his breeding programmes will not necessarily lead to the selection of the potentially best genotypes thereby failing to exploit fully the inherent potential of natural populations. Although it is appreciated that there are numerous alternative methods for yield assessment and their comprehensive evaluation would require large scale and very elaborate experimentation, nevertheless it was thought that some inferences might be drawn from looking at some of the results which were obtained using a few different methods in operational breeding projects.

It is emphasised that the present data, offered for consideration, were drawn from trials, which were part of breeding programmes which had the main objective of supplying simultaneous information on a few natural populations and on a limited number of genotypes within these populations with the ultimate aim of providing material for the constitution of synthetic varieties. It was thought however, that the data, collected in connection with the evaluation of the plant material were also suitable to throw some light on the comparative value of the various management techniques used in connection with yield assessment.

MATERIALS AND METHODS

Two species were included in the projects : Italian ryegrass Lolium multiflorum Lam. and cocksfoot Dactylis glomerata L. Available data relate to the following trial conditions :

A/ Spacing

- 1) **Spaced plants** = 60 x 60 cm spacing, to provide non competitive conditions
- 2) **Close plants** = 12 x 12 cm spacing, to provide competitive conditions
- 3) **Mini-plots** = 5 x 5 cm spacing, to provide 63 plants per one 48 x 38 cm size plot in simulated sward conditions.

B/ Cutting

- 1) **According to general development** : everything cut on the same calendar date, when the medium early material of the trial reached the required stage of development.
- 2) **According to individual development of populations and genotypes.**

All cuttings were carried out at 8 cm above ground, with a "Little Wonder" (Webb) hedge cutter. Plants were helped up by hand and a wooden frame was used to ensure uniform stubble height. The cutting stage was chosen as the 7th day after the recorded ear emergence date.

Fertilizer was given at the rate of 300 N, 50 P and 130 K kg/ha for spacing 1, and 650 N, 100 P and 300 K kg/ha for spacings 2 and 3. P and K were given in 3 equal dosages in March, July and October, N was applied in 6 equal quantities : the first in March and after each cut subsequently.

Ear emergence dates were recorded as follows :

for spacing 1, The appearance of the third inflorescence was observed,

for spacing 2, The time of emergence of the third head was estimated at the time of cutting by measuring the distance of the tips of the inflorescence from the base of the flag leaf and calculating the probable ear emergence date - whether predating or backdating on the basis of 0.7 cm mean elongation growth/day. This assessment followed the pattern of the author's previous work (Emecz 1967) but the actual figure of 0.7 cm was derived from daily measurements on the trial material.

for spacing 3, Ear emergence was measured by the N.I.A.B. technique for 50% emergence.

D.M. yields were obtained from material dried in oven at 80°C for 12 hours,

in plants and 50% ear emergence in plots.

The effect of spacing and of cutting treatments upon the yield of individual plants is rather obvious and well known, efforts were therefore directed to examine possible treatments x genotype interactions. It was considered that the analysis of variance would give insufficient guidance on the possible ultimate influence on selection, should such interaction effects exist, consequently the following aspects were examined :

- a) Possible treatment effects on ear emergence dates.
- b) Possible treatment effects on varietal constitution of the 10 best plants selected from the same populations under various treatments.
- c) Possible existence of regression of ear emergence date upon total D.M. production, using ear emergence as the independent and D.M. yield as the dependent variable.
- d) Possible differences in yield performance of the polycrossed progeny of material selected from the same populations under different treatment.
- e) Possible effects of treatments upon the yield performance of the same progenies

RESULTS AND DISCUSSION

Data are available on cocksfoot with spacing 1 and 2, under cutting management 1. 150 plants were used in each spacing. These belonged to 5 natural populations, denoted by A, B, C, D and E each being represented by 30 genotypes. Plants were established in early April in Hartman pots, which were kept out of doors. Originally 7 seeds were sown per pot but at the 4 week stage these were thinned out and only the best seedling was vigour. Field planting took place at the 10 week stage. In the seeding year, 2 cuts were taken at approximately the 20 cm height stage. The first full year produced 4 cuts. The first was taken 10 days after the mean emergence date of the medium population, subsequent cuts followed the 20 cm height requirement.

There was greater variation in ear emergence dates within populations than between them, but this did not effect the experimental aim to focus attention on the treatment x genotype interaction.

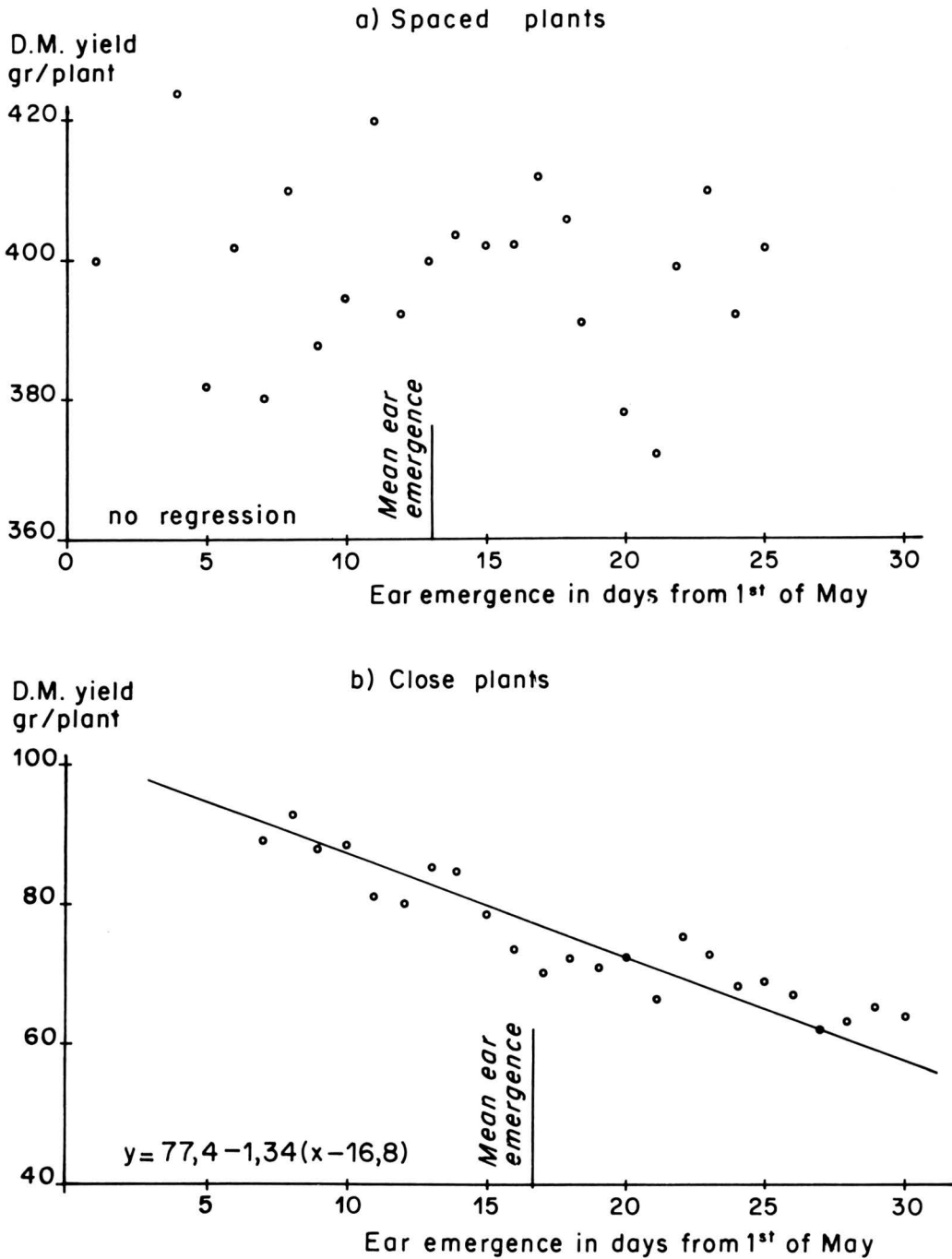
Fig. 1 shows that spacing appears to have affected ear emergence date as both the commencement of ear emergence and the mean ear emergence date were later in the close plants.

Spacing had an effect upon the relationship between ear emergence date and D.M. yield as well. There was no measurable correlation between these features in spaced plants while in close plants ear emergence date had a negative linear regression upon total D.M. yield.

The effect of spacing on the ear emergence dates is in accordance with the

FIGURE I

Relationship between ear emergence date and total D.M. yield in cocksfoot when cut at «general development»



Yield figures represent the total of 6 cuts.
Points represent the mean of plants which emerged on the same day.

Table 1 . Analysis of regression of ear emergence date upon D.M. yield of cocksfoot in close (competitive) spacing and under a "general development" cutting system.

| Source of variance | DF | SS | MS | Source of variance | DF | SS | MS |
|--|-----|----------|--------|--------------------|-----|---------|----------|
| Between groups (Emergence dates) | 24 | 10,307.9 | 429.5* | | 1 | 8825.3 | 8825.3** |
| Within groups (Same ear emergence date) | 125 | 34,251.7 | 274.0 | | 23 | 1482.6 | 64.5 |
| | | | | Residual | 125 | 34251.7 | 274.0 |

(The formula for grouped date was used to give different weights for groups of different size).

* P = 0.05

** P > 0.001

Table 2 . Yield data of cocksfoot plants in two spacings during individual performance test and in subsequent polycross progeny tests in mini-plots.

| Individual performance test | | Progeny test with polycrossed seed, in mini-plots | | | |
|-----------------------------|---------------------------------|---|---|--------------------------------|--------------------------------------|
| Origin of material | Mean D.M. yield gr/plant/6 cuts | % improvement | Origin of material | Mean D.M. yield gr/plot/6 cuts | % improvement over original material |
| Spaced plants ° | 376.0 | | <p>Original unselected seed mixture → F₁ progeny → F₁ progeny</p> <p>L.S.D. at 0.05</p> | 251.2 | |
| Selected 10 best genotypes | 633.6 | | | 277.4 | 10.4 |
| Difference in yield | 257.6 | 68.5 | | 294.7 | 17.3 |
| Close plants ° | 77.4 | | | 14.5 | |
| Selected 10 best genotypes | 121.2 | | | | |
| Difference in yield | 43.8 | 56.6 | | | |

° mean of 150 plants

★ mean of 5 replicates

reports of Calder & Cooper (1961), who also found that wider spacing promoted ear emergence in cocksfoot, and was not due to the different techniques in recording. The effect of spacing in relation to the regression of ear emergence date upon D.M. yield is probably a novelty but is very marked. Table 1 shows that the variation in yield of the plants of the same ear emergence date was quite high which is understandable in unselected material, nevertheless the differences between dates reached significance at 0.05 level. Most of the variation between the mean yield of the ear emergence dates is explainable by the regression, and no significant variation left which is not explainable by the linear association.

Spacing seemed to have had affected the outcome of the selection and the subsequent performance of the progeny as well : Table 2 shows that when the best 10 genotypes were selected, the improvement over the mean of the trial was a somewhat higher percentage in spaced plants while the realization of yield improvement during the first year of the polycross progeny test is greater with the plants derived from close plants. The differences are substantial enough to warrant some attention.

The composition of the 10 best genotypes also varied with spacing : in spacing 1 it was made up of 3, 5 and 2 genotypes of population A, D, E respectively, while in spacing 2 eight genotypes were derived from B and two came from E.

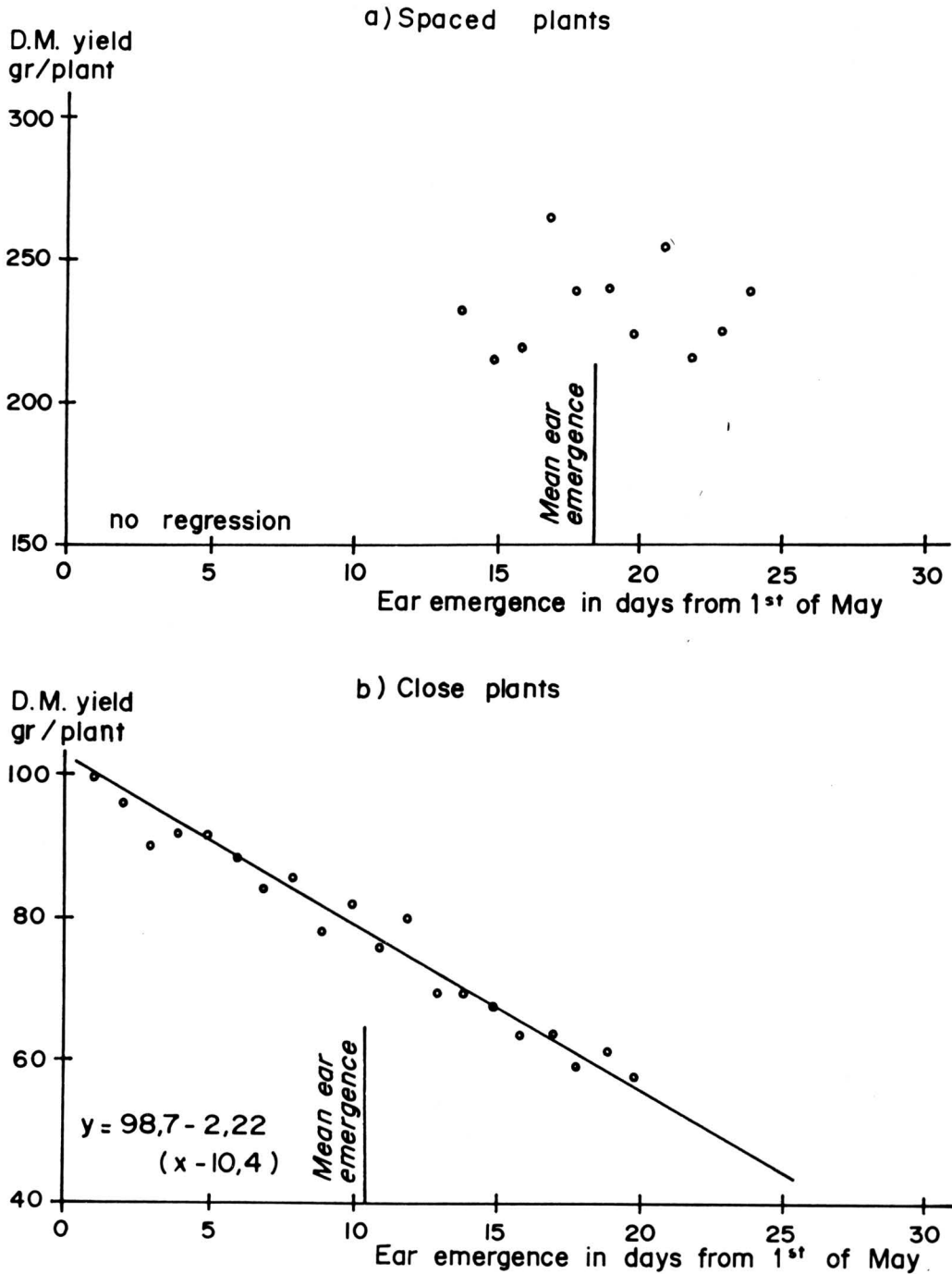
Similar data were also available on Italian ryegrass with the same treatments, but there were 10 natural populations, denoted as A, B, C, D, E, F, G, H, I, J, with 20 genotypes in each. Plants were established in August and planted out to the field at the 6 weeks stage. There was one cut in the autumn of the seeding year and 5 cuts the following year. Results are displayed in Fig. 2 and Table 3. Fig. 2 shows that the general pattern of the spacing effect was very similar to that in cocksfoot. The noteworthy difference was that ear emergence date was promoted by the close spacing and not delayed. Such an effect was observed by the author in earlier works (Emecz 1963, 1966). The association between the ear emergence dates and the total D.M. yield was very much the same as in cocksfoot i.e. no regression in spacing 1 while most of the variation was explainable by a negative linear regression in spacing 2.

The effect of the outcome of the selection was also similar, though even more noticeable. The 10 best genotypes in spacing 1 were made up by 3, 3, 2, 1 and 1 from populations C, E, H and J respectively. In spacing 2, they were mainly derived from population G, (eight genotypes) while F and J also contributed 1-1 genotype. Table 4 shows that the improvement in the yield of the 10 best genotypes over the overall mean was again greater in spacing 1, while the realization of yield potential in the first year of the polycrossed progeny was again greater in the selection from spacing 2.

The analysis exhibited an unusual feature as the residual mean squares were significantly greater in both species than the mean squares representing the deviation from the regression. It would be difficult to identify the exact causes, but it would appear that the effect of ear emergence date was rather

FIGURE II

Relationship between ear emergence date and total D.M. yield in Italian ryegrass when cut at «general development»



Yield figures represent the total of 6 cuts.
Points represent the mean of plants which emerged on the same day.

Table 3 . Analysis of regression of ear emergence date upon D.M. yield of Italian ryegrass in close (competitive) spacing under a "general development" cutting system.

| Source of variance | DF | SS | MS | Source of variance | DF | SS | MS |
|---|-----|----------|-----------|---|-----|---------|------------|
| Between groups (Emergence date) | 19 | 20,878.1 | 1098.9*** | Regression ↙ ↘ Deviation from regression | 1 | 20628.1 | 20628.1*** |
| | | | | | | | |
| Within groups (same ear emergence) date | 180 | 6,731.3 | 37.4 | | 180 | 6731.3 | 37.4 |

The formula for grouped date was used to adjust for different group sizes

*** $P \geq 0.001$

Table 4 . Yield data of Italian rye grass plants in two spacings during individual performance test and in subsequent polycross progeny test in mini-plots.

| Individual performance test | | Progeny test with polycrossed seed in mini-plots | | | |
|-----------------------------|---------------------------------|--|--|--------------------------------|--------------------------------------|
| Origin of material | Mean D.M. yield gr/plant/6 cuts | % improvement | Origin of material | Mean D.M. yield gr/plot/6 cuts | % improvement over original material |
| Spaced plants ° | 240.8 | | → Original unselected seed mixture ★ → F ₁ progeny ★ | 321.3 | |
| Selected 10 best genotypes | 365.6 | | | 355.6 | 10.7 |
| Difference in yield | 124.8 | 51.8 | F ₁ progeny ★ L.S.D. at 0.05 | 393.9 | 22.6 |
| Close plants ° | 98.7 | | | 22.7 | |
| Selected 10 best genotypes | 134.7 | | | | |
| Difference in yield | 36.0 | 36.5 | | | |

° mean of 200 plants

★ mean of 5 replicates

similar on all genotypic variation within date. The latter was very considerable, probably due to the use of unselected material of widely different origin.

A separate test, involving 11 populations of Italian ryegrass, (including those used in the other test) with 170 genotypes each, was grown at spacing 1 but under cutting treatment 2. The results of this are displayed on Fig. 3. It shows similarity to a curvilinear regression of ear emergence date upon total D.M. yield. Several polynomials were fitted and the quadratic, cubic, quartic and quintic were each significant at the 0.1% level, but in each case the R^2 (a measure of how much of the total variation in the dependant variable, D.M. yield - is explainable by the polynomial in ear emergence date) was low. The highest R^2 was in the quintic model, but even this only reached 0.047. This meant that the polynomial model was of no great use. Using logged values of the yield data did not result in any significant improvement in the fitting.

It is noteworthy however that the alteration in the time of cutting effected the type of relationship between emergence date and total D.M. yield which showed a definite advantage for the medium early genotypes under cutting 2, while under cutting 1 there was no definite pattern at all. Some importance of this may be reflected by a further trial in which 40 genotypes of the same material were involved. These were selected to represent ten groups, with 4 genotypes in each. The 4 genotypes within a group had identical ear emergence dates, while there was a 4 day difference of ear emergence between groups. The groups were used separately in a polycross trial. Seed was harvested bulked within group but kept separately for each group. 18 mini-plots were established from the seed of each group. These were used in cutting treatments 1 and 11, giving 9 replicates for each. Results are shown in Fig. 4 and Tables 5 and 6. Both, the overall yield and the best line varied with the treatments, both being higher under cutting 11. The identity of the best line also changed as the earliest line yielded most in cutting 1 while the 5th line was the highest yielder under cutting 11.

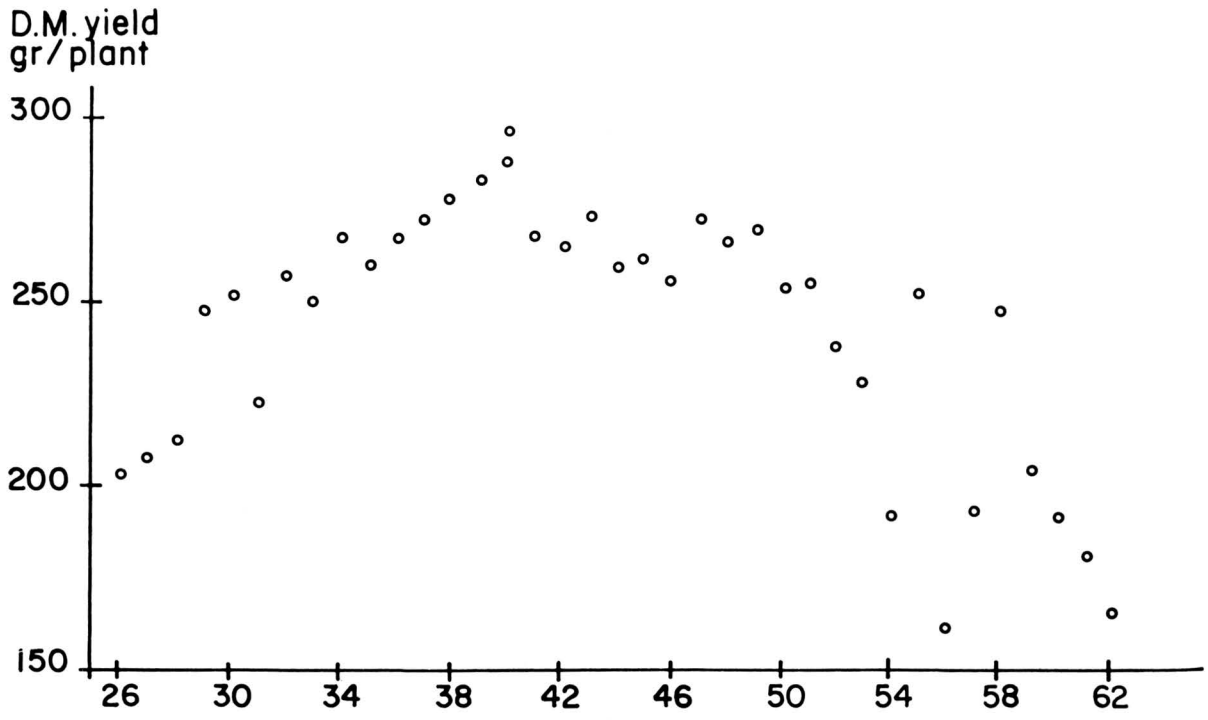
The quality of the forage also must have been affected by the treatments and it should be expected that in cutting 1 the % DDM varied quite drastically from line to line, while in cutting 11 it was probably quite similar for most or even all lines. However it is just an assumption as D.D.M. was not examined.

Admittedly, the period, covered by the trial, was much longer than normally included in one group of a yield assessment trial but it helped to show up the differences more distinctly. Furthermore, even if only 3-3 lines are pooled, the groups would only cover 8 days, nevertheless in the 1st and second groups the best line would vary with the cutting treatment.

The cutting treatment affected the regression of ear emergence dates upon the total D.M. yield ; under cutting 1, the regression was negative and linear while under cutting 11, it was a polynomial, the cubic, quartic and quintic giving equally good fits. In both experiments the regression was highly significant (see Table 6). The R^2 value in the cutting 11 treatment implied that

FIGURE III

The relationship between ear emergence and D.M. yield in Italian ryegrass under «individual development» cutting treatment.



ear emergence in days from 1st of April
Yield figures represent the total of 6 cuts
Points represent the mean of plants which emerged on the same day

Regression analysis (quintic)

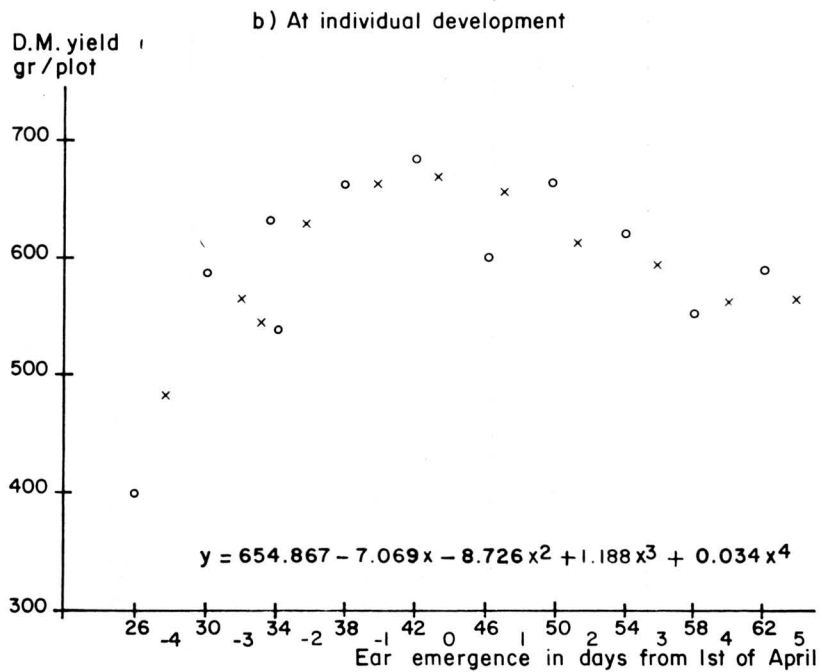
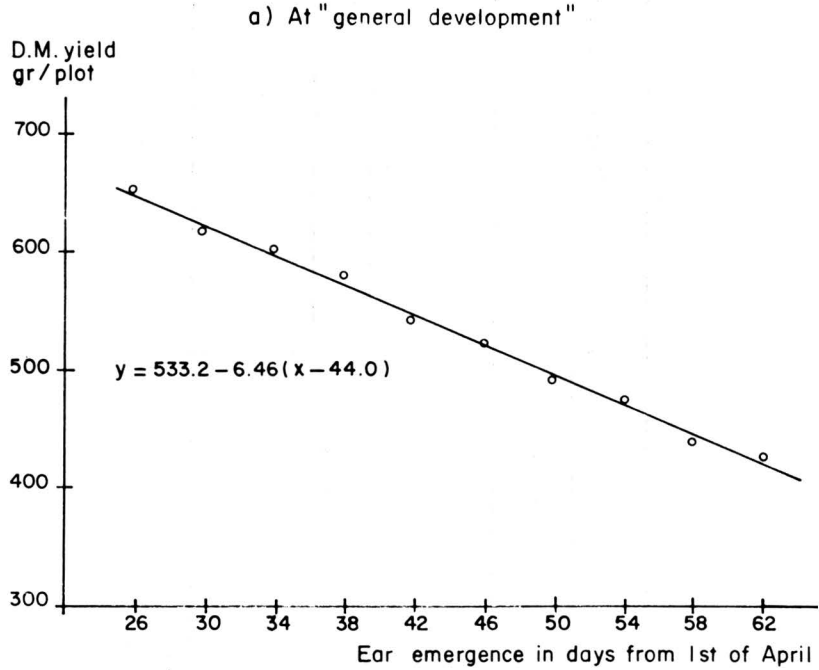
| Source of variance | DF | SS | MS | F |
|--------------------|------|-----------|--------|---------|
| Regression | 5 | 235.100 | 47.020 | 18.46 * |
| Residual | 1864 | 4.748.000 | 2.547 | |
| Total | 1869 | 4.983.100 | | |

$R^2 = 0.047$

* significant at 0.01

FIGURE IV

Regression of ear emergence date upon D.M. yield of Italian ryegrass grown in mini-plots under 2 cutting treatments



Crosses represent fitted data of quartic polynomial
Points represent the mean of 9 mini-plots.

Table 5 . Total D. M. yield of Italian ryegrass lines, grown in mini-plots, under different cutting treatments

| Origin of material | D.M. yield in gr/plot | | D.M. yield of best line gr/plot |
|-----------------------|-----------------------|------------|--|
| | cutting I | cutting II | |
| Original seed mixture | 510.2 ° | 516.4 ° | <hr style="width: 50px; margin: 0 auto;"/> 650.1 ° |
| 10 selected lines | 533.2 ★ | 587.0 ★ | |
| L s d at 0.05 | 16.3 | 13.7 | 28.4 |

° represent the mean of 9 replicates

★ represent the mean of 90 plots

Table 6 . Analysis of regression of ear emergence date upon yield of Italian ryegrass lines grown in plots

a) Under "general development" cutting treatment.

| SOURCE OF VARIANCE | D.F. | S.S. | M.S. | V.R. |
|---------------------------|------|---------|---------|------|
| Regression | 1 | 496,329 | 496,329 | 2057 |
| Deviation from regression | 8 | 1,583 | 198 | |
| Residual | 80 | 12,528 | 157 | |

Total 89 510,440

b) Under "individual development" cutting treatment

| SOURCE OF VARIANCE | D.F. | S.S. | M.S. | V.R. |
|--------------------|------|---------|---------|------|
| Line | | | | |
| Cubic polynomial | 3 | 422,523 | 140,841 | 6.3 |
| Remainder | 6 | 133,828 | 22,304 | |
| Residual | 80 | 16,729 | 232 | |

Total 89 573,080

$$R^2 = 0,7373$$

the variation in D.M. yield is accounted for - to a considerable extent - by the polynomial in the ear emergence date. The exact fitting of a polynomial is of no great practical value ; the real importance lies in the identification of the peak of performance which has a direct influence on the outcome of the selection.

The results are in harmony with previous reports : The N.I.A.B. report (1969) on Italian ryegrass has shown that the yield is reduced by about 1% for each day delay in ear emergence, under a cutting I type of management, giving a negative linear regression. The author's former results (1966) on the other hand indicated a polynomial relationship between ear emergence date and yield, when cutting was adjusted to developmental stage of each population individually.

CONCLUSIONS

The results indicate that the management, applied during individual performance test as well as during the first progeny assessment trial could have a substantial influence on both the genotypic constitution of the ultimate selection and the subsequent realization of the inherent yield potential of this selection.

The regression analysis, involving the ear emergence date and the total yield, gave a useful type of information on the likely differences in the ultimate selection groups as constituted on the basis of differential management during individual performance test. It appeared that some of the management tended to obscure the true potential of the medium maturity group in the trial, thereby leading to the selection of potentially inferior genotypes.

The data do not give an accurate reflection on the possible improvement over the mean of the original population as heavy selection pressure was applied in the early stage for seedling vigour, thereby probably eliminating many of the poorer performers. Earlier results (1965) of the author have indicated that a poor performance in the seedling stage was usually associated with low performance in the seeding year, and often also in the first full year.

The apparent effect of spacing and cutting management could have a feasible explanation in the possible change in the limiting factor which is likely to result from such changes. Water, light, nutrient supply are all more likely to become limiting under competitive conditions, while temperature might be the main limiting factor in wide spacing. A simultaneous cutting, adjusted to the medium group, tends to favour the early plants or populations as regards D.M. yield as they take greater advantage of the higher growth rate normally associated with the reproductive development of the tillers, up to the stage of anthesis (Emecz 1962). Individual cutting on the other hand allows a greater manifestation of the real inherent yield potential.

It appears from the data, that if simultaneous cutting is essential, the

breeder could gain some advantage from competitive spacing during individual performance tests. Even greater benefit may result if individual cutting can be applied to plants in wide spacing. It would be logical to assume that the combination of individual cutting time with competitive spacing could further increase the precision of the yield assessment, but this management is rather complicated to organize.

While it would be too hasty to claim that the results discussed provide sufficient guidance to use the possible management practices during the breeding programme to full advantage, the regularly emerging similar patterns and the extent of the differences obtained from different approaches suggest that breeders who would completely ignore the implications may miss valuable opportunities in exploiting the inherent capabilities of existing populations.

REFERENCES

- Calder, D.M. & Cooper, J.P. 1961. The effect of spacing and nitrogen level on floral initiation in cocksfoot. *Nature*, Lond. 191 195-6.
- Emecz, T.I. 1962. The effect of meteorological conditions on anthesis in agricultural grasses. *Ann.Bot. Lond. N.S.* 26 159-172.
- Emecz, T.I. Breese, E.L. Lewis, E.J. 1964. Tetraploidy in Italian ryegrass. *Annu. Rep. Welsh Pl. Breed. Sta.* 1963. 29-33.
- Emecz, T.I. 1966. Yield assessment in the progeny test phase of grasses. *Proc. Int. Symp. Var. Test. Budapest.* 1966.
- Emecz, T.I. 1968. A selection programme in cocksfoot. *Annu. Rep. Welsh Pl. Breed. Sta.* 1967, 46-9.
- N.I.A.B. 1969. Farmers leaflet. Recommended varieties of grasses. Cambridge.

MORPHOLOGICAL CHARACTERISTICS AND EXPRESSION OF VIGOUR

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Most of the work of breeding forage crops is made in spaced plants nurseries, whereas the desired cultivars must be designed to be used in dense swards. Are the yields in both conditions in good correlations? Is it possible to find morphological characteristics linked to yield in swards, and less susceptible to competition? Such characteristics would be most important for breeding.

METHODS

A set of genotypes or cultivars has been studied at varying densities: spaced plants (70 x 70 cm or 50 x 50 cm), "micro-trials" (2 x 20 cm, 5 x 20 cm or 10 x 20 cm) and "swards" (rows 20 cm apart). For each density, the yield and the number and size of organs were studied.

As a first step, correlations have been established between yield in swards and different characteristics in other densities.

As a second step, all data obtained will be submitted to multivariate analysis, in order to get selection indices for yield.

RESULTS PER SPECIES

a) **Lucerne** (M.T. Chesneaux, P. Guy, A. Porcheron)

For 25 F₂ hybrids, four characteristics have been measured on spaced plants: yield, height, width and number of stems.

The best spaced plant character to predict the annual sward yield is the height. Width and number of stems are negatively correlated with each other and are of no use for such a prediction (table 1).

The study of correlations between characters within a given density also leads to the same conclusions:

- Height is the most representative factor of the yield. It is the less susceptible one to competition.

- Width and number of stems are always negatively correlated with each other and not correlated to yield.
- The first cut yield gives a precise idea of the potential annual yield.

Although there is no strong genotype x density interactions, these results show that selection in spaced plants for sward yield may become more accurate, by working at the same time on yield and height. The most adapted type for dense conditions seems to be a very high one (this character is very stable and heritable), with middle-wide but close tufts and rather thick stems.

b) **Cocksfoot** (J.C. Bertholleau, J.P. Gachet, A. Gallais)

In the seeding year, no genotype x density interaction has been observed. But an orthogonal regression analysis has shown two important indices to predict sward yield : an index of general vigour (number and size of tillers) and an index of photosynthetic efficiency (habit as measured by % of erect plants, and leaf width).

At the spring cuts of following years, there is also a good correlation between spaced plants yields and sward yields ($r = .80^*$), but mostly due to a slight earliness variation between genotypes, although chosen in a same group of earliness. The influence of developmental stage on vigour has thus to be eliminated.

With equal earliness, the partial correlation between yields in the two conditions is much lower and non-significant. At the first cut, the number of reproductive tillers is the most important factor ; at the second cut, it is the mean weight of the vegetative tillers.

The total yield of the two cuts shows a very good correlation with leaf length ($r = .86^*$) as measured at the first cut.

In another trial, the correlation between yields at the two densities, as corrected by earliness, appears to vary according to developmental stage : this will be further specified.

Summer and autumn cuts have been to a large extent dependent upon environment. Nevertheless, the same two factors as in seeding year appear to be the most important ones.

As a whole, some sets of characters, measured on spaced plants, come steadily out as favourable to sward yield : number and weight of the reproductive tillers, size of the vegetative ones, leaf length, erect habit. For total annual yield, habit and leaf length have a good predictive value ($r = .77^*$ and $.68^*$).

In order to be adapted to competition, a cocksfoot genotype must have an erect habit, long leaves and stems, and many stems in aftermath (such stems have never been observed in swards).

c) **Meadow fescue** (M. Gillet, J. Jadas-Hecart)

Yield, number and mean weight of tillers have been studied on 12 varieties in sward and spaced plants. In the latter condition, tuft area has also been measured.

None of the spaced plant characteristics has been correlated with any sward one.

Data will be further studied with multivariate analysis.

d) **Tall fescue** (M. Gillet, J. Jadas-Hecart)

23 varieties have been studied in spaced plants and swards. There was a range of different earliness, the influence of which was eliminated by partial correlations. The size of organs (stems, different leaves) has been measured. Partial correlation calculations was applied to these data, in order to separate their own influence from each other.

In the seeding year, there were positive correlations between yields at the two densities ($r = .58^*$ and $.87^*$ for two cuts, respectively). Spaced plants leaf length was also a good criterion of sward yield ($r = .79^*$ and $.60^*$).

Of the following year, only the first, reproductive cut has yet been analysed.

For this cut, when the influence of all morphological characteristics has been eliminated, there still is a good correlation between the yields of the two stands. But it disappears under the influence of the morphological characters : actually, for a given yield in spaced plants, a big size of all organs reduces the sward yield ; nevertheless this size is correlated with a good "nursery" yield : probably they are all influenced by the general vigour of the plant.

For this first, reproductive cut, the best variety would have in spaced plants a good vigour with little organs. Such a variety must thus have numerous stems.

Other cuts have not yet been fully analysed, but we have shown that the breadth of leaves at all cuts are in good correlation with each other. This holds equally for length of leaves but the last one before head, which is rather more linked to breadth, as if under floral influence, this leaf tended to a fixed length/breadth ratio, fully realised in glume.

e) **Italian rye-grass** (P. Mansat, J.C. René)

10 ecotypes of a same earliness have been studied in swards, "micro-trials" (2 cm and 5 cm between plants on the row) and spaced plants nursery : this work is completed by measures made on other micro-trials.

Table 1 . Correlations between sward yield and spaced plants characteristics in lucerne

| Spaced plants Swards | Height | 1 st cut yield | Annual yield | Stem width | Stem number |
|--------------------------------|--------|-------------------|-----------------|---------------|----------------|
| 1 st cut G.M. yield | .78* | .71* | .54 | .14 | .22 |
| Annual G.M. yield | .83* | .71* | .73* | | |

Table 2 . Correlation for yield between nursery and 3 other densities in Italian Ryegrass

SW = Sward

Mi 2cm } Microtrials (see text)
Mi 5cm }

| | 13/08/63 (year of sowing) | 13/05/64 (earling stage) | 23/06/64 (earling regrowth) | 21/10/64 (vegetative regrowth) |
|---------|------------------------------|-----------------------------|--------------------------------|-----------------------------------|
| sw | .76** | .73** | .65* | .69* |
| Mi 2 cm | .88** | .87** | .79** | |
| Mi 5 cm | .86** | .78** | .67* | |

* Significant at 5 % level

** Significant at 1 % level.

- Within sward, the yield is not correlated to the number of tiller but to their mean weight for 3 cuts and to the area of individual leaves at the 2 first ones. Number and weight of tillers are of course negatively correlated.

- Within micro-trial, the yield is also correlated with individual leaf area and not to tiller number, at all cuts, for a set of diploid and tetraploid clones.

- Within nursery, the yield is linked with all characters : weight of tillers, area of leaves or number of tillers. Spacing allows for expression of all these characters.

It appears that weight of tiller and leaf area are more stable characters than number of tiller.

Which ones, measured on spaced plants or micro-trials, are in relation to sward yield ?

None of the micro-trial data gives a repeated correlation with it. On the other hand, on the two last 1964 cuts, length and area of nursery leaves are clearly correlated with sward yield, whereas the number or mean weight of tillers was not (Unfortunately, the size of leaves was not measured at the first cut).

Nevertheless, nursery yield is highly correlated with sward and micro-trial yield (table 2).

Van Bogaert has shown that the vigour of individual plants, when eye-noted, was in good correlation with weight of the same plants. The selection criterion in spaced plants is thus simple : general vigour and leaf size.

Some observations made on perennial ryegrass lead to similar conclusions : the weight of organs is more important than their number, at least when the latter is not too high. For example, for Devon Eaver, which has very numerous little tillers, the yield is made by their number. But between Primevère, Raidor and Reveille with middle tillering, the weight of organs is more important.

CONCLUSION

This work has not yet been fully analysed. But the present data show that similar trends appear in most cases :

- there is a positive correlation between vigour at different densities,

- in most cases genotypes with big organs are more adapted to competition than those with numerous organs. This is true, either for alfalfa, where all cuts are fully reproductive, or for vegetative cuts of grasses (reproductive in case of italian ryegrass),

- for grasses, it seems that the ability to make numerous reproductive stems in spaced plants is linked with a good vigour in dense conditions, even if the sward is, there, only vegetative.

INTRA AND INTERGENOTYPIC COMPETITION IN MEDICAGO SATIVA

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INTRODUCTION

In this paper we report the results of our second step of experiments to find out the competition effects on some quantitative characters of lucerne (*Medicago sativa*).

In our first series (4) the study was effected :

- a) on seedlings of 28 and 48 days in greenhouse (at two water levels and two densities : 200 and 500 seeds per metre) ;
- b) on fully grown plants transplanted in field at the density of 300 plants per square metre, after 90 days of greenhouse growing.

The competition effects were measured as "resistance to competition" taking the spaced plants as reference.

From these studies we concluded that :

- a) at the same numerical density, the genotype which has a higher yield in pure plot increases its yield in mixture ; on the contrary, the lower yielding genotype reduces its yield in mixture ; we explained these results by means of the "biological density" ;
- b) all the mixtures on trial presented a lower yield than the most vigorous constituent in pure stand. This result does not justify the assertion according to which, to neutralize the negative competition effects, the use of mixtures should be considered with varieties having a good aptitude to cooperate for yield.

The aim of the present work is to test the validity of these results on wider material grown closer to field conditions.

MATERIAL AND METHODS

The following 10 genotypes were used :

Variety : Florida - 1558 S₀ - 1558 S₁ - 1564 S₀ - 1564 S₁ - 1564/4425 S₂ -
1564/4425/5385 S₃

Variety : Friulana- 649 S₀ - 862 S₀ - 862/3375 S₂ - 1753 S₀

These genotypes were studied in the following 5 experiments :

Exp. 1 - 1558 S₀ and 1564 S₀ in pure stand and in mixture at fifty per cent

Exp. 2 - 1558 S₁ and 1753 S₀ in pure stand and in mixture at fifty per cent

Exp. 3 - 862 S₀ and 862/3375 S₂ in pure stand and in mixture at fifty per cent

Exp. 4 - 649 S₀, 1558 S₀ and 1564 S₀ in pure stand and in mixture at 33.3 per cent of each component

Exp. 5 - 1564 S₀, S₁, S₂, S₃ in pure stand and in mixture at 25 per cent of each component.

Each genotype, except 862 S₀ and 862/3375 S₂, was also grown as spaced plants. The competition growing (pure stand and mixture) was at the density of 200 seeds per metre (=1000 plants a square metre). The spaced plants were 30 x 15 cm^s apart. This density, at the two first cuttings, is roughly equivalent to a density of 4 plants per square metre if transplanted 90 days after sowing.

The seeds were calibrated (1.5 to 1.6 mm.) and pierced by a needle after 12 hours in Petri dishes, to avoid the effects of size and hardness ; in this way, all the seeds were at the same rate of swelling at sowing time.

In each experiment 850 seeds were sown : 50 seeds as spaced plants and 800 in the competition plots, 400 in pure stand and 400 in mixture.

Soil homogeneity, both in structure and in quality, was obtained by excavating a bunker 2.10 metres wide, 50 cm. deep and 11.00 metres long, and filling it with well mixed soil. Experiment n. 3 was carried out in different soil ; nevertheless, the results of this experiment agree very well with the others, as we shall see later.

The seeds were planted in rows 2 metres long, separated by tin strips 2 metres long and 40 centimetres deep : this arrangement allowed us to give each row the same amount of soil and water (the experimental field was covered by a transparent plastic roof).

In the two-component mixtures the genotypes systematically alternated along the row. In the others the component genotypes were randomized.

In each row the experimental data were collected only on the 200 plants of the central metre. Three cuttings were effected. At the two first cuttings,

the data were collected on per-plant basis. As the mortality at the first cut was very low (Table 1), each mean value represents a sample going from 197 to 200 plants. The data collected on per-plant basis were : dry matter weight, height, number of stems ; at the second cut the percentage of blooming plants and the total number of inflorescences per plant were collected too. At the third cutting only the total production of the pure plots and the mixed plots was registered. The experimental arrangement used for the two-component mixtures permitted the total production of each single component to be revealed.

RESULTS

Competition effects on the average values

Resistance to competition

The averages per plant are reported in the Tables from 2 to 5. As in the previous experiments, the competition effects have been evaluated as "resistance to competition" by

$$\frac{x_c}{x_i} \cdot 100$$

where x_i = character mean value in spaced plants and x_c = character mean value in pure stand (intra-genotypic competition) or in mixture (inter-genotypic competition).

Dry matter weight - From the values plotted in figure 1 we can observe :

- 1) the resistance to competition is related to the nature of genotype. At the density in study it shows very low values (under 10 in pure stand), which sharply decrease at the second cut for all the genotypes both in pure stand and in mixture. This is because the increase of the dry matter weight in the spaced plants is much greater than in pure stand or in mixture.
- 2) among all the genotypes on trial, only 649 S₀ (exp. 4) shows no difference between the resistance value of pure stand and that of mixture. The increase or decrease of resistance to competition in the mixture is not related to the resistance values in pure stand, but to the dry matter mean values of genotypes in pure stand : the genotype which is more productive in pure stand increases its resistance to competition if grown in mixture ; the contrary occurs for the less productive genotype.

This performance is the same in all the experiments and for the two cuttings.

Particularly :

- in the two-component mixture, competition benefits the genotype having the

Table 1 . Percentage of mortality and minimal plants

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | | 5 | | | |
|-------------------------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₁ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| Percentage of mortality | | | | | | | | | | | | | |
| 1rst Cutting | | | | | | | | | | | | | |
| Spaced plants | 2 | - | - | 4 | - | - | - | 2 | - | - | - | - | - |
| Pure stand | - | - | - | 1 | - | 4 | 1 | - | 2 | - | 2 | 3 | 3 |
| Mixture | - | 1 | 1 | - | 4 | 2 | - | 1 | - | - | - | - | 2 |
| 2nd Cutting | | | | | | | | | | | | | |
| Spaced plants | 2 | - | - | 4 | - | - | - | 1 | - | - | - | - | - |
| Pure stand | - | 6 | 5 | 4 | 2 | 11 | 4 | 6 | 6 | 6 | 6 | 19 | 9 |
| Mixture | 3 | 7 | 3 | 13 | 14 | 14 | 2 | 2 | 4 | 2 | 7 | 16 | 9 |
| Percentage of minimum plants | | | | | | | | | | | | | |
| 1rst Cutting | | | | | | | | | | | | | |
| Spaced plants | - | - | - | - | - | - | - | - | - | - | - | 24 | 4 |
| Pure stand | - | - | - | - | 9 | 15 | - | - | - | - | - | 1 | - |
| Mixture | - | - | - | - | 5 | 27 | - | - | - | - | - | - | - |
| 2nd Cutting | | | | | | | | | | | | | |
| Spaced plants | - | - | - | - | - | - | - | - | - | - | - | - | 2 |
| Pure stand | 4 | 4 | 3 | 6 | 16 | 22 | 3 | 4 | 5 | 5 | 6 | 6 | 3 |
| Mixture | 3 | 6 | 3 | 3 | 8 | 53 | 1 | 1 | 7 | 3 | 8 | 4 | 3 |

Note - The minimal plants are those with both height and weight values too small to be registered.

Table 2 . Dry matter weight - mean values (g.)

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | | 5 | | | |
|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₁ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| 1rst Cutting | | | | | | | | | | | | | |
| Spaced plants | 5.43 | 5.64 | 4.74 | 4.50 | - | - | 5.58 | 5.43 | 5.64 | 5.64 | 3.65 | 3.62 | 2.78 |
| Pure stand | 0.36 | 0.34 | 0.35 | 0.34 | 0.27 | 0.19 | 0.36 | 0.45 | 0.41 | 0.43 | 0.34 | 0.36 | 0.27 |
| component values | 0.41 | 0.28 | 0.44 | 0.20 | 0.39 | 0.15 | 0.36 | 0.53 | 0.38 | 0.76 | 0.25 | 0.24 | 0.14 |
| mean | 0.34 | | 0.32 | | 0.28 | | 0.43 | | | | 0.35 | | |
| 2nd Cutting | | | | | | | | | | | | | |
| Spaced plants | 11.64 | 9.97 | 9.40 | 8.53 | - | - | 11.59 | 11.64 | 9.97 | 9.97 | 6.76 | 9.72 | 7.54 |
| Pure stand | 0.42 | 0.37 | 0.43 | 0.37 | 0.44 | 0.29 | 0.44 | 0.49 | 0.42 | 0.50 | 0.30 | 0.49 | 0.39 |
| component values | 0.51 | 0.29 | 0.58 | 0.18 | 0.69 | 0.19 | 0.42 | 0.61 | 0.35 | 0.93 | 0.18 | 0.30 | 0.15 |
| mean | 0.41 | | 0.39 | | 0.48 | | 0.45 | | | | 0.41 | | |

Table 3 . Height - mean values (cm.)

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | | 5 | | |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₁ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| 1rst Cutting | | | | | | | | | | | | |
| Spaced plants | 84 | 70 | 83 | 68 | - | - | 75 | 84 | 70 | 53 | 49 | 43 |
| Pure stand | 57 | 56 | 60 | 57 | 36 | 30 | 65 | 62 | 57 | 50 | 55 | 55 |
| Mixture component values | 58 | 50 | 65 | 47 | 37 | 27 | 64 | 69 | 62 | 48 | 51 | 43 |
| mean | 54 | | 56 | | 33 | | 65 | | | 54 | | |
| 2nd Cutting | | | | | | | | | | | | |
| Spaced plants | 118 | 108 | 123 | 112 | - | - | 120 | 118 | 108 | 91 | 91 | 82 |
| Pure stand | 50 | 50 | 56 | 52 | 40 | 31 | 58 | 54 | 50 | 44 | 54 | 58 |
| Mixture component values | 54 | 43 | 61 | 39 | 45 | 28 | 57 | 64 | 51 | 37 | 48 | 35 |
| mean | 49 | | 51 | | 38 | | 57 | | | 49 | | |

Table 4 . Number of stems - mean values

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | | 5 | | | |
|--------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₀ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| 1st Cutting | | | | | | | | | | | | | |
| Spaced plants | 4.96 | 3.82 | 4.00 | 4.07 | - | - | 5.28 | 4.96 | 3.82 | 3.82 | 4.87 | 5.24 | 5.34 |
| Pure stand | 1.00 | 1.01 | 1.00 | 1.02 | 1.09 | 1.22 | 1.02 | 1.02 | 1.05 | 1.11 | 1.08 | 1.14 | 1.07 |
| component values | 1.01 | 1.02 | 1.00 | 1.01 | 1.25 | 1.05 | 1.02 | 1.02 | 1.05 | 1.14 | 1.06 | 1.03 | 1.05 |
| mean | 1.01 | | 1.01 | | 1.16 | | 1.03 | | | | 1.07 | | |
| 2nd Cutting | | | | | | | | | | | | | |
| Spaced plants | 7.78 | 10.00 | 7.28 | 8.11 | - | - | 9.70 | 7.78 | 10.00 | 10.00 | 10.19 | 10.26 | 10.71 |
| Pure stand | 1.39 | 1.43 | 1.41 | 1.41 | 1.51 | 1.57 | 1.47 | 1.54 | 1.62 | 1.75 | 1.64 | 1.72 | 1.66 |
| component values | 1.54 | 1.45 | 1.57 | 1.21 | 2.00 | 1.33 | 1.51 | 1.65 | 1.62 | 1.81 | 1.59 | 1.41 | 1.52 |
| mean | 1.50 | | 1.40 | | 1.72 | | 1.59 | | | | 1.59 | | |

Table 5 . Percentage of blooming plants and average number of inflorescences per plant.

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | | 5 | | | |
|--------------------------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₁ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| Percentage of blooming plants | | | | | | | | | | | | | |
| Spaced plants | 100 | 98 | 100 | 98 | - | - | 100 | 100 | 98 | 98 | 100 | 80 | 93 |
| Pure stand | 15 | 20 | 21 | 16 | 25 | 12 | 28 | 19 | 22 | 26 | 14 | 30 | 3 |
| Mixture | 23 | 11 | 31 | 4 | 28 | 7 | 21 | 38 | 25 | 51 | 3 | 16 | 2 |
| Number of inflorescences | | | | | | | | | | | | | |
| Spaced plants | 27 | 34 | 22 | 18 | - | - | 29 | 27 | 34 | 34 | 22 | 32 | 14 |
| Pure stand | 0.39 | 0.50 | 0.63 | 0.42 | 0.50 | 0.21 | 0.65 | 0.63 | 0.86 | 0.82 | 0.37 | 0.75 | 0.05 |
| Mixture | 0.75 | 0.25 | 1.08 | 0.05 | 0.60 | 0.09 | 0.60 | 1.57 | 0.70 | 1.97 | 0.08 | 0.32 | 0.02 |

FIGURE I

Resistance to competition - dry matter weight
(P = pure stand; M - mixture)

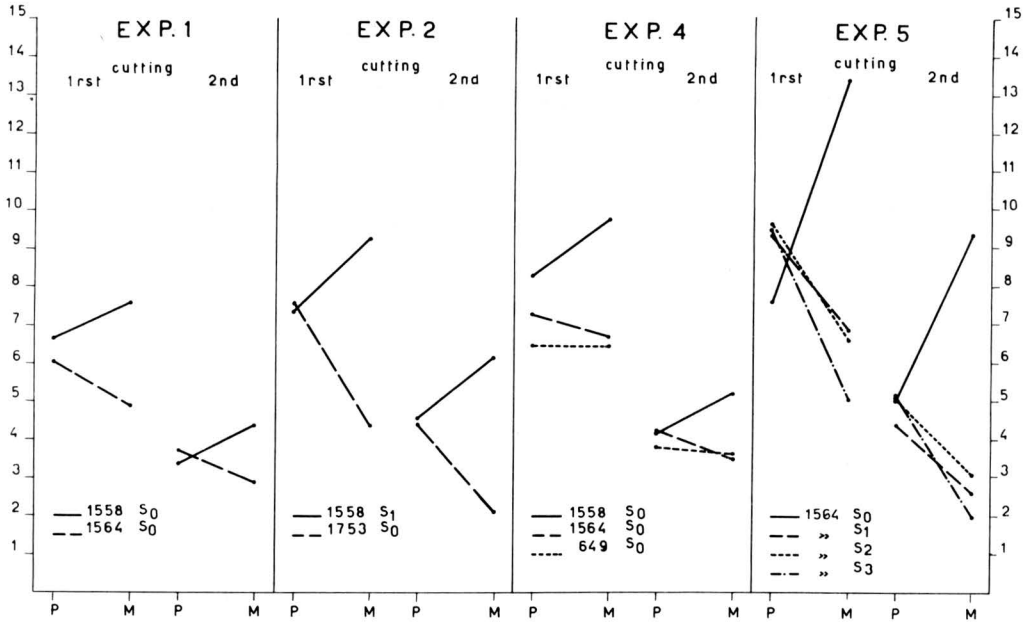


FIGURE II

Resistance to competition - height

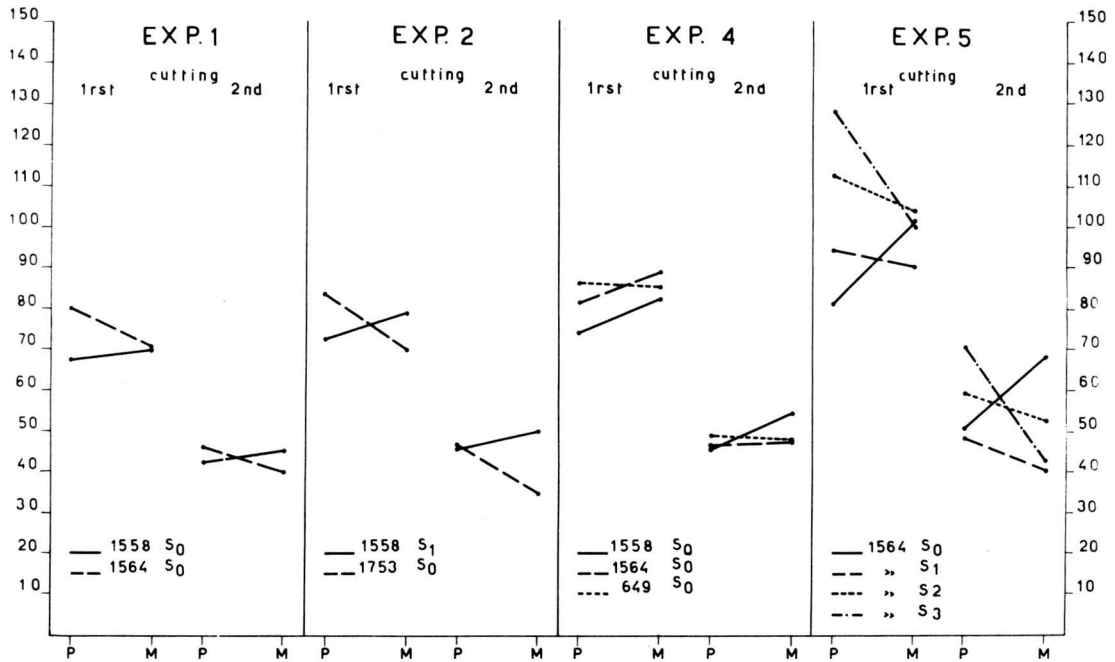


FIGURE III

Resistance to competition - number of stems

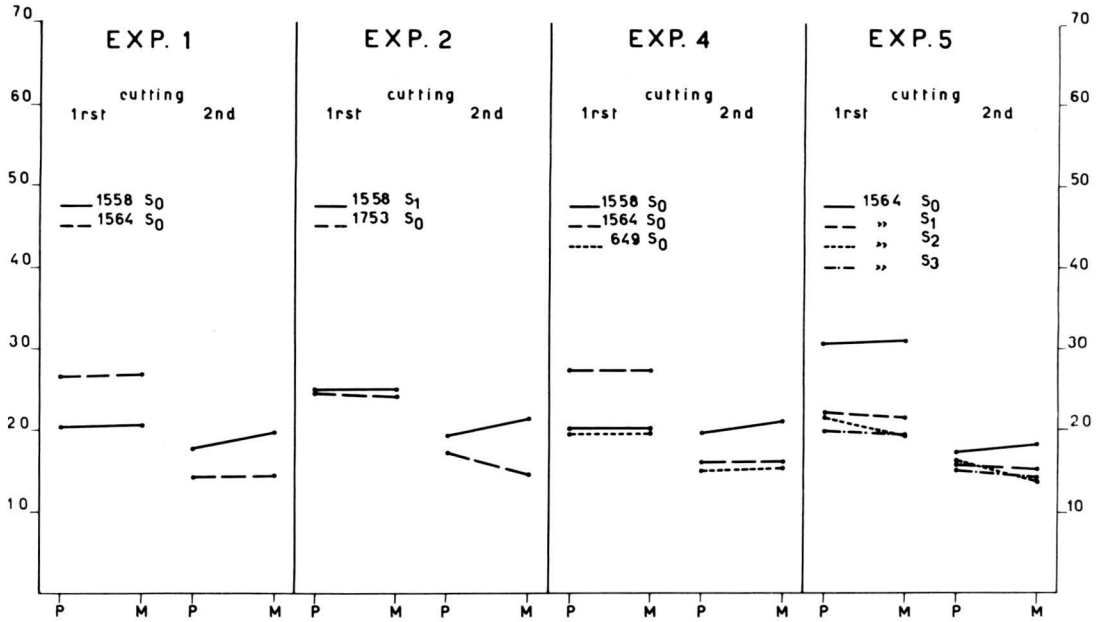
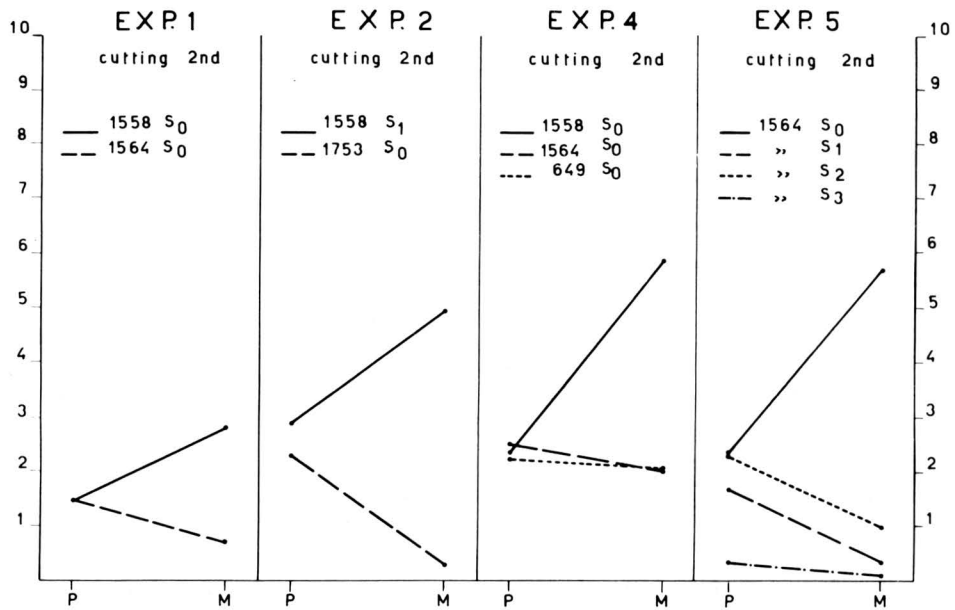


FIGURE IV

Resistance to competition - number of inflorescences



greater average in pure stand and it is to the disadvantage of the weaker genotype, making the difference between the genotypes become significant ;

- in the three-component mixture, the genotype 649 is "indifferent" to the situation of intergenotypic competition (mixture), both at the first and at the second cutting, although it has a lower average than the genotype 1564 (first cut) which is depressed by the mixture condition ;
- in the four-component mixture, only the genotype having the greater average is benefited, the three others are drastically depressed.

These results agree very well with those of the first step of research carried out at the density of 300 plants per square metre.

Height (Figure 2). As in the previous experiments, the effects of competition on this character are much lower than on the yield. Furthermore, these effects are not always depressive : the genotypes 1564/4425 S_2 and 1564/4425/5385 S_3 show, at the first cut, resistance values over 100 in pure stand. Concerning the comparison of resistance values in pure stand or in mixture, the observations made for the dry matter weight are still valid. On this point the results are therefore different from those of the cited experiment at the density of 300 plants per square metre, in which the resistance to competition of the genotypes was the same both in pure stand and in mixture.

Number of stems (Figure 3). This character is more susceptible than the plant height and less susceptible than the dry matter weight. At the first cutting the resistance is quite similar in pure stand and in mixture for all the genotypes, except 1564/4425 S_2 (exp. 4). At the second cutting, on the contrary, the more vigorous genotypes (greater dry matter weight) show a greater resistance in mixture than in pure stand condition.

From the results of dry matter weight, plant height and number of stems, we can deduce that the competition effect is strong also on another plant character : the stem thickness.

Number of inflorescences (Figure 4). This is the character which is most susceptible to competition. Genotypes with a high dry matter weight gain great benefit from the mixture.

Relation between mixture and pure stand - Sensibility of the characters

The position of the studied characters as regards the resistance to competition is the following : 1) plant height ; 2) number of stems ; 3) dry matter weight ; 4) number of inflorescences. This classification completely agrees with that obtained in previous research.

By inspecting the relationship between mixture and pure stand we can discover the sensibility of the studied characters to intergenotypic competition at the

density of 1000 plants per square metre. In Table 6 the genotype values are evaluated by

$$\frac{x - x_0}{x_0}$$

where x_0 is the character value in pure stand (intragenotypic competition) and x its value in mixture (intergenotypic competition).

The following considerations should be made :

- 1) All in the mixtures the greater variation is shown by the number of inflorescences ; in order follow the dry matter weight, plant height and number of stems, the genotypes 862 S₁ and 862/3375 S₂ form an exception, as the number of stems is more sensible than the plant height.
- 2) The range of dry matter weight variation is not justified by the variations in plant height and number of stems. This seems to indicate the existence of a great sensibility of the stem thickness.
- 3) As regards the studied characters, the increase or the decrease of the dry matter weight in the mixture vs the pure stand is reached at the first cutting only through the concurrence of the plant height and, in a lesser degree, of the number of stems (with the exception of experiment n. 3).

In conclusion, the number of stems is more affected by competition than the plant height when the spaced plants are taken as reference whereas, when the comparison is made between mixture and pure stand at the same density of 1000 plants per square metre, the number of stems results less sensible than the plant height. At the density of 300 plants per square metre, on the contrary, it still remains more sensible than the plant height (Table 11). This seems to indicate that as the numerical density increases, the rôle of plant height increases too and that of the number of stems decreases : when this latter character has reached the minimum limit, the plant reacts with a modification of height and stem thickness. It is presumable that the minimum limit of the number of stems is related not only to the density of growing but also to the genotype nature.

These observations seem to indicate that each genotype has its own form or shape, depending on the number of stems, the height, the thickness of the stems and the foliage. There may be an "ideal form" of the lucerne plant which would give maximum yield. In this case, it would be necessary not only to create varieties resembling this ideotype, but also to discover the cultivation methods which would render the newly created form as similar as possible to the chosen model. Obviously, there would need to be more than one ideal type of lucerne ; the "type" which gives maximum yield in ventilated hay would very likely prove mediocre in dehydrated products.

Table 6 . Mixture vs pure stand (per-plant mean values)

| CHARACTERS | Dry matter weight | | Height | | Number of stems | | Number of inflorescences |
|---------------------|-------------------|--------|--------|--------|-----------------|--------|--------------------------|
| | 1rst | 2nd | 1rst | 2nd | 1rst | 2nd | 2nd |
| Experiment 1 | | | | | | | |
| 1558 S ₀ | + 0.14 | + 0.31 | + 0.01 | + 0.08 | 0.00 | + 0.10 | + 0.92 |
| 1564 S ₀ | - 0.18 | - 0.22 | - 0.11 | - 0.14 | + 0.01 | + 0.01 | - 0.50 |
| Experiment 2 | | | | | | | |
| 1558 S ₁ | + 0.26 | + 0.35 | + 0.08 | + 0.09 | 0.00 | + 0.11 | + 0.71 |
| 1753 S ₀ | - 0.41 | - 0.51 | - 0.18 | - 0.25 | - 0.01 | - 0.14 | - 0.88 |
| Experiment 3 | | | | | | | |
| 862 S ₀ | + 0.44 | + 0.56 | + 0.04 | + 0.12 | + 0.14 | + 0.33 | + 0.20 |
| 862 S ₂ | - 0.22 | - 0.35 | - 0.10 | - 0.11 | - 0.14 | - 0.16 | - 0.56 |
| Experiment 4 | | | | | | | |
| 649 S ₀ | 0.00 | - 0.05 | - 0.02 | - 0.02 | 0.00 | + 0.02 | - 0.08 |
| 1558 S ₀ | + 0.18 | + 0.24 | + 0.11 | + 0.18 | 0.00 | + 0.07 | + 1.49 |
| 1564 S ₀ | - 0.07 | - 0.17 | + 0.09 | + 0.02 | 0.00 | 0.00 | - 0.19 |
| Experiment 5 | | | | | | | |
| 1564 S ₀ | + 0.76 | + 0.86 | + 0.21 | + 0.34 | + 0.02 | + 0.03 | + 1.40 |
| 1564 S ₁ | - 0.27 | - 0.40 | - 0.04 | - 0.16 | - 0.02 | - 0.04 | - 0.78 |
| 1564 S ₂ | - 0.34 | - 0.39 | - 0.08 | - 0.12 | - 0.10 | - 0.19 | - 0.57 |
| 1564 S ₃ | - 0.49 | - 0.62 | - 0.22 | - 0.40 | - 0.02 | - 0.09 | - 0.60 |

Table 7 . Observed vs estimated mixture and vs maximum pure stand value

| | Observed <u>vs</u> estimated mixture | | Observed mixture <u>vs</u> maximum pure stand | |
|---------------------|--------------------------------------|----------|---|----------|
| | per-plant | per-plot | per-plant | per-plot |
| Experiment 1 | | | | |
| 1rst cutting | - 0.03 | - 0.02 | - 0.06 | - 0.05 |
| 2nd " | + 0.07 | + 0.05 | - 0.05 | - 0.06 |
| 3rd " | - | - 0.08 | - | - 0.08 |
| Experiment 2 | | | | |
| 1rst cutting | - 0.08 | - 0.09 | - 0.09 | - 0.10 |
| 2nd " | - 0.03 | - 0.04 | - 0.10 | - 0.11 |
| 3rd " | - | - 0.01 | - | - 0.05 |
| Experiment 3 | | | | |
| 1rst cutting | + 0.21 | + 0.15 | + 0.03 | - 0.04 |
| 2nd " | + 0.29 | + 0.16 | + 0.09 | - 0.09 |
| 3rd " | - | + 0.31 | - | + 0.21 |
| Experiment 4 | | | | |
| 1rst cutting | + 0.04 | + 0.05 | - 0.05 | - 0.05 |
| 2nd " | 0.00 | + 0.06 | - 0.09 | - 0.02 |
| 3rd " | - | + 0.11 | - | + 0.06 |
| Experiment 5 | | | | |
| 1rst cutting | 0.00 | 0.00 | - 0.09 | - 0.20 |
| 2nd " | - 0.03 | - 0.01 | - 0.08 | - 0.19 |
| 3rd " | - | + 0.22 | - | - 0.16 |

Note - The estimated mixture value was calculated as average of the component genotypes in pure stand.

Behaviour of the genotypes

The three experiments with two-component mixtures all gave the same results (Table 6). When two genotypes are grown together, one is improved and the other deteriorates. This is shown in the dry matter weight, in the inflorescences, and in the height. Regarding the dry weight, in the mixture in exp. 1, the loss in 1564 S₁ is almost completely compensated for by the gain in 1558 S₂; in exp. 2, the loss of 1753 S₁ is not compensated for by the loss in 862/3375 S₂. So only in the first experiment it seems to be there an additive effect. As regards the height in exp. 1 and 3, there were clear cases of additive effects. There was a very large gap between loss and gain in the amount of inflorescences. To conclude, we found no cases of either reciprocal disadvantage, or of reciprocal advantage for any of the characters studied, contrary to results obtained by Chalbi (1). In exp. 4, using three components, the most noteworthy result was the behaviour of the genotype 649 S₁ in which there was no change of any importance for any of the characters under study. The other two genotypes acted in more or less the same way as in the two-component mixture in exp. 1. The presence of the genotype 649 S₁ seems to have the effect of balancing 1564 S₁ and 1558 S₂. In fact, when compared to exp. 1, the genotype 1558 S₂ gains less, while the genotype 1564 S₁ deteriorates less (dry matter).

In the mixture of 4 components, only the genotype 1564 S₁ shows a distinct gain in all characters, while the other three components deteriorate. However, a test carried out on 300 plants per square metre, with twelve components (3), gave these three results for the dry matter weight : advantage for the genotypes which are most vigorous when grown in purity, no effect on some genotypes of medium value, and deterioration in the less vigorous genotypes.

Comparison of the observed with the estimated mixture and the most productive genotype.

The results concerning the dry matter weight may be resumed as follows :

- a) **Two-component mixtures** (Table 7). In exp. 1, the observed mixture show no univocal response when compared with estimated value, whereas it is always less than the most productive genotype. In exp. 2, the observed mixture is always less than the estimated one, even though only slightly. What is more evident is the waste which occurs to the benefit of the most productive component. These results can be easily explained by bearing in mind that, in a mixture, the genotype 1558 S₁ gains much less than 1753 S₂ loses. In exp. 3, the observed mixture is always significantly higher than the estimated one. Except for the third cutting the observed mixture is better in single per-plant production but worse in per-plot production, when compared to the most productive component. These results depend on two facts : 862 S₁ gains much more in a mixture than 862/3375 S₂ loses, and the mortality rate of S₂ plants in mixture is high after the first cutting. The result of the third cutting can be explained easily by the fact that S₁ in mixture has 52.59 gm. production as against 7.91 gm. for S₂.

- b) **Mixtures containing more than 2 components** (Table 7) : in exp. 4 the most significant feature was that, at the third cutting, the observed mixture not only exceeded the estimated one, but also the most vigorous component as for the per-plot production. This was caused by high mortality amongst the weaker genotypes, so that the most productive genotype remains, in purity, at a much higher density than in the mixture. This also explains the results of exp. 5, where, however, the observed mixture is less than the most productive genotype.

In conclusion, the observed and estimated mixtures are more or less equal (except for exp. 3) where the mortality rate is almost non-existent (first cutting) ; the observed mixture is always less than the most productive component.

Effects of competition on variability

- 1) Plants grown at 1000 per square metre show an increase of C.V. in dry matter weight, height and number of stems (Table 8-10) over spaced plants. These results agree with those of previous experiments (300 pl/m²).
- 2) Genotypes which increase their yield in mixture have a C.V. for dry matter weight lower than in pure stand ; the opposite is true for genotypes whose yield decreases in mixture. The behaviour of 862/3375 S₂ is explained by the fact that this genotype has a much higher percentage of dead and minimal plants in mixture than in pure stand.
- 3) In all the exp.s, the C.V. of the mixtures are almost always higher than those of pure stand.

DISCUSSION

If two genotypes, different both in vigour and earliness, are grown at the same density, their biological density will, at a determined point, be found to differ. It will be higher for the more vigorous genotype.

We feel that it is the difference in biological density which influences the behaviour of the genotypes in mixture, when the numerical density causes competition among the plants. To be more precise, the genotype which is more vigorous in pure stand (higher dry matter weight) increases its own dry matter weight in mixture, because it passes from a higher to a lower biological density ; the genotype which is weaker in pure stand becomes still more weaker in mixture, because it passes to a higher biological density. This interpretation seems to apply to all the mixtures, that is, even for those with more than two components, and not only in lucerne but also in red clover, as we have found in other, not yet published experiments.

By this hypothesis on the variation of biological density, we can explain the phenomenon of intergenotypic competition in lucerne without falling back on ideas about "competition ability", and "aggressive characters", etc. We feel

Table 8 . Dry matter weight - C.V.

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | 5 | | | |
|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₁ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| 1rst Cutting | | | | | | | | | | | | |
| Spaced plants | 33 | 51 | 33 | 37 | - | - | 34 | 33 | 51 | 53 | 51 | 34 |
| Pure stand | 97 | 99 | 93 | 94 | 84 | 86 | 104 | 98 | 99 | 102 | 108 | 84 |
| Mixture | 99 | 108 | 86 | 124 | 75 | 87 | 92 | 86 | 103 | 102 | 112 | 94 |
| component values | | | | | | | | | | | | |
| mean | 105 | | 106 | | 91 | | 94 | | | 115 | | |
| 2nd Cutting | | | | | | | | | | | | |
| Spaced plants | 47 | 47 | 44 | 39 | - | - | 44 | 47 | 47 | 59 | 53 | 49 |
| Pure stand | 106 | 96 | 98 | 113 | 116 | 115 | 110 | 125 | 122 | 112 | 110 | 80 |
| Mixture | 98 | 113 | 89 | 133 | 89 | 111 | 119 | 107 | 127 | 106 | 108 | 89 |
| component values | | | | | | | | | | | | |
| mean | 108 | | 117 | | 114 | | 124 | | | 133 | | |

Table 9 . Height - C.V.

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | | 5 | | | |
|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₁ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| 1rst Cutting | | | | | | | | | | | | | |
| Spaced plants | 10 | 21 | 14 | 15 | - | - | 14 | 10 | 21 | 21 | 21 | 19 | 13 |
| Pure stand | 40 | 42 | 37 | 38 | 29 | 30 | 38 | 41 | 43 | 38 | 42 | 44 | 34 |
| Mixture | 39 | 46 | 35 | 48 | 29 | 30 | 37 | 34 | 40 | 27 | 45 | 44 | 42 |
| component values | | | | | | | | | | | | | |
| mean | 43 | | 43 | | 33 | | 37 | | | | 43 | | |
| 2nd Cutting | | | | | | | | | | | | | |
| Spaced plants | 9 | 17 | 11 | 11 | - | - | 12 | 9 | 17 | 17 | 13 | 9 | 13 |
| Pure stand | 54 | 55 | 49 | 57 | 42 | 40 | 52 | 60 | 62 | 52 | 60 | 57 | 38 |
| Mixture | 51 | 61 | 48 | 64 | 31 | 44 | 56 | 52 | 64 | 35 | 64 | 60 | 56 |
| component values | | | | | | | | | | | | | |
| mean | 57 | | 58 | | 41 | | 58 | | | | 60 | | |

Table 10 . Number of stems - C.V.

| EXPERIMENTS | 1 | | 2 | | 3 | | 4 | | 5 | | | | |
|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | 1558 S ₀ | 1564 S ₀ | 1558 S ₁ | 1753 S ₀ | 862 S ₀ | 862 S ₂ | 649 S ₀ | 1558 S ₀ | 1564 S ₀ | 1564 S ₀ | 1564 S ₁ | 1564 S ₂ | 1564 S ₃ |
| 1rst Cutting | | | | | | | | | | | | | |
| Spaced plants | 32 | 33 | 30 | 35 | - | - | 24 | 32 | 33 | 33 | 33 | 33 | 30 |
| Pure stand | 0 | 10 | 0 | 12 | 28 | 34 | 14 | 12 | 21 | 29 | 26 | 31 | 24 |
| Mixture | 10 | 12 | 0 | 7 | 37 | 21 | 15 | 12 | 20 | 31 | 22 | 16 | 21 |
| component values | | | | | | | | | | | | | |
| mean | 11 | | 5 | | 33 | | 16 | | | | 24 | | |
| 2nd Cutting | | | | | | | | | | | | | |
| Spaced plants | 37 | 48 | 26 | 26 | -- | -- | 33 | 37 | 48 | 33 | 36 | 37 | 32 |
| Pure stand | 51 | 60 | 46 | 48 | 43 | 49 | 50 | 46 | 46 | 49 | 42 | 44 | 44 |
| Mixture | 46 | 53 | 45 | 38 | 45 | 43 | 49 | 47 | 56 | 38 | 43 | 43 | 45 |
| component values | | | | | | | | | | | | | |
| mean | 50 | | 45 | | 49 | | 51 | | | | 43 | | |

Table 11 - Mixture vs pure stand at the density of 300 transplanted plants in a square metre (per-plant mean values)

| CHARACTERS | Dry matter weight | | Height | | Number of stems | |
|---------------------|-------------------|--------|--------|--------|-----------------|--------|
| | 1rst | 2nd | 1rst | 2nd | 1rst | 2nd |
| Genotypes | | | | | | |
| 176 S ₀ | + 0.31 | + 0.34 | + 0.08 | + 0.03 | + 0.11 | + 0.35 |
| S ₁ | - 0.13 | - 0.35 | - 0.02 | - 0.03 | - 0.05 | - 0.24 |
| 1564 S ₀ | + 0.37 | + 0.41 | + 0.05 | + 0.03 | + 0.12 | + 0.21 |
| S ₁ | - 0.28 | - 0.27 | 0.00 | - 0.03 | - 0.11 | - 0.21 |
| 1767 S ₀ | + 0.09 | + 0.35 | - 0.05 | - 0.02 | + 0.10 | + 0.15 |
| S ₁ | - 0.12 | - 0.13 | 0.00 | - 0.07 | - 0.01 | - 0.15 |
| 1558 S ₀ | + 0.20 | + 0.29 | + 0.01 | + 0.02 | + 0.11 | + 0.17 |
| 1564 S ₀ | - 0.17 | - 0.18 | 0.00 | 0.00 | - 0.07 | - 0.07 |

that a genotype in mixture does not increase its own weight because it is aggressive and possesses better competition ability, but because it finds a lower biological density in mixture than in pure stand. Thus it follows that the question to be answered is not what characters govern competition, but how the morphophysiological structure of a genotype reacts to variations in biological density within a determined level of numerical density. We found that : a) all the characters we measured react to variations in the biological density : earliness, dry matter weight, height, number of stems ; b) at a density of 1000 plants per square metre the amount of inflorescences and the dry matter weight were affected strongly, the height a good deal, and the number of stems scarcely at all. This is explained by the fact that as the numerical density gradually increases - and if there is no mortality - the number of stems tends to equal value 1 for all genotypes. At a density of 300 per square metre the dry matter weight, the number of stems and the amount of inflorescences are susceptible, while the height is not affected at all.

Other authors (2, 6) state that characters affected by intergenotypic competition are of a numerical or multiplicative nature, while characters associated with the elongation of plant tissues remain unaffected. We find this to be only partly true. In fact, the height is just as much influenced as the characters of numerical nature, as for example the number of stems, but at different density levels.

Our hypothesis not only permits a correct interpretation of results, but is also strengthened by the fact that none of the characters we studied played a leading role in the behaviour of genotypes in mixture. According to Yamada, one of the characters most closely related to competition ability (the one which is least influenced by competition) is height, which controls the intake of light. However, in exp.s 1,4,5, the comparison of results obtained in pure stand and in mixture revealed that height has no effect at all on the behaviour of genotypes in mixture. In fact, some genotypes, which in pure stand are taller than, or at least as tall as, more vigorous types, not only show a decline in vigour in mixture, but also undergo a notable reduction in height.

The number of stems was almost the same value for all the genotypes in pure stand, even though it is very different in spaced plants. In previous experiments we saw that earliness at 30-40 days after sowing played an important rôle in the behaviour of genotypes in mixture ; in the fully-grown plant this character is of no importance. In fact, we saw in exp. 1 and 4 that the most early plant (amount of inflorescences per plant) in pure stand showed not only a decrease in vigour and in inflorescences when placed in mixture, but also dropped behind its competitors.

To conclude, the characters as plant height, number of stems, and earliness are not able, if taken singly, to influence the behaviour (competition ability, according to other authors) of genotypes in mixture. The only character of any importance is the vigour - measured as dry matter weight - which expresses the total value of the plant. If our hypothesis is valid :

- a) the discussion of genetic control in competition (5,6) automatically collapses
- b) it is easy to calculate the behaviour of single genotypes in mixture from the value of dry matter weight in pure stand, especially when the mixture has only two components.

CONCLUSION

These results confirm, and, at the same time, qualify those obtained in previous experiments. We summarize as follows :

- 1) All the characters studied are influenced by competition "tout court" (intra and intergenotypic). In intergenotypic competition, the susceptibility of height and number of stems depends on the levels of numerical density.
- 2) At the same numerical density, the higher yielding genotype in pure plot increases its yield in mixture ; on the contrary, the lower yielding genotype, in mixture, reduces its yield. We feel that is the result of the differing biological density of each component and is not caused by higher or lower competition ability. The characters as plant height, number of stems, and earliness are unable, singly, to govern the behaviour (competition ability) of the plants in mixture. This behaviour is influenced only by the vigour of the plant, which is its global value and is measured by the dry matter weight.
- 3) When testing two components in mixture, there was neither reciprocal damage nor reciprocal advantage for any of the studied characters.
- 4) All the mixtures studied gave a lower yield than the most productive component in pure stand. Therefore the aim of breeding does not seem to be that of creating varieties able to co-operate in a mixture but to create homogeneous varieties highly yielding in pure stand. We feel that choice of plants should not only be carried out in conditions similar to field ones, but based above all on weight, as the simpler characters, such as height and number of stems, when at high density, depend to a great extent on the vigour of each individual plant.

SUMMARY

We report the results of our second step of experiments to find out the competition effects on some quantitative characters of lucerne (Medicago sativa).

Ten genotypes were used. Each genotype was grown both as spaced plants and at the density of 200 plants per metre (= 1000 plants a square metre) in pure stand and in mixture.

The seeds were calibrated (1.5 to 1.6 mm.) and pierced by a needle after 12 hours in Petri dishes, to avoid the effects of size and hardness ; in this way, all the seeds were at the same rate of swelling at sowing time.

We summarize the results as follows :

- 1) All the characters studied are influenced by competition "tout court" (intra and inter-genotypic). In intergenotypic competition, the susceptibility of height and number of stems depends on the levels of numerical density.
- 2) At the same numerical density, the higher yielding genotype in pure plot increases its yield in mixture ; on the contrary, the lower yielding genotype, in mixture, reduces its yield. We feel that this is the result of the differing biological density of each component and is not caused by higher or lower competition ability. The characters as plant height, number of stems and earliness are unable, singly, to govern the behaviour (competition ability) of the plants in mixture. This behaviour is influenced only by the vigour of the plant, which is its global value and is measured by the dry matter weight.
- 3) When testing two components in mixture, there was neither reciprocal damage nor reciprocal advantage for any of the studied characters.
- 4) All the mixtures studied gave a lower yield than the most productive component in pure stand. Therefore the aim of breeding does not seem to be that of creating varieties able to co-operate in a mixture but to create homogeneous varieties highly yielding in pure stand. We feel that choice of plants should not only be carried out in conditions similar to field ones, but based above all on weight, as the simpler characters, such as height and number of stems, when at high density, depend to a great extent on the vigour of each individual plant.

REFERENCES

- Chalbi, E., 1970. Mise en évidence d'aspects génétiques dans les associations de géotypes chez la luzerne. V^o Colloque Ecologie - Paris. (1)
- Nguyen Van, J., 1969. Structures, sensibilités et modalités d'action des caractères d'agressivité. Ann. Amél. Plantes, 4, 391-404. (2)
- Rotili, P., 1970. Gli effetti della competizione intergenotipica nel test di progenie da polycross in erba medica Quad. Spet. Ist. Forag. - Lodi n. 2. (3)
- Rotili, P., Zannone, L., 1970. Effetti della competizione sui caratteri quantitativi dell'erba medica. Quad. Sper. Ist. Forag. - Lodi n. 2. (4)
- Sakai, K.I., 1955. Competition in plants and its relation to selection. Cold Spring Harbor Symp. Quant. Biol. 137-157. (5)

Yamada, T., Horiuchi, S., 1960. Of the bias of quantitative characters and the change of their distribution in a population due to interplant competition. 8th Intern. Grass. Congr., 297-301. (6)

VARIABILITY OF COMPETITION RESPONSES

FOR 14 ALFALFA "GENOTYPES"

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SUMMARY

After some definitions related to biological competition, its establishing process and factors are reviewed, pointing out the importance of genetic variability studies according to this situation. The results of such a study applied to 14 genotypic structures of alfalfa are presented, four competition pressures being confronted. The data are submitted to separate analysis, for several cuts, and to global analysis. Vigour being evaluated by means of first unifoliate leaf area, vigour-production correlations are calculated. A measure of phenotypic stability in the various conditions is given.

INTRODUCTION

In a dense sward, an organism is submitted to two forces : one physical, the other biological (effectively available nutrients per individuals). If the whole level is superior to the needs, each individual finds its account in ; if it is inferior, each individual tends to obtain the maximum from it. Needs coincidence increase the competition, phenomenon that "occurs when each of two or more organisms seeks the measure it wants of any... factor.... when the.... supply.... is below the combined demand". (Donald, 1963).

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This is occurring at the interspecific level as at the intraspecific one. In this later case, the simultaneity condition is maximum. A competition between parts of a same individual is possible and it is necessary to point out that the presence of individuals do not lead automatically to competition.

Two manifestations of it are interesting :

1. the plastic deviations ; if $Y_{i/i}$ is the character value in pure, the deviation will be :

$$\frac{\Delta Y}{Y_{i/i}} = \frac{Y_{i/j} - Y_{i/i}}{Y_{i/i}} \quad (Y_{i/j} = \text{measure on } i \text{ in presence of } j) ;$$

2. the plant population evolution

The variables of it are = nutrients, water and light. The space competition is rare. According to the Clements' definition : "... all the advantages... of competing species are epitomized in ... : amount and rate". The speed of mineral nutrients absorption, from the environment, explains the ability. The relative amount of elements acts on competition intensity. The ability changes according to soils. The water competition depends from the radicular structure, in grass - legume associations. This competition, at last, acts in the root-aerial organs balance. The entanglement of roots gives a picture of that kind of competition.

The competition for light is expressed, from the point of view of ability, by the limb pattern. Each individual exposes the maximum of leaves to the light flux. Then a gradient is established in the plant. Many research workers, particularly Kasanaga and Monsi have studied this problem ; it may be "..... that the difference is only a millimetre, yet this may be decisive". The rapidly growing plant domines.

Interactions exist between these competitions :

Interactions between competitions for two biotypes A and B

Competition only for :

light

nutrients

Effect =
direct

| | |
|---|---|
| Intrusion of A in the light environment of B \Rightarrow B \downarrow | Intrusion of A in the nutrients environment of B \Rightarrow B \downarrow |
| B has a reduced capacity to exploit the nutrients | B has a reduced capacity to exploit the light supplies |

indirect

Important factors are : population density and individual abilities. The schema from figure 1 allows to analyse the first : between B and C, the density is of medium level, corresponding to the one in crops. Whereas the yield of one plant is a fonction of its genotype, in a dense sward, the genotype x environment interaction is predominant. Donald (1963) has found that if plants in these conditions grow with a low rate, in spaced plants an intraplant competition exists; these later yield more seeds in the whole but fewer per inflorescences. The yield, related to density, is often expressed by :

$$\frac{1}{w} = Ad + B \quad \text{with} \quad \begin{array}{l} w = \text{yield/plant} \\ d = \text{density} \\ A \text{ and } B = \text{constants} \end{array}$$

Whereas the adapted individuals survive, the other have a decreasing population, if the competition is severe. The interaction with the environment is important. There would be no relation between competitive ability and any characters. The competition variance is high when genotypic variance is low. The ability can be partitioned in two subability : one for survival, the other for action. A character, then, is decomposed in the following manner (Gallais, 1970) :

$$Y_{i/j} = \mu + \alpha_i + \beta_j + T_{i/j}$$

μ = mean of all (i + j) associations studied

α_i = mean production effect of i in presence of all other biotypes studied

β_j = mean partner effect of j on all other biotypes studied

$T_{i/j}$ = interaction effect

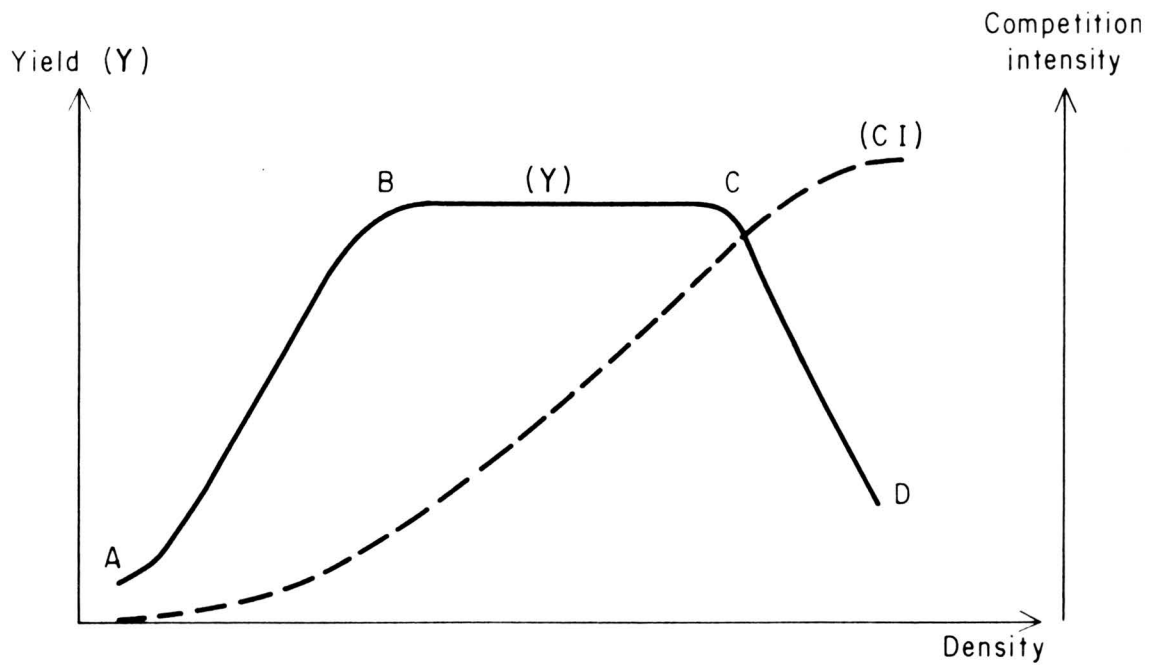
This model allows to study the competition with more accuracy.

The spaced plants performances seem independant from competition ability. This ability has a low heritability. The major component of this characteristic is "aggressivity" or partner or acting effect.

It seems important to emphasize that there is no linkage between ability and yield. Harlan and Martin have found such a no dependance in barley. Let us remind, also, that some authors reject the term "competition", and for others, in a pure line, the individuals should be never in competition ! This should be the case in homogeneous varieties. However, in a recent experiment, alfalfa clones, tested at two competition levels : homocompetition (propagule from one clone in confront with others from the same) and heterocompetition (propagule from one clone with others from different clones), have manifested no clear evidence of differences between the two situations.

FIGURE I

Density effects on yield per unit area and competition intensity



At last, if two organisms differ for ability, the best will domine, as well by antagonism as by cooperation.

This work is a study of 14 alfalfa "genotypes" with a view to estimate their competitive - or according to a suggestion of Breese - their ecological ability.

MATERIAL AND METHODS

1 - Plant material

14 alfalfa genotypes were used (Table 1).

TABLE 1
Characteristics of alfalfa genotypes used

| Number | Name | Earliness in comparison with control | Dry matter production in comparison with control (2) |
|--------|--------------------------------|--------------------------------------|--|
| 1 | Cardinal | equal | slightly inferior |
| 2 | Du Puits (Control) | | 100 |
| 3 | Luciole | equal | slightly inferior |
| 4 | Europe | equal | slightly superior |
| 5 | St Leger | earlier | 94 |
| 6 | FD 100 | equal | 100 |
| 7 | Mulasserie | equal | ? |
| 8 | Orchésienne | equal | 100 |
| 9 | 64-50 (HD ⁽¹⁾ , F2) | earlier | 83 |
| 10 | 64-44 (HD, F3) | equal | 99 |
| 11 | 64-49 (HD, F3) | earlier | 100 |
| 12 | 61-17 (HD, F2) | earlier | 97 |
| 13 | 64-43 (HD, F2) | equal | 93 |
| 14 | 61-41 (HD, F2) | equal | 85 |

(1) HD = Two-way hybrid

(2) in field trials

The extreme categories of seed, leading to an initial capital effect, were eliminated.

2 - Experimental design

Four conditions were realized :

- a) Spaced plants (A),
- b) Intragenotypic competition or pure stand (B), square repartition (2,4 cm),
- c) Intergenotypic competition (C), genotypes 1 to 5 and 7 to 14 confronted with the same genotype (F.D. 100),
- d) Interspecific competition (D) by mean of Italian rye-grass. Each plant was surrounded by four "aggressors" (except on borders),
- e) Collected data : production (25 plants) at the opening of flower buds (three cuts), the flowering being among the distinguishable critical stages. The yield was gross evaluated excepted at the second cut (stem and leaf separated). It should be as interesting to pay attention to flower numbers.

On the other hand, the vigour of each genotype was estimated by area of the first unifoliate leaf.

- f) Experimental working out : the experiment was conducted in greenhouse. The four competition conditions was allotted at random between four growing tanks.

3 - Methods of analysis

- a) Preliminary analysis : at this stage, the different conditions are handled separately,
- b) Global analysis : the different results from each condition were grouped. The genotype 6, competitor in C environment, is excluded from this analysis, conducted according to the hierarchical model, the replication x competition interaction being not individualized. In fact, it is the genotype x competition interaction that is important here. For the different structures, according to their adaptability, diverse responses may be conceived (we will have the opportunity to return to this point),
- c) Phenotypic stability analysis : for each genotype, the regression is calculated between its productivity and the performances, of the whole set of genotypes. The genotypes with a coefficient near 1 have a mean stability. A genotype with a low coefficient yields often more than the mean in unfavourable conditions,
- d) Distribution studies (evaluation of the distribution symmetry of leaf areas, using the g_1 coefficient).

The performances of each genotype, confronted with different competition, are thus described in several manners.

RESULTS

They are condensed in Table 2 for dry matter yield. All variance analysis are included in the next paragraph.

DISCUSSION

1. Partial analysis

a) Production ability (resistance to aggression) of producer genotypes

- Spaced plants (Table 3) : The various genetical structures, in almost absence of competition (light aerial competition), express clearly their potentialities, in the given conditions. The hybrid progenies have moderate performances (Figure 2). In the last part of result analysis, we will have the opportunity to turn on this point.

- Intrageneric competition or pure stand (Table 3) : It is a matter here of competition effect due to the meeting of identical genotypes. The volume per plant is in fact different in pure stand and in spaced plants ; for an entirely similitude, it should be necessary that the exploited volume by each plant were identical in A and B (which should come with 13 plants per pot and not with 49). The partial analysis shows that there is no detectable differences between genotypes. A combined analysis of the three cuts allows to distinguish three sets of genotypes (Figure 3). The most salient facts concern : the stability of "Du Puits" and the retrogression of "Mulasserie".

- Intergeneric competition (Table 3) : Anew, only, the combined analysis of the three cuts give appearance to a "genotype" effect. Several genotypes perform better in that situation than in pure stand (particularly 1 and 14), the others have either an inferior production (4, 5, 8, 9, 11, 13) or an equal one (2, 3, 7, 10, 12). (Figure 3).

- Interspecific competition (Table 3) : the analysis of the first cuts does not reveal any differences ; at every one of them, the "genotypic" variance increases. At the third cut, the ranking is significant. Two explanations are possible for the domination decrease of rye-grass accompanying this fact :

- reversal of the situation to the benefit of the legume,
- rye-grass having invaded the "chemical space" has exhausted it.

Several cuts are necessary, but the genotype x cut interaction never is significant.

The competition effects may be measured in terms of "competition resistance", taking the spaced plants as reference (Table 4).

Table 2 . Dry matter yield per cuts ($\log_{10} x$) \times 1000 $x = 10^{-2}$ grams
(Sum of two replications)

| Environment | Spaced plants (A) | | | Intragenotypic competition Pure sward (B) | | | Intergenotypic competition (C) | | | Interspecific competition (D) | | | 1 + 2 + 3 | | | | |
|-------------|-------------------|-------|-------|---|-------|-------|--------------------------------|-------|-------|-------------------------------|-------|-------|-----------------------|-----------------------|-----------------------|-----------------------|--|
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | A | B | C | D | |
| Genotypes | | | | | | | | | | | | | | | | | |
| 1 | 5 714 | 5 716 | 6 814 | 5 067 | 5 042 | 5 778 | 5 202 | 5 477 | 5 779 | 4 470 | 4 972 | 5 596 | 18 244 | 15 887 | 16 458 | 15 038 | |
| 2 | 5 700 | 5 944 | 7 090 | 5 485 | 5 519 | 6 154 | 5 420 | 5 518 | 5 937 | 4 465 | 4 780 | 5 602 | 18 734 | 17 158 | 16 875 | 14 847 | |
| 3 | 5 703 | 5 712 | 6 802 | 5 426 | 5 465 | 6 090 | 5 481 | 5 460 | 5 762 | 4 801 | 5 034 | 5 608 | 18 217 | 16 981 | 16 703 | 15 443 | |
| 4 | 5 716 | 5 602 | 6 887 | 5 458 | 5 358 | 5 793 | 5 352 | 5 240 | 5 645 | 4 781 | 4 871 | 5 517 | 18 205 | 16 609 | 16 237 | 15 169 | |
| 5 | 5 607 | 5 473 | 6 606 | 5 550 | 5 217 | 5 945 | 5 409 | 5 269 | 5 619 | 4 783 | 4 816 | 5 582 | 17 686 | 16 712 | 16 297 | 15 181 | |
| 7 | 5 672 | 5 831 | 7 114 | 5 194 | 5 108 | 5 733 | 5 089 | 5 164 | 5 769 | 4 574 | 4 746 | 5 486 | 18 617 | 16 035 | 16 022 | 14 806 | |
| 8 | 5 607 | 5 695 | 6 858 | 5 277 | 5 227 | 6 051 | 5 212 | 5 252 | 5 445 | 4 049 | 4 399 | 5 348 | 18 160 | 16 555 | 15 909 | 13 796 | |
| 9 | 5 618 | 5 607 | 6 779 | 5 424 | 5 129 | 5 933 | 5 182 | 5 278 | 5 587 | 4 622 | 4 647 | 5 182 | 18 004 | 16 486 | 16 047 | 14 451 | |
| 10 | 5 432 | 5 290 | 6 387 | 5 298 | 5 277 | 5 805 | 5 151 | 5 334 | 5 646 | 4 589 | 4 874 | 5 592 | 17 109 | 16 380 | 16 131 | 15 055 | |
| 11 | 5 455 | 5 598 | 6 802 | 5 200 | 5 203 | 6 080 | 4 838 | 4 997 | 5 487 | 4 524 | 4 808 | 5 347 | 17 855 | 16 483 | 15 322 | 14 679 | |
| 12 | 5 615 | 5 365 | 6 612 | 5 392 | 5 238 | 5 939 | 5 427 | 5 440 | 5 756 | 4 557 | 4 456 | 5 165 | 17 592 | 16 569 | 16 623 | 14 178 | |
| 13 | 5 693 | 5 513 | 6 710 | 5 324 | 5 228 | 5 875 | 5 212 | 5 102 | 5 522 | 4 467 | 4 522 | 5 137 | 17 916 | 16 427 | 15 836 | 14 126 | |
| 14 | 5 508 | 5 759 | 7 122 | 5 249 | 5 239 | 5 843 | 5 223 | 5 580 | 5 891 | 4 729 | 4 836 | 5 171 | 18 389 | 16 331 | 16 694 | 14 736 | |
| Total | 73040 | 73105 | 88583 | 69344 | 68250 | 77019 | 68198 | 69111 | 73845 | 59411 | 61761 | 70333 | $\Delta_{0,05} = 153$ | $\Delta_{0,05} = 197$ | $\Delta_{0,05} = 257$ | $\Delta_{0,05} = 203$ | |

Table 3 . Variance analysis : cut by cut

| Origin of variation | Sum of squares | Degrees of freedom | Mean squares | Calculated F | F 0.05 | Variation coefficient |
|---------------------|----------------|--------------------|--------------|--------------|--------|-----------------------|
|---------------------|----------------|--------------------|--------------|--------------|--------|-----------------------|

Spaced plants (Condition A)

| | | | | | | |
|-------------------|-----------|----|----------|--------------|------|--------|
| 1st cut genotypes | 57 411.8 | 12 | 4 784.3 | <u>3.343</u> | 2.69 | 1.35 % |
| 2d cut genotypes | 199 963.4 | 12 | 16 661.4 | <u>4.152</u> | 2.69 | 2.25 % |
| 3d cut genotypes | 273 516.1 | 12 | 22 793.0 | <u>3.108</u> | 2.69 | 2.51 % |

Pure stand (Condition B)

| | | | | | | |
|-------------------|-----------|----|---------|-------|------|--------|
| 1st cut genotypes | 112 725.6 | 12 | 9 394.9 | 2.071 | 2.69 | 2.53 % |
| 2d cut genotypes | 106 812.5 | 12 | 8 901.0 | 0.993 | 2.69 | 3.61 % |
| 3d cut genotypes | 109 872.5 | 12 | 9 156.0 | 1.170 | 2.69 | 2.99 % |

Intergenotypic competition (Condition C)

| | | | | | | |
|-------------------|-----------|----|-----------------|-------|------|--------|
| 1st cut genotypes | 182 538.7 | 12 | 15 211.6 | 1.857 | 2.69 | 3.45 % |
| 2d cut genotypes | 178 521.1 | 12 | 14 876.8 | 1.202 | 2.69 | 4.18 % |
| 3d cut genotypes | 136 489.1 | 12 | 11 274.1 | 0.737 | 2.69 | 4.37 % |

Interspecific competition (Condition D)

| | | | | | | |
|-------------------|-----------|----|----------|--------------|------|--------|
| 1st cut genotypes | 238 486.5 | 12 | 19 373.9 | 2.021 | 2.69 | 4.34 % |
| 2d cut genotypes | 224 104.5 | 12 | 18 675.4 | 2.299 | 2.69 | 3.79 % |
| 3d cut genotypes | 221 034.5 | 12 | 18 419.5 | <u>4.000</u> | 2.69 | 2.51 % |

FIGURE II

Rank, cut by cut of genotypes (Sum of two repetitions) and evolution of ranking

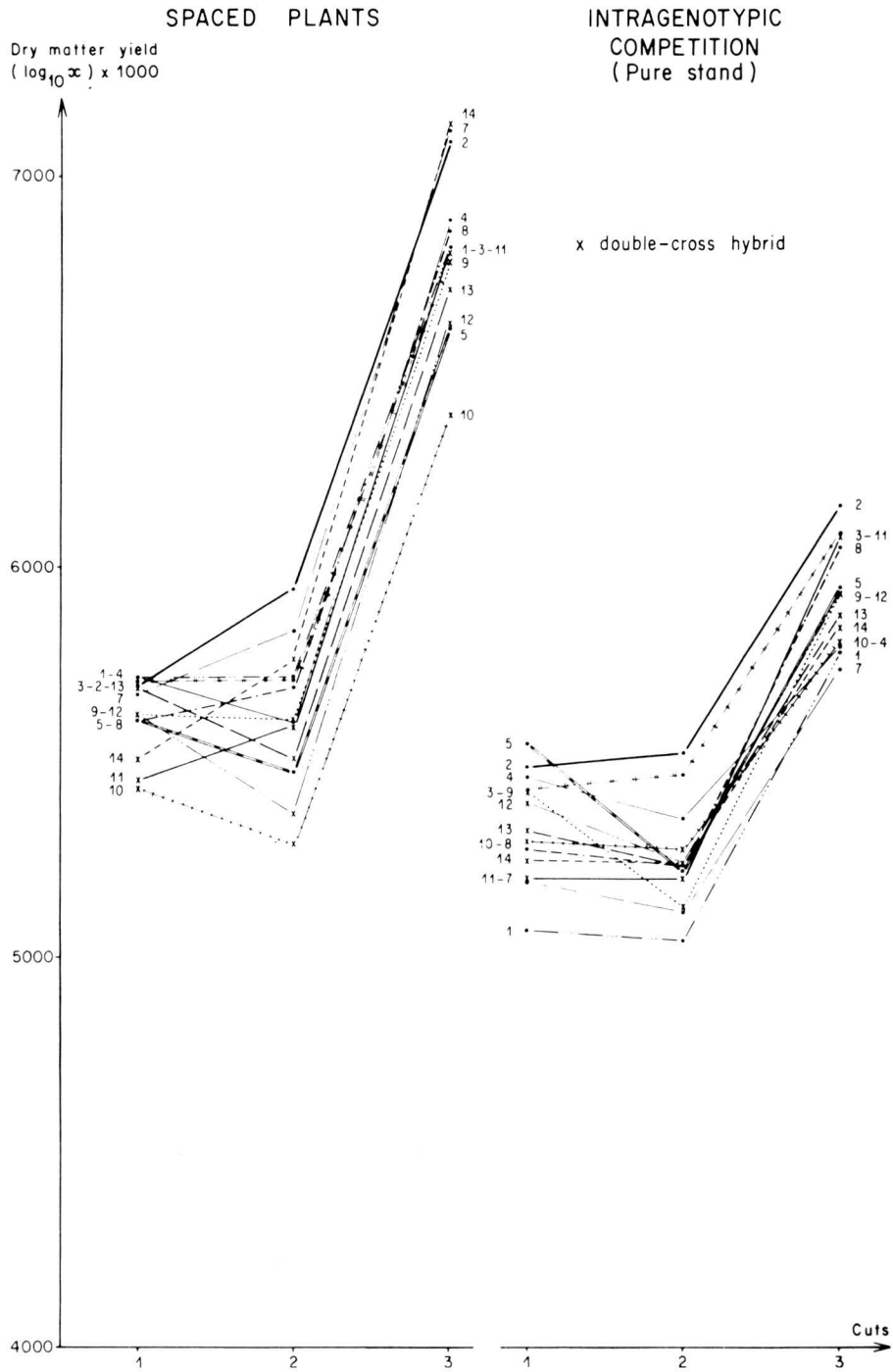


FIGURE II (bis)

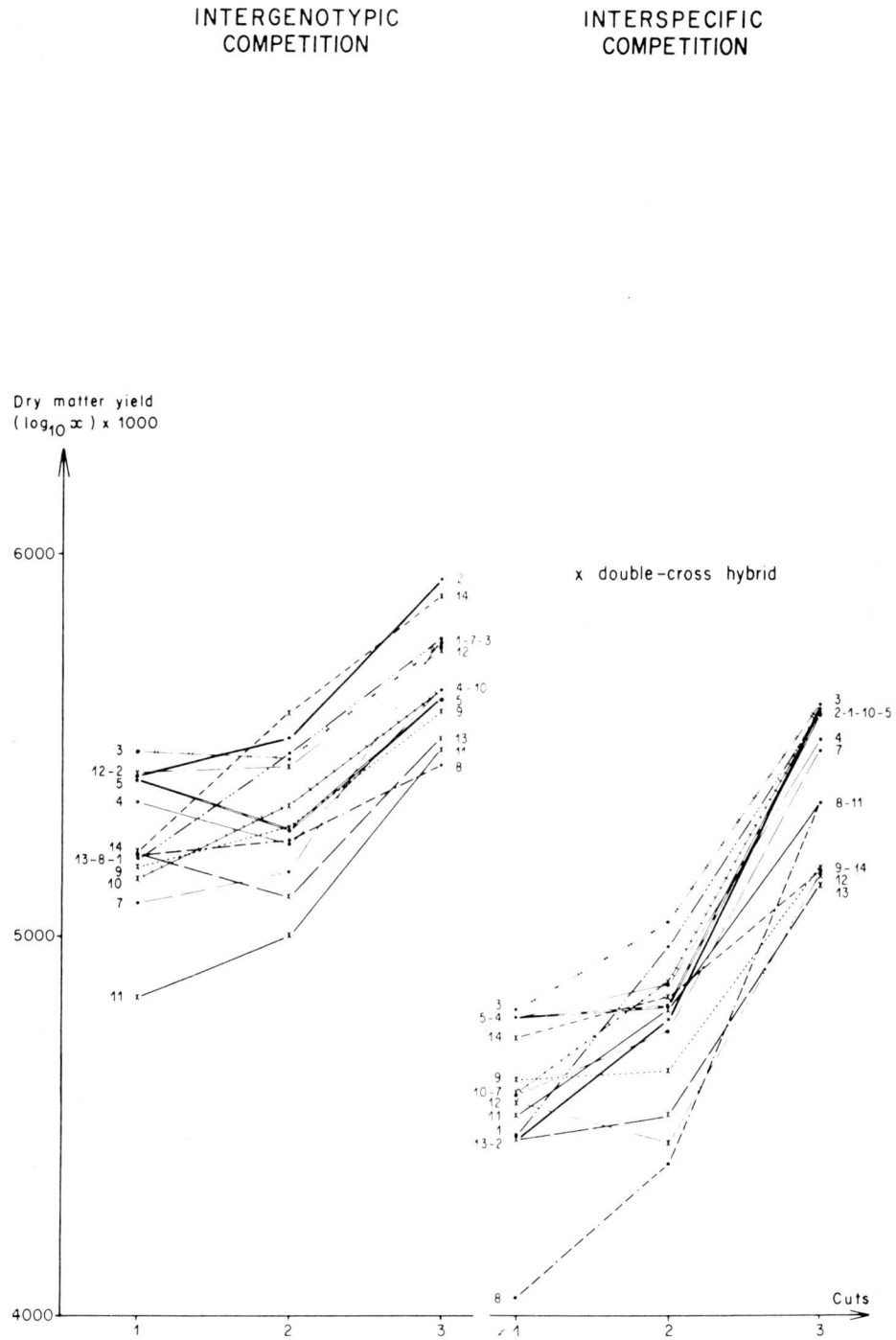


FIGURE III

Ranking (for the sum of three cuts) of genotypes in each environment
(sum of the two repetitions)

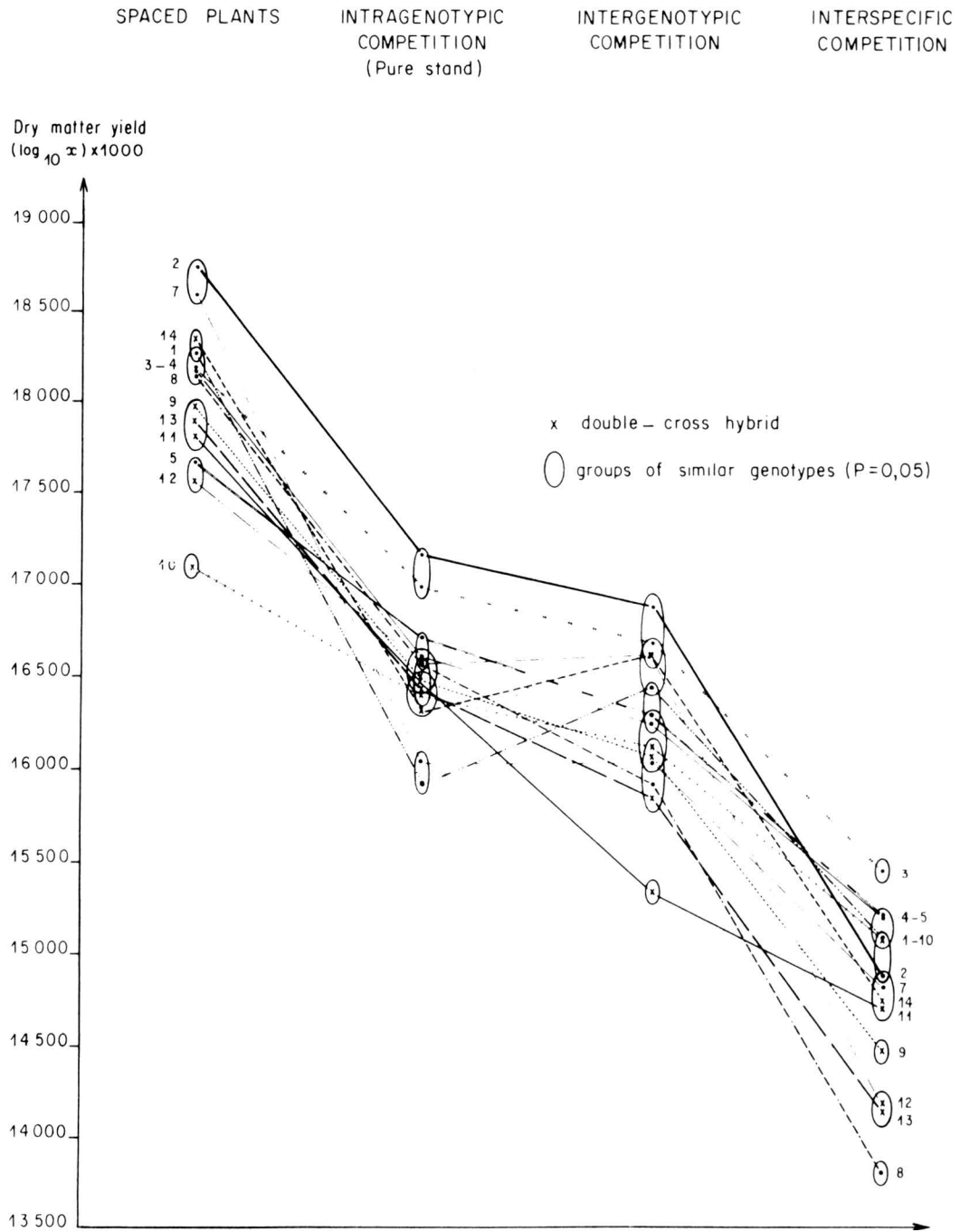


Table 4

"Competition resistance" $\left(\frac{x}{\text{spaced plants}} \right)$

| G | $\frac{B}{A}$ | $\frac{C}{A}$ | $\frac{D}{A}$ | \bar{x}_G |
|-------------|---------------|---------------|---------------|-------------|
| 1 | 0,87 | 0,90 | 0,82 | 0,86 |
| 2 | 0,91 | 0,90 | 0,79 | 0,87 |
| 3 | 0,93 | 0,91 | 0,84 | 0,89 |
| 4 | 0,91 | 0,89 | 0,83 | 0,88 |
| 5 | 0,94 | 0,92 | 0,85 | 0,90 |
| 7 | 0,86 | 0,86 | 0,79 | 0,84 |
| 8 | 0,91 | 0,87 | 0,75 | 0,84 |
| 9 | 0,91 | 0,89 | 0,80 | 0,87 |
| 10 | 0,95 | 0,94 | 0,87 | 0,92 |
| 11 | 0,92 | 0,85 | 0,82 | 0,86 |
| 12 | 0,94 | 0,94 | 0,80 | 0,89 |
| 13 | 0,91 | 0,88 | 0,78 | 0,86 |
| 14 | 0,88 | 0,90 | 0,80 | 0,86 |
| \bar{x}_C | 0,91 | 0,90 | 0,81 | |

b) Competitor studies

There is an absence of "genotypic" effect on F.D. 100 ; the explanation of it is that its domination may be due to an "intrinsic" superiority. For rye-grass, there is only a very important "cut" effect, followed by a grass regression.

c) Comparison mixtures vs. pure stands

The mutual effects, of "FD 100" and of the other genotypes, may be expressed in relative bias :

Table 5 . Variance analysis of the producer genotypes (in the four environment) : cut by cut

| Origin of variation | Sum of squares | Degree of freedom | Mean squares | F calculated | F 0.05 | Variation coefficient |
|--------------------------------------|----------------|-------------------|--------------|--------------|--------|-----------------------|
| | | <u>1st cut</u> | | | | |
| Total | 4 743 815 | 103 | | | | |
| genotypes | 299 391 | 12 | 24 944 | 4.29 | 1.95 | |
| environments | 3 846 579 | 3 | 1 282 193 | 220.50 | 2.80 | |
| genotypes × environments | 291 844 | 36 | 8 177 | 1.58 | 1.65 | |
| Replications (into the environments) | 27 026 | 4 | 6 756 | 1.16 | 2.58 | |
| Residue | 279 036 | 48 | 5 813 | | | |
| | | <u>2d cut</u> | | | | 2.40 % |
| Total | 3 684 964 | 103 | | | | |
| genotypes | 304 180 | 12 | 25 348 | 3.03 | 1.95 | |
| environments | 2 548 850 | 3 | 849 617 | 101.53 | 2.80 | |
| genotypes × environments | 405 219 | 36 | 11 256 | 1.34 | 1.65 | |
| Replications (into the environments) | 25 044 | 4 | 6 251 | - | 2.58 | |
| Residue | 401 671 | 48 | 8 368 | | | |
| | | <u>3d cut</u> | | | | 3.49 % |
| Total | 8 416 327 | 103 | | | | |
| genotypes | 251 920 | 12 | 20 993 | 2.39 | 1.95 | |
| environments | 7 222 195 | 3 | 2 407 398 | 273.66 | 2.80 | |
| genotypes × environments | 488 995 | 36 | 13 583 | 1.54 | 1.63 | |
| Replications (into the environments) | 30 941 | 4 | 7 735 | - | 2.58 | |
| Residue | 422 276 | 48 | 8 797 | | | |
| | | | | | | 2.11 % |

- reciprocal disadvantages (5 in first cut, 3 in third cut),
- antagonism (6 and 8 respectively),
- reciprocal advantages (2 and 2).

(A time displacement of the regrowth precludes the use of the second cut).

The response variability shows how complex are the reciprocal actions. Then it is important to follow the evolution during several cuts. Also, the study of several characters should specify the compensations.

In intergenotypic competition, the dry matter production of "F.D. 100" is important. "Cardinal" is either favoured or indifferent. Several mixtures are superior to the most productive component in pure stand ; the advantage varies :

$$(7 + 6) = 5 \%, (13 + 6) = 6 \%, (1 + 6) \text{ and } (12 + 6) = 7 \%, (5 + 6) = 9 \%, \\ (14 + 6) = 19 \%$$

All the genotypes are disadvantaged in presence of the grass (but it is not possible to calculate bias in this case).

2. Global analysis

a) "Environment" effect and responses of producer genotypes

Although competition acts greatly, differences between genotypes remain ; but the effects are only partly additive (Table 5).

b) Leaf percentage

When the bias are analysed at the level of foliar contribution to total weight, it is noted a relation between leaf percentage and etiolation.

3. Vigour - production correlation

The alfalfa genotypes have been measured for leaf area. On the basis of the calculated parameter g_1 , the normality was tested :

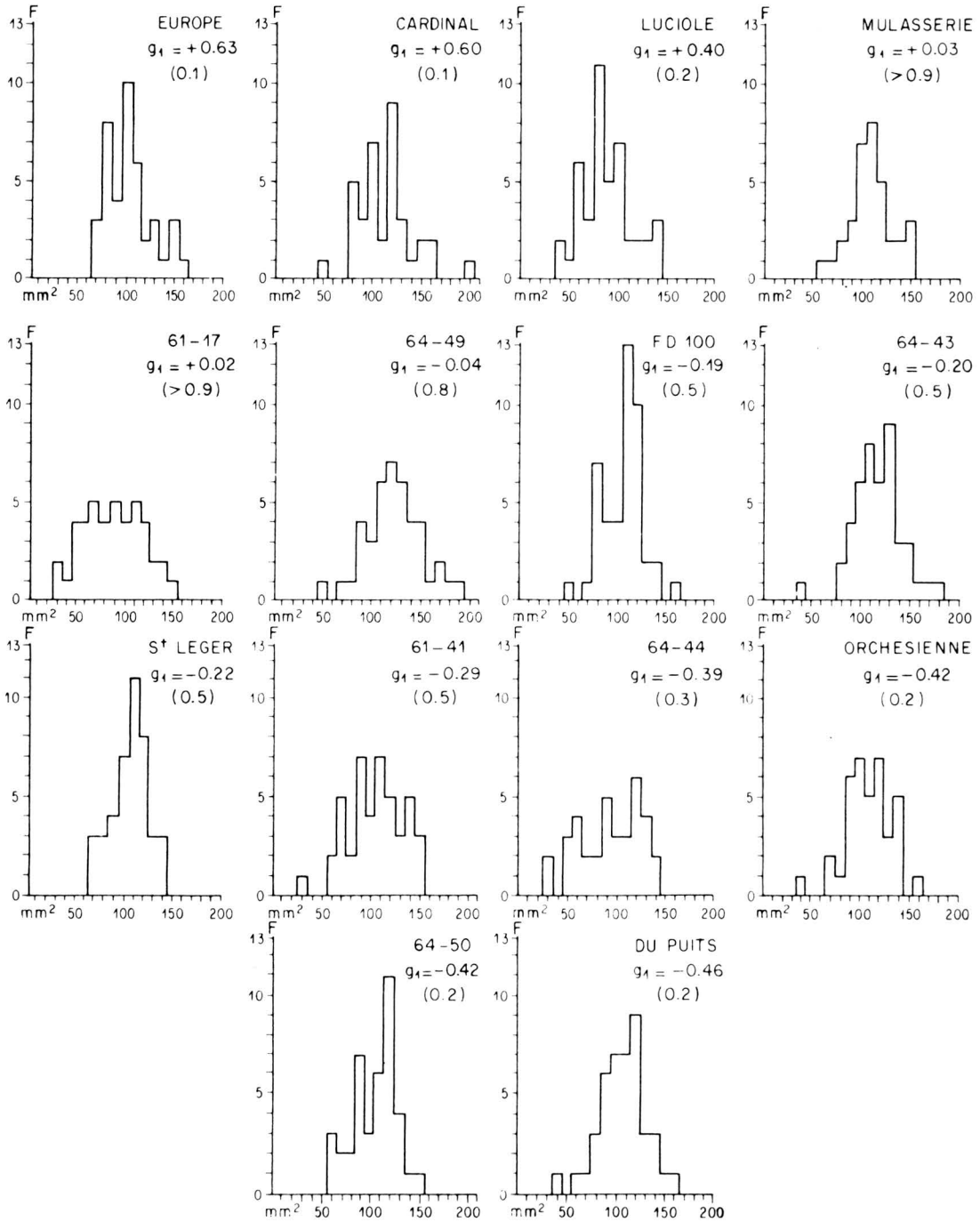
- a - if $g_1 = 0$, the distribution is normal,
- b - if $g_1 > 0$, the mode is inferior to the mean, numerous individuals are weak ;
- c - if $g_1 < 0$, it is the contrary.

For the whole set of genotypes, the results of the figure 4 are obtained ; are differentiated :

a - the genotypes "Mulasserie", 61-17 and 64-49, the individuals of which are

FIGURE IV

Distribution characteristics for leaf areas
(Between brackets = P 0.05)



normally distributed,

- b - the genotypes "FD 100", 64-63, "St Léger" and 61-41 which have a quasi normal distribution,
- c - the genotypes "Europe", "Cardinal" and "Luciole" with an excess of weak individuals,
- d - the genotypes 64-44, "Orchésienne", 64-50, "Du Puits", with an excess of strong individuals.

The figure 5 gives the relations between the mean leaf area of a genotype, and the skewness of the distribution (taking into account, or not taking, the g_1 sign), from one hand, and the dry matter production at the first cut. In spaced plants, excluding the genotypes 64-49 (greatest leaf area associated to smallest production) and "Luciole" (smallest area associated to a good production), the correlation coefficient between vigour and production is : + 0,725 ; the relation is negative in competition.

4. Phenotypic stability in the different environments

It appears interesting to synthetize the response of each genotype in competition by its stability (regression of each genotype yield on the mean yield in each condition). This method of analysis has been described elsewhere. Three of the 13 linear regressions are illustrated on figure 6 A (Table 6). 7 coefficients are inferior to 1, of which only three in a significant manner at the 0,05 point. They characterize stable genotypes towards changes in competition pressure and specifically adapted to severe environments (figure 6B). The "Du Puits" variety, with a coefficient significantly superior to 1, shows sensitiveness to changes in competition pressure and specific adaptability to low competitions. "Orchésienne" is more unstable too. Among mean stability genotypes ($b = 1.00$), it is possible to distinguish those with high yields, such "Europe" and "61-41", showing a good general adaptability to competition, and those with a low yield, such all the two-way hybrids except "61-41" and "64-44", badly adapted to all competition conditions.

It is necessary to underline that the stability notion is relative to the condition sample studied. The same regression coefficient is not obtained considering the three cuts or only the first two for the conditions B, C and D and the first for A (Table 6). This is clear above all for the genotype "61-41" (n° 14) of which the stability is improved by time.

FIGURE V

Relation between response to competition and vigour

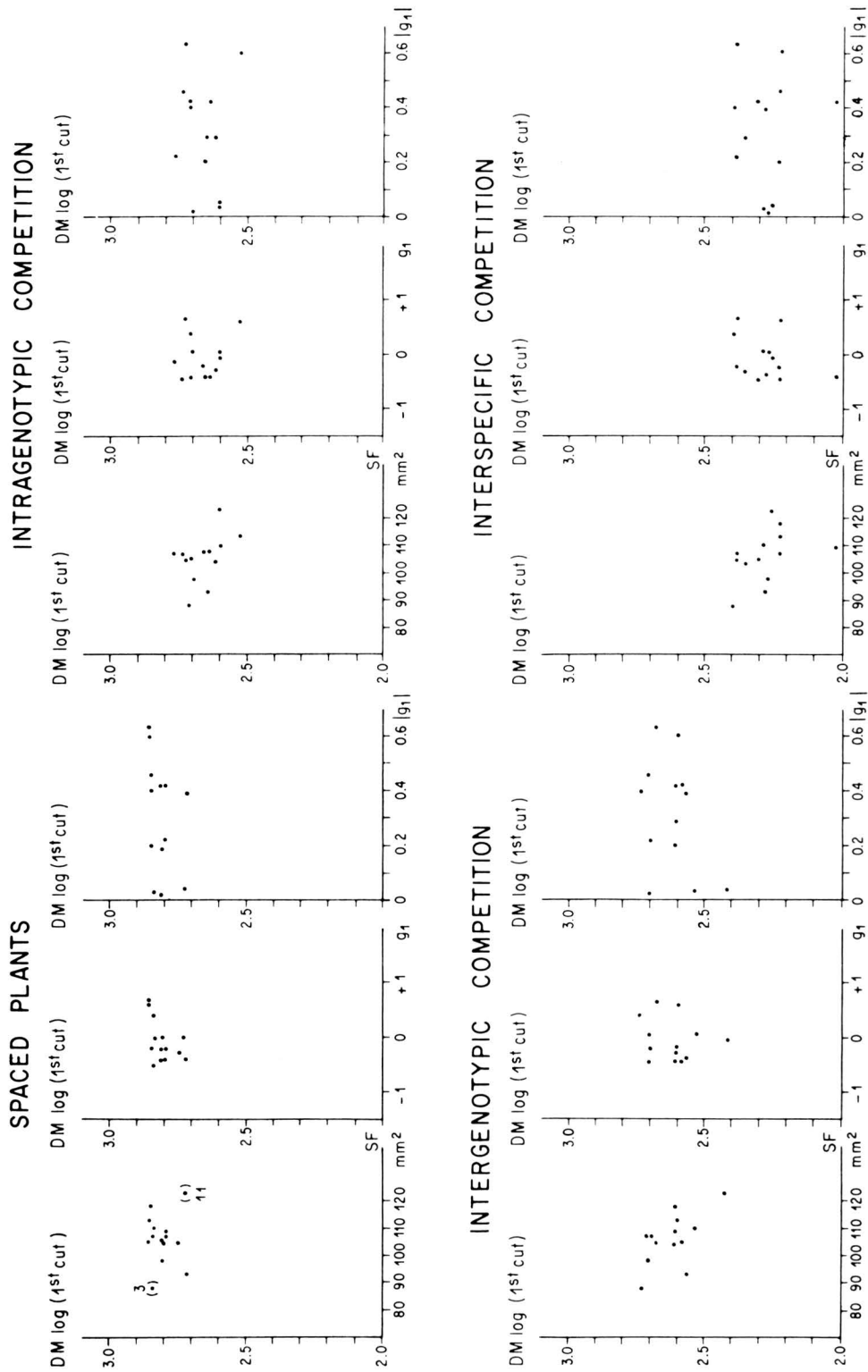
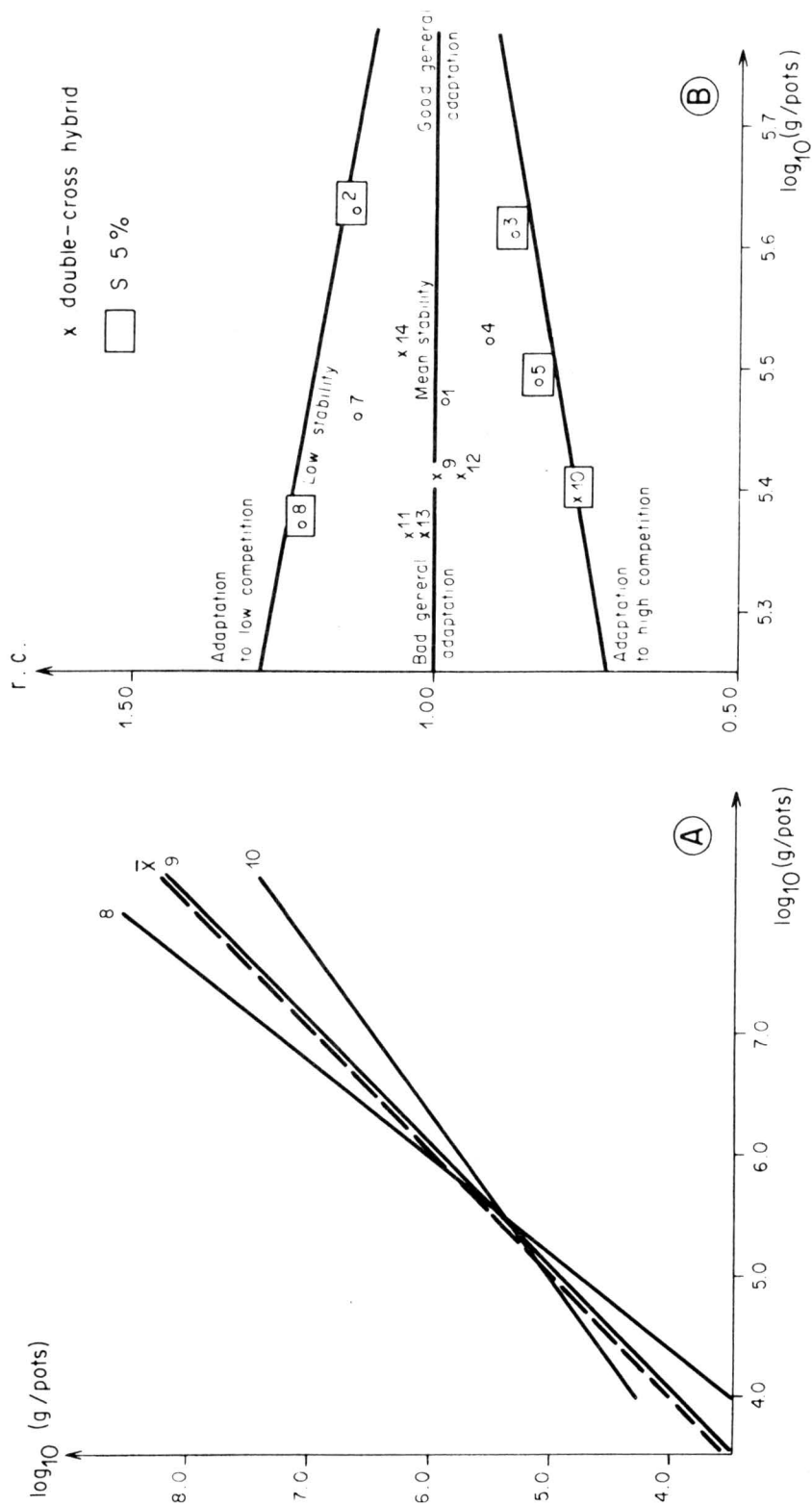


FIGURE VI

Social homeostasis of α alfalfa genotype set



(A) = Linear regressions showing the relation between the individual yields of 3 genotypes and the population mean, \bar{X} , of 13 genotypes grown in different competition conditions

(B) = Relation between genotypic adaptation (regression coefficient) and mean yield for 13 genotypes

Table 6

Phenotypic stability of a set of alfalfa genotypes

G = genotypes

\bar{Y}_i = mean yield

b = regression coefficient for the three cuts in all conditions

S_b = standard error of b

$P_{0,05}$ = signification of b for the departure from 1

b_P = regression coefficient for two cuts in conditions B, C and D an one in A.

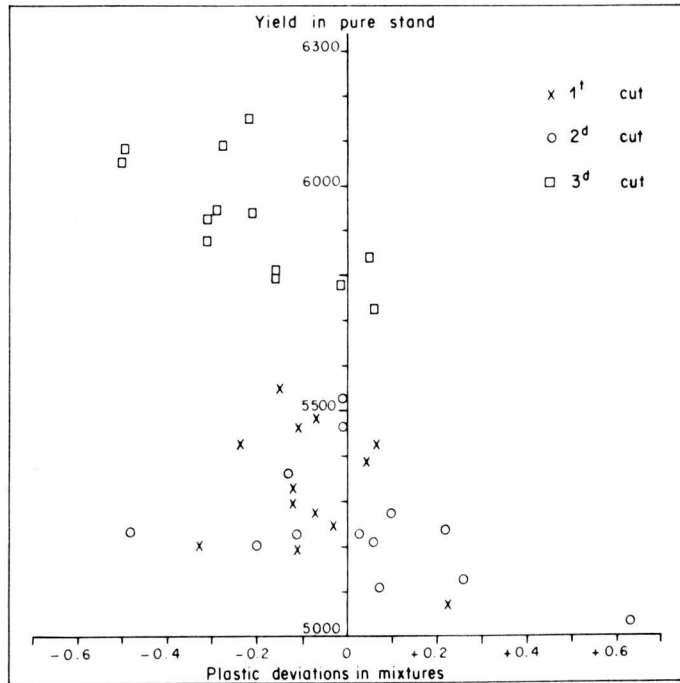
| G | \bar{Y}_i | b | S_b | P = 0.05 | b_P |
|----|-------------|-------|-------|----------|-------|
| 2 | 5.634 | 1.139 | 0.049 | S | 1.132 |
| 3 | 5.612 | 0.872 | 0.023 | S | 0.843 |
| 4 | 5.518 | 0.915 | 0.047 | NS | 0.882 |
| 14 | 5.512 | 1.056 | 0.091 | NS | 0.827 |
| 5 | 5.489 | 0.827 | 0.055 | S | 0.883 |
| 1 | 5.468 | 0.986 | 0.088 | NS | 0.833 |
| 7 | 5.456 | 1.128 | 0.076 | NS | 0.980 |
| 9 | 5.415 | 0.996 | 0.044 | NS | 1.052 |
| 12 | 5.413 | 0.961 | 0.086 | NS | 1.256 |
| 10 | 5.389 | 0.767 | 0.062 | S | 0.842 |
| 8 | 5.368 | 1.223 | 0.074 | S | 1.464 |
| 11 | 5.361 | 1.036 | 0.084 | NS | 0.782 |
| 13 | 5.358 | 1.025 | 0.052 | NS | 1.227 |

Table 7 . Effect of competition on heritability of yield

| Nature of competition | Cuts | | | \bar{x} |
|-----------------------|------|------|------|-----------|
| | 1 | 2 | 3 | |
| Spaced plants | 0.76 | 0.80 | 0.75 | 0.77 |
| Intragenotypic | 0.67 | 0.49 | 0.53 | 0.56 |
| Intergenotypic | 0.65 | 0.54 | 0.42 | 0.54 |
| Interspecific | 0.66 | 0.69 | 0.79 | 0.71 |
| \bar{x} | 0.68 | 0.63 | 0.62 | 0.65 |

FIGURE VII

Relation between yield in pure stand and plastic deviations in mixture, of the same numerical density



CONCLUSIONS

The genetic variability is buffered at the levels of intragenotypic, intergenotypic and interspecific competition. This may be expressed in terms of heritability, in the "narrow" sense, of which the part played by it in the confidence to allow to phenotypic value as guide of the reproductive value is important. This phenotypic value includes the reproductive value, from one hand, and, from the other, a residue which comprises, here, the deviations owing to competition. It is necessary to recall that it is a property not only from one character (here, the yield) but also from the environment to which individuals are submitted. The heritability calculation is an evaluation of genetic variance in per cent of total variance. If the effect of competition on this criterion, applied to yield, is analyzed (Table 7), it is intraspecific competition that acts the more. It is possible to remark, incidentally, that h^2 decreases from cut to cut.

According to Rotili and Zannone (1970), at the same numerical density, the best genotypes in pure stand increase their yield in mixtures. This is not obvious in the condition of the experiment described here (Figure 7).

In the experimental design followed, the genotype x competition interaction is significant only at the 10 % point ; and if the first unifoliate leaf express the vigour, there is no relation between this latter and competition performance.

But it is interesting to note that genotypes differ in stability ; this information on regression analysis is to add to the others accumulated at present with various success. A good stability associated with a high yielding ability is an interesting "character". In fact, the three varieties "Du Puits", "Luciole", "Europe", have the best performances ; but the responses of five hybrids from the six require a further attention.

REFERENCES

- Donald, C.M., 1963. Competition among Crop and Pasture Plants. *Adv. in Agro.*, 15, 1-118.
- Gallais, A., 1970. Application d'un modèle d'analyse des relations d'associations binaires. V^o Coll. Ecologie, Paris, 35 p. ronéo.
- Rotili, P. and Zannone, L., 1970. Intra and intergenotypic competition in *Medicago sativa*. *Eucarpia*, Fodder Crops Section, Lusignan, 14 p. ronéo.

Editions S. E. I.
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