# RATIONAL METHODS FOR BREEDING CROSS-FERTILIZERS

BY

## S, J. WELLENSIEK



Mededelingen van de Landbouwhogeschool Deel 48 – Verhandeling 7

H. VEENMAN & ZONEN — WAGENINGEN — 1947

479782

## CONTENTS

I. Introduction	. 229
II. Vegetative reproduction of type and the use of clones in breeding-metho	ds
until the end of 1944	. 230
§ 1. The vegetative reproduction of rve	. 230
δ 2. The use of rve clones in breeding-methods	231
UI Methods for breeding a rve variety resistant to eelworm	233
8.1 Problem and general plan of work	233
5.2 The carrying out of the backcrossings	
8.3 The breeding for homozygoesis of the characteristic of eelwor	. 400 ·
y 5. The breading for homolygesis of the characteristic of conwor	
$\begin{array}{c} 1 \\ 1 \\ 2 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\$	· 2011
93.1. Stateling-point	. 2014 025
g 3.2. Mass sciences alloction	. 200 025
g 3.5. Peuigree selection	. 200
9 3.4. Comparison between mass selection and pedigree selection	ЭП 238
§ 3.5. Methods with use of clones	. 240
§ 3.5.1. Method 1: Test crossing and vegetative maintenan	ce
of the clones, p. 240; § 3.5.2. Method 2: Test-crossing and par	<b>Γ</b> -
crossing, p. 241; § 3.5.3. Method 3: Pair-crossings with gen	8-
rative testing, p. 242; § 3.5.4. Method 4: Diallel crossin	g,
p. 243; § 3.5.5. Method 5: Inbreeding by means of se	if-
fertilization, p. 244; § 3.5.6. Mutual comparison of the	ne -
methods, p. 245; § 3.5.7. Supplementary remarks, p. 24	6.
IV. Rye breeding in general	. 246
§ 1. Extension from eelworm resistance to other characters	. 246
§ 2. The scheme of breeding for high yield: test-crossing and pai	ľ-
crossing	. 248
V. The method of repeated back-crossing	. 251
§ 1. The method as such $\ldots$ $\ldots$ $\ldots$ $\ldots$ $\ldots$ $\ldots$	. 251
§ 2. The carrying out of the back-crosses with regard to the selection	n,
in various cases	. 252
<ul> <li>§ 2.1. Dominance or recessiveness of the desired characterist</li> </ul>	ic
in connection with self- or cross-fertilization	. 252
§ 2.2. Complete valuation and selection before or after flowering	ig 252
§ 2.3. Grouping of the different cases.	. 252
§ 3. The selection of homozygotes after back-crossing	. 254
§ 3.1. The problem	. 254
§ 3.2. The selection of AA from $Aa + aa$ .	. 254
§ 3.2.1. Self-fertilizers, p. 254; § 3.2.2. Cross-fertilizers which	h
can be evaluated before flowering, p. 254; § 3.2.3. Cros	5-
fertilizers which cannot be evaluated before flowering, p. 25	5.
§ 3.3. The selection of a from $AA + Aa$	. 255
§ 3.3.1. Self-fertilizers, p. 255; § 3.3.2. Cross-fertilizers which	h
can be evaluated before flowering, p. 255; 8.3.3.3. Cros	s-
fertilizers which cannot be evaluated before flowering p. 25	5
VI. Discussion of results: The breeding of cross-fertilizers in general	256
VII. Summary	259
Literature cited	261

## 631.52:581.162.32

# RATIONAL METHODS FOR BREEDING CROSS-FERTILIZERS

## by

## S. J. WELLENSIEK

(Received for publication on March 12th 1947) (Instituut voor Plantenveredeling and Laboratorium voor Tuinbouwplantenteelt, Wageningen, Netherlands)

#### I. INTRODUCTION

When we compare the technic of breeding of various crops, no matter what their nature may be, we see that the choice or the making of very variable starting material for the selection proper do not present special difficulties. Neither does the selection itself, which in essence is the recognition of desired types. The most complicated and hence the most interesting problems are met with when we attempt to breed true the selected types. However, the crops which, as a rule, are reproduced vegetatively (typical cases: potato, strawberry) and the self-fertilizers (flax, pea) do no longer present special difficulties. The most arduous problems are encountered in those cross-fertilizers which are not commonly reproduced vegetatively and the very core of these difficulties is the impossibility of completely regulating the pollination. The situation is considerably simplified when the plants in question bear fruit more than once, for in this case we can continue our work with individuals of which a preceding progeny test has demonstrated that they are genetically good (Cyclamen).

A typical case of a cross-fertilizing plant which offers all the complications connected with this condition from the point of view of breeding, is rye. When at the end of 1938 I was charged to evolve new breeding methods for this crop, the idea of vegetative propagation immediately presented itself. The great advantage of this would be that the plant could then be essentially assimilated to plants bearing fruit more than once.

The method of vegetative reproduction was soon found. The obtained clones, however, could, as a rule, not be maintained for more than 2 years. Yet, by means of these clones, it was possible to improve the breeding technic to a considerable extent. How this was to be done most rationally was not obvious at first. By concentrating all attention on the many experimental possibilities which the use of clones allowed, a calm theoretical reflection remained wanting.

When during the evacuation in the winter of 1944/45 the practical work perforce had to be abandoned, I pondered over the theoretical aspects of rye breeding. These meditations proved not only a prophylactic against mental degeneration, but also bore fruit. Especially the method of breeding a variety of rye resistant to eelworms was elaborated. The results were surprising and transcended the scope of the limited initial problem. They afforded an insight in the most rational breeding method which in general can be obtained with the aid of clones in crossfertilizing plants. In the last year of the war the vast material used in the rye breeding experiments was almost completely lost. However, there has now been developed a rather refined breeding technic, all the separate phases of which have proved themselves in previous experiments to be easily executed. The publication which follows aims at expounding the basis of this method. At the same time the importance of a theoretical consideration regarding plant breeding problems is emphasized; problems which in my opinion have been much neglected.

230

After a summary of the literature already published and in which the state of the problem up to 1944 is presented, the way of breeding eelworm resistant rye will first be evolved, because it actually constitutes the basis to the subsequent development. Afterwards, building on the results established in the preceding step, the procedure to be followed in rye breeding in general will be dealt with. Then the method of repeated back-crossings is discussed in its generality. Of this method the breeding of eelworm resistant rye is a special case. In order to throw a light on the importance of a theoretical examination of breeding problems, this method will be elaborated for all crops divided in a few categories. At the end a general survey is given of the improvement of cross-fertilizers with the previously established results as basis.

#### 11. VEGETATIVE REPRODUCTION OF RYE AND THE USE OF CLONES IN BREEDING-METHODS UNTIL THE END OF 1944

#### § 1. The vegetative reproduction of rye

Rye can be easily multiplied vegetatively by means of division. This has been applied by HEEMSTRA (unpublished), by MUNERATI (10)<sup>1</sup>) in 1924 and by KOWARSKY (see AUST, 1, p. 84) in 1939. They worked with rye sown at the normal time and obtained only a few descendants from one plant. For the sake of completeness I also mention RIEBESEL (13), who in 1937 applied vegetative reproduction in species crosses of cereals and who claimed that this caused otherwise sterile plants to become fertile. My own rather extensive clone material of wheat  $\times$  rye hybrids by no means confirms this opinion.

SOPHIA AUST (1) gave in 1941 an account of vegetative multiplication in rye. Sown in September in pots in the open air, the plants were placed half December in the greenhouse with a temperature of  $10-12^{\circ}$  C, where they were divided. She obtained with the better clones of her material 20 plants on the average and a maximum of 87 plants.

None of the above mentioned research workers has used vegetative reproduction in order to draw the clones thus obtained into a breeding scheme.

My first experiments (18) were made in 1939 with material sown in the autumn of 1938. The divided plants immediately began to shoot and a satisfactory multiplication was not obtained. Meanwhile a periodic seedtime experiment in which during one whole year weekly sowings were made, demonstrated that winter rye sown in March still yields plants producing shoots, but that plants sown in May remain bushy and tiller strongly which is the ideal condition for vegetative multiplication. April is a transitional month. Plants sown on the 8th of May 1940 were multiplied vegetatively the same year. This could be performed twice with the clone plants which means that three multiplications in all were made. From 130 clones about 100 plants to the clone were obtained with a maximum of 330 plants, no attempt being made to attain the maximum (19). The number of plants per clone could be considerably increased by sowing already in the middle

3) Numbers in parentheses refer to the list of literature on p. 261.

[4]

of April — eliminating all plants that developed shoots —, and by excessive manuring with N (REINDERS 11). REINDERS (12) also found that germinating the seed at  $22-23^{\circ}$  C or at  $30^{\circ}$  C sometimes produces a favorable effect, because the sowing can be done earlier. The effect of this treatment, however, is too slight for regular application. Clones of 500 or even 600 plants were made in later years, but in most cases a much smaller number of plants per clone will do.

231

Vegetative multiplication by itself, consequently, no longer constitutes a problem, which is due mainly to the practice of spring-sowing of winter rye. A different thing is the maintenance of clone plants in vegetative condition. After wintering on the field, that is to say after having been exposed to the vernalizing influences of low temperature and short days, the clone plants shoot, which precludes the possibility of keeping them in a vegetative condition or multiplying them vegetatively, because after shooting, flowering and seed formation death inevitably follows, at least in "ordinary", non-perennial rye, which is the only cultivated rye in the Netherlands.

Because of the great importance of keeping plants in a vegetative condition, means were sought to prevent the shooting of clone plants. At first two ways of intervening seemed promising (19, pp. 430–433). To begin with, when shooting commenced, while the ear was not yet visible externally and had a length of 0,5 cm, a longitudinal section was made in the enveloping leaf-sheath and the ear was removed. This operation is easily performed after some practice. The only instrument needed is a sharp-pointed knife. It amounts to cutting the top of the plant and the usual result ensues, growth in length is arrested and tillers develop. Because these tillers also soon begin to form ears, they too should be operated upon. However, a simple expedient was found to retard strongly the growth in length of the tillers by giving the plants a daily illumination of 8 hours only. Ear formation is stimulated by a short day, but shooting requires a long day.

The combined application of removal of the ear and short-day treatment produced good results in some cases and it has been possible to keep clone plants alive for 3 years. But this succeeded only with a few individuals of some clones. To be used in a breeding scheme this method has too uncertain effects. Miss Dr D. E. REINDERS tried to improve the method but practically important results were not secured. A considerable handicap in her work was the difficulty to grow the plants during the winter under artificial light and high temperature. Black-out and current rationing made illumination experiments well-nigh impossible and coal shortage prevented the attaining of high enough a temperature. We must leave the question whether it be possible to maintain for practical purposes clones alive for more than 2 years to the future for answer. In a breeding scheme a span of life of 2 years only for the plants can be reckoned with.

#### § 2. The use of rye clones in breeding-methods

When the possibility of a satisfactory vegetative multiplication of winter rye after spring sowing was demonstrated I immediately drew up two selection schemes with the aid of clones (18). In the first scheme the Petkus reserve seed method (see LAUBE, 5 and 6) was applied in principle, but individual plants were replaced by clones. We start from a few clones which are grown together, but isolated from other rye, so that they only can fertilize each other. Before flowering as thorough a selection as possible is made, but objective criteria are wanting for this work. The most important thing is a strong tillering capacity of which the number of plants per clone constitutes a function. After grain setting and matu-

[5]

ration a second clonal selection is performed based mainly on the yielding capacity. Only the seed of the best clones is kept. Part of this seed is sown on test plots that are not isolated; another part is saved as reserve seed. When, after a progeny test, a final selection is made, the reserve seed of the clones finally selected is sown for isolated multiplication or the whole procedure is repeated with the reserve seed as starting material. The seed from the plants of the test plots is not considered for further selection purposes, because it has been contaminated by pollen of plants not qualified for selection. The use of reserve seed means a a control of the pollination in the plants which develop from this seed.

The importance of crossing in pairs, such as was done in my second scheme, has been pointed out for the first time by Von SENGBUSCH (15, pp. 85–88; 16) in 1939 and 1940. By crossing plants of a cross-fertilizer two by two the individual selection is approximated as much as possible without there being a danger of deterioration due to inbreeding. Now pair crossings of 2 rye plants yields too little grain to do anything with on sufficiently large a scale. But through the use of clones this attractive method becomes possible. Instead of isolating all clones together from other rye, they are isolated two by two, while in other respects the first scheme is followed. Good results were obtained by planting  $2 \times 25$  plants of 2 clones in a mixture on plots of 1 m<sup>2</sup>. The distance between these plots was first 50 m, later on 30 m.

Both ways preserve the advantage of the Petkus method: in the increase of selected material pollination is controlled by means of the reserve seed device. Only plants that have proved to belong to the hereditary good group are allowed to participate in this pollination. New advantages are:

I. The selection of mother plants is replaced by a selection of clones which is much more precise, because in the judging of a clone many scores of individuals permit to determine the influence of the environment.

2. Because clones of numerous plants produce much more seed than single plants do, the judging of the progenies can be done on a considerably larger scale. Besides, the use of pair crossing has the great advantage that the control of the pollination goes much farther at first, involving one clone only.

In 1942 it was demonstrated experimentally that the two schemes developed in 1940 did not entail practical difficulties (19), while in 1943 a few further details were settled (20, pp. 461-466). Experience gained since confirm the practicability and indications were secured pointing especially to the value of pair crossings. The difficulty, however, was the ulterior handling of the material selected in one series. A renewed application of the scheme of crossing in pairs seemed obvious at first, but convincing evidence was secured that this led to loss of vigor due to inbreeding. This is not surprising, as the starting material for the second series of selections which comes from one crossing in pairs consists of sisters and brothers. Because numerical data were lost during the evacuation of Wageningen I am unfortunately not in a position to offer further particulars, but as already stated, they were very convincing.

Loss of vigor due to inbreeding, however, occurs also when the ordinary Petkus method, modified by the use of clones is followed. In this procedure the material for the second series of selection is derived from one clone which has been pollinated by several clones and consequently represents half-sisters and half-brothers. Loss of vigor is less pronounced in this case, due to the less close relationship than with sister  $\times$  brother pollination, but indications of deterioration were also detected in this material.

**23**2

[6]

In itself loss of vigor caused by inbreeding is not ultimately fatal; it can be offset by means of crossing unrelated material. The difficulty, however, is that we don't know to what degree the deterioration differs from one family to another, which makes the basis of comparison unreliable.

I think that I have now found the right method which constitutes as it were the completion of the previously applied procedures which were not yet sufficiently thought out theoretically. The new conceptions will be treated in the following.

#### III. METHODS FOR BREEDING A RYE VARIETY RESISTANT TO EELWORM

#### § 1. Problem and general plan of work

In some soils eelworms cause such severe damage that the variety Petkus cannot be cultivated owing to its great susceptibility. The Dutch land variety "Ottersumsche" is quite resistant and hence is cultivated on fields infested by eelworms, but it yields much less than Petkus. According to my own experience its yield is about 70 % of Petkus.

The creation of a new variety out of descendants of a cross between "Petkuser" and "Ottersumsche" which will combine the productivity of Petkus to the eelworm resistance of Ottersum rye suggests itself. Efforts in this direction made by KOESLAG and STIELTJES remained without results. For literature see SEINHORST (14, p. 2).

In collaboration with Ir J. W. SEINHORST, who took charge of the determination of resistance, I began work towards the ends outlined above (21, p. 6). Soon it was possible to ascertain that the  $F_1$  of the cross Petkus × Ottersum was resistant. This cross was made between members of a clone, other plants of which had been tested as to eelworm resistance. The Petkus parent clone hence was proven to be susceptible, the Ottersum parent clone to be resistant. The  $F_1$  being resistant showed that the hybridization had been successful and that resistance is dominant.

The task set now was to obtain a new rye variety resistant to eelworms but otherwise approaching Petkus. The indicated method to attain this aim is the method of repeated backcrossing. In our case dominance of resistance and the possibility of determining susceptibility before flowering facilitated the work.

The general procedure then is as follows. The  $F_1$  of the cross Petkus × Ottersum is crossed back to Petkus. Among the descendants of this backcross a segregation will occur in susceptible and resistant individuals. The latter can be detected at an early stage. They are again backcrossed to Petkus and the procedure already outlined is repeated. When a sufficient number of backcrossings has been performed, so that it may be assumed that the hybrids through repeated introduction of genic material of Petkus rye have become very similar to this variety, breeding to obtain constancy is undertaken. This is done in the first place in connection with resistance to eelworm, but also for yield and other practically important characters.

#### § 2. The carrying out of the backcrossings

It is not the intention to describe the practical execution of the work in all its details. Only a few words will be said about performing the backcrossing, because this part is closely connected with the general tenor of this paper which points to the importance of the application of vegetative multiplication as part of a selection method.

233 -

[7]

The backcrossings can be made artificially just as this has been done with the first crosses of the parental varieties between themselves. This method, however, is complicated and therefore time consuming.

It is much simpler to leave pollination to nature and allow spontaneous crossing to take its course. This can be done by means of space isolation of 2 plants to be crossed. In this way, however, a relatively small number of kernels is secured. This drawback is surmounted by vegetative multiplication of both plants to be crossed and by letting fertilization occur between 2 clones. Loss of time occasioned by the execution of vegetative multiplication after spring sowing need not be feared now, for by sowing immediately after the harvest, say in August, a vegetative multiplication sufficient for this case can be accomplished.

But there is a consideration of very different nature which caused me to renounce the use of spontaneous cross-fertilization between 2 clones. Petkus rye, used as the parent variety in the backcrossings, being a variety of a cross-fertilizer, is far from homozygous, so that from plant tot plant considerable genetic differences may occur. By allowing only one clone of Petkus rye to partake in the backcrossing, we too much put all our eggs in one basket. It, therefore, is recommendable to use a certain number of Petkus seedlings instead of one clone.

Very satisfactory results were obtained by flanking 1 or 2 rows planted to a clone of the  $F_1$  Petkus × Ottersum on both sides by Petkus seedlings. The length of the rows was put at 1 m, the distance in the row at 10 cm and the distance between the rows 20 tot 25 cm. Such a small plot can easily be isolated from other plots of flowering rye. If the plants belonging to one clone are intersterile, the kernels develop on the clone plants after fertilization through pollen of the surrounding Petkus seedlings.

The same end may be attained by planting the clone to be cross-pollinated in a large field of Petkus rye. If one desires to cross different clones with Petkus, they can be placed at a mutual distance of 50 m, which certainly affords sufficient isolation.

#### § 3. The breeding for homozygosis of the characteristic of eelworm resistance

#### §3.1. Starting-point.

We have now arrived at the most important aspect of the method expounded in this study: the production of a true breeding new variety when the process of backcrossing has been completed. We shall confine ourselves to eelworm resistance and represent this character as simplified as possible from a genetical point of view by denoting resistance in Petkus by the pair of recessive genes aa and the resistance in Ottersum rye by the deminant pair AA. It is true that only the dominance of resistance has been demonstrated experimentally, but not its monofactorial nature. For a theoretical comparison of different methods a monofactorial difference may be assumed. Though reality may not correspond to this assumption, yet the essential differences between the various methods remain valid.

The original cross Petkus  $\times$  Ottersum then is  $aa \times AA$ , the F<sub>1</sub> is Aa and the backcrossings are of the type  $Aa \times aa$ , the progeny of which segregates into  $(A + a) a \rightarrow Aa + aa$  that is to say 1 resistant to 1 susceptible. The susceptible aa individuals are eliminated at an early stage and the next backcrossing is again  $Aa \times aa$ .

The last backcrossing also segregates into Aa + aa. The task now is the breeding of AA, the homozygous resistant form. After a susceptibility test the aa plants

235

are removed from the Aa + aa population. Mutual fertilization of the remaining Aa's produces  $(A + a) (A + a) \rightarrow AA + 2 Aa + aa$  from which population the aa's are again eliminated. The problem then is how in the most rational way the AA's can be separated from the AA + 2 Aa population and made the starting point of a new variety.

We shall first investigate what can be attained by means of mass selection and by means of pedigree selection. Then we shall develop a few more rational methods.

#### § 3.2. Mass selection.

Let the starting population of the composition AA + 2Aa be denoted the generation  $F_{n+1}$ . In this population the gametic ratio is A + A + 2A + 2a = = 4A : 2a = 2A : a. Complete panmixis, as we may expect from absolute crossfertilizers, produces zygotes in the proportion  $(2A + a)^2 = 4AA + 4Aa + aa$ . The *aa*'s are eliminated in a susceptibility test and the population 4AA + 4Aa, which we shall designate for the purpose of the outline to be developed presently as 2AA + 2Aa, remains, representing the  $F_{n+2}$ .

The gametic ratio of the  $F_{n+2}$  is 6A: 2a = 3A: a. The zygotic ratio – the  $F_{n+3}$  – becomes  $(3A + a)^3 = 9AA + 6Aa + aa$  of which after removal of aa remains 9AA + 6Aa or 3AA + 2Aa.

Continuing this way we find for the composition of the  $F_{n+1}$ ,  $F_{n+2}$  etc. the relations of AA to Aa respectively 1:2, 2:2, 3:2, 4:2, etc.; the general formula for the  $F_{n+p}$  being p AA: 2 Aa. Because in the general formula the sum of the coefficients of the terms is p + 2, p individuals being AA to 2 Aa, the

# % of Aa is $\frac{2}{p+2} \times 100$ .

For the gametic ratios of the generations  $F_{n+1}$ ,  $F_{n+2}$ , etc., the relations of A tot  $\alpha$  are respectively 2:1, 3:1, 4:1, 5:1, etc., with the general formula for the  $F_{n+p}$  represented by (p+1): 1. Hence the zygotic ratio of the  $F_{n+p}$  is given by the formula  $\{(p+1)+1\}^2 = (p+1)^2 + 2(p+1) + 1$ , formula essentially the same as the one given bij SIRKS (17, p. 19).

In this zygotic ratio we are especially interested in the % of undesired aa's. The expansion shows that among the  $\{(p + 1) + 1\}^2 = (p + 2)^2$  zygotes 1 is aa, hence the % of the aa zygotes produced by the  $F_{n+p}$  is  $\frac{1}{(p+2)^3} \times 100$ . This means that the  $F_{n+20}$  still produces approximately  $0,2^{\circ}/_{0}$  or  $2^{\circ}/_{00}$  undesired zygotes.

The relations derived above have been given together with a few obvious additions in the columns 2, 3, 6, 9, 10 and 13 of table 1 on p. 238. We shall return to these tables in § 3.4. From the numbers in column 13 we readily conclude that the decrease of the % of aa's is very slow.

#### §3.3. Pedigree selection.

Just as in the case of mass selection the starting population in the  $F_{n+1}$  is of course AA + 2Aa. The gametic series formed by these plants is A : A : 2A : 2a or a ratio 2A : a. As contrasted with mass selection, in pedigree selection we save the seed of every plant separately. We must know what the composition is of the families obtained through individual sowing of seed from the  $F_{n+1}$ -plants having the genotypes AA or Aa.

The question is easily solved when we ask ourselves what gametes are formed by the mother plants and by which gametes they are fertilized. In total panmixis the latter correspond always to the gametes produced by the population as a whole.

We then find that the mother plants AA only produce A gametes which are exposed to fertilization by a 2A + a mixture as the above derived ratio shows. The result is  $A(2A + a) \rightarrow 2AA + Aa$ . The mother plants Aa, producing two kinds of gamestes A and a, give rise to families of the composition  $(A + a) (2A + a) \rightarrow 2AA + 2A + aa$ .

(A + a)  $(2A + a) \rightarrow 2AA + 3Aa + aa.$ 

Now if we submit both families 2AA + Aa (coming from the mother plant AA) and 2AA + 3Aa + aa (coming from the mother plant Aa) to a susceptibility test we find in the first case all plants phenotypically resistant, but in the second case the susceptible aa plants segregate in a ratio 5 resistant (2AA + + 3Aa) to 1 susceptible (aa). Hence we can distinguish the Aa-plants of the original population by their progeny from the AA-plants. Because we desire exclusively AA, we eliminate the families of the Aa-plants. For after removing the susceptible aa's from the families 2AA + 3Aa + aa a proportion 2AA: : 3Aa remains which is much less favorable in relation to the undesirable Aa than in the families issued from the AA-plants which give a relation 2AA : 1 Aa. To prevent loss of vigor due to inbreeding, these families are sown for further selection in a mixture.

The practical procedure of pedigree selection hence is: to secure seed from a number of individual plants of the initial population, to subject the families coming from this seed to a susceptibility test and continue the work only with families which do not exhibit any segregation of susceptible plants.

The last mentioned families which represent the  $F_{n+2}$  then have the composition 2 AA : Aa. The gametic ratio of these plants is 5 A : a. Consequently an AA-plant will give as family  $A(5A + a) \rightarrow 5 AA + Aa$ , while an Aa-plant yields a family  $(A + a)(5A + a) \rightarrow 5 AA + 6 Aa + aa$ . After taking out aa the family becomes 5 AA + 6 Aa, which is a far less favorable composition than 5 AA + Aa given by the AA-family.

In the  $F_{a+3}$  we only continue with 5 AA + Aa families. Because here the gametic ratio is 11 A : a an AA-plant gives a family A (11 A + a)  $\rightarrow$  11 AA + + Aa, while an Aa-plant gives a progeny (A + a) (11 A + a)  $\rightarrow$  11 AA + + 12 Aa + aa.

We now have sufficient data for establishing general formulae. Compare in connection with the following paragraphs columns 4, 5, 7, 11, 12 and 11 of table 1 on p. 238 to which we shall return in § 3.4.

First of all we found as composition of the generations  $F_{n+1}$ ,  $F_{n+2}$ ,  $F_{n+3}$ , the proportion of AA to Aa rendered by 1:2,2:1,5:1. If we write this succession as 1:2, 4:2, 10:2, we see that the continuation is 22:2, 46:2 etc.; that is to say the first term is the double of the corresponding term of the previous generation increased by 2. As sum of the terms we have the series 3, 6, 12, 24, 48 etc. or  $3\cdot 2^0, 3\cdot 2^1, 3\cdot 2^2, 3\cdot 2^3, 3\cdot 2^4$  etc. For the  $F_{n+p}$  the sum of the terms consequently is  $3\cdot 2^{p-1}$  and because the last term of the ratio of AA to Aa plants is always 2 in the notation here followed, the general formula for the proportion in the  $F_{n+2}$  becomes  $(3\cdot 2^{p-1}-2): 2$ . The % of undesirable Aa's is  $\frac{2}{3\cdot 2^{p-1}} \times 100$ .

If we now turn our attention to the proportion of the gametes A to a produced by the generations  $F_{n+1}$ ,  $F_{n+2}$ ,  $F_{n+3}$ , then we find respectively the ratios

236

[ 10 ]

2:1, 5:1, 11:1. Continuation of this succession furnishes 23:1, 47:1 etc., that is the first term is twice the corresponding term of the previous generation increased with 1. The sums of the two terms of these proportions are equal to those of the succession of proportions of AA to Aa, so that the general formula for this sum becomes also  $3 \cdot 2^{p-1}$ . Furthermore, because the last term in the gametic ratios is always 1 the general formula for the gametic ratio of A to a in the generation  $F_{n+p}$  reads  $(3 \cdot 2^{p-1} - 1) : 1$ .

From this last formula we obtain a relation between the zygotes AA, Aa and aa in the  $F_{n+p}$  expressed by the formula  $(3 \cdot 2^{p-1} - 1)^2 : 2 \times (3 \cdot 2^{p-1} - 1) : 1$ with a sum of terms equal to  $(3 \cdot 2^{p-1})^2$ . Because the undesirable *aa* always occurs once, the % of *aa* is  $\frac{1}{(3 \cdot 2^{p-1})^2} \times 100$  which with reference to column 14 of table 1 can also be written  $\frac{1}{(2^{p-1})^3 \cdot 3^2} \times 100$ . When we apply this formula to the  $F_{n+4}$  we find 0,2 % of undesirable *aa*'s, the same value that mass selection yields only after 20 generations. Hence pedigree selection is much more effective and we should be inclined to attach great value to this method, especially when used for several generations. Two objections, however, should be raised against continued appeal

to pedigree selection. To begin with, one never gets rid of the undesirable  $\alpha\alpha$ 's entirely within a reasonable number of generations, no matter how rapid their percentual decrease may be in the successive years. In this connection the second difficulty becomes serious. For a rational procedure it is practically impossible to go beyond the  $F_{n+4}$  and that because of the great number of plants necessary to the susceptibility test. Let us consider this point more in detail.

In the  $F_{n+1}$  we found for the relation resistant : susceptible in the segregating families 5 : 1. With a total of 60 plants per family the expectation is consequently 50 : 10 with a standard error of  $\pm 2,9$ . The number of 60 plants consequently is enough to have a reasonable chance that the segregating recessives will indeed show up.

In the  $F_{n+4}$  the proportion resistant : susceptible in the segregating families becomes 47 : 1. Hence in a total of 480 plants to the family the expectation is 470 : 10 with a standard error of  $\pm$  3,1. A number of plants smaller than 480 does not yield reliable results. However, it is possible to obtain 480 descendants of one mother plant, so that this condition may be satisfied, apart from the comprehensiveness of the susceptibility test.

In the  $F_{n+5}$ , however, the situation is different, for we now have to handle not less than 960 plants. The expectation then is 950 : 10 with a standard error of  $\pm$  3,1. Such a large number cannot be attained for the progeny of every mother plant. We could, in order to increase the seed production, apply vegetative multiplication, but also in connection with the size of the susceptibility investigation the method then becomes very complicated.

The conclusion is that the method of pedigree selection is applicable up to and including the  $F_{n+4}$ . The result then is a population in which out of every 1000 plants 2 are susceptible on the average. A similar result is obtained in mass selection after 20 generations.

Pedigree selection, hence, is not unsatisfactory for practical purposes. But we never are sure to get rid of the undesirable recessives. We shall presently find that other methods can be developed which in a short time yield an entirely reliable result. § 3.4. Comparison between mass selection and pedigree selection. The above derived conclusions relative to the course of mass selection and pedigree selection are important enough form a general point of view to warrant summing them up in a more easily surveyed form. This is done in table 1 and in graphs 1 and 2.

#### TABLE 1.

Comparison	between	mass	selection	and	pedigree	selection	for	AA,	starting	from	the
, <b>·</b> ·			populati	on A	À + 2 A	$a, F_{n+1}$	•		•	•	

2	3	4	5	6	7
<u>.</u>	ratio A.	· %	Aa		
mass	Σ.	pedigree	Σ	mass	pedigree
1:2	3	1:2	3	$\frac{2}{3} = 67\%$	$\frac{2}{3} = 67\%$
2:2	4	4:2	6	$\frac{2}{4} = 50\%$	$\frac{2}{6} = 33\%$
3:2	5	10:2	12	$\frac{2}{5} = 40\%$	$\frac{2}{12} = 17\%$
4:2	6	22:2	24	$\frac{2}{6} = 33\%$	$\frac{2}{24} = 9\%$
5:2	7	46:2	48	$\frac{2}{7} = 29\%$	$\frac{2}{48} = 4\%$
6:2	8	94:2	96	$\frac{2}{8} = 25\%$	$\frac{2}{96} = 2\%$
p:2	p + 2	(3.2p-1-2):2	3.2p-1	$\frac{2}{2+2} \times 100$	$\frac{2}{3.2p-1} \times 100$
	2 mass 1 : 2 2 : 2 3 : 2 4 : 2 5 : 2 6 : 2 p : 2	$\begin{array}{ c c c c c c } 2 & 3 & \\ \hline & ratio A \\ \hline mass & \Sigma \\ \hline 1:2 & 3 \\ 2:2 & 4 \\ 3:2 & 5 \\ 4:2 & 6 \\ 5:2 & 7 \\ 6:2 & 8 \\ \hline p:2 & p+2 \\ \end{array}$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	2       3       4       5       6         ratio $AA : Aa$ % A         mass $\Sigma$ pedigree $\Sigma$ mass         1 : 2       3       1 : 2       3 $\frac{2}{3} = 67 \%$ 2 : 2       4       4 : 2       6 $\frac{2}{4} = 50 \%$ 3 : 2       5       10 : 2       12 $\frac{2}{5} = 40 \%$ 4 : 2       6       22 : 2       24 $\frac{2}{5} = 33 \%$ 5 : 2       7       46 : 2       48 $\frac{2}{7} = 29 \%$ 6 : 2       8       94 : 2       96 $\frac{2}{8} = 25 \%$ p : 2       p + 2 $(3.2p-1-2):2$ $3.2p-1$ $\frac{2}{2} \times 100$

8	9	10	11	12	13	14	
F	İ	gametic r	atio A : a	% of undesired zygotes aa			
	mass	Σ	pedigree	Σ	mass	pedigree	
n + 1	2:1	3	2:1	3	$\frac{1}{3^4} = 11 \%$	$\frac{1}{3^3} = 11 \%$	
n + 2	3:1	• 4	5:1	6	$\frac{1}{4^2} = 6\%$	$\frac{1}{6^2} = 3\%$	
n + 3	4:1	5	11:1	12	$\frac{1}{5^3} = 4\%$	$\frac{1}{12} = 0.7 \%$	
<u>n</u> + 4	5:1	6	23:1	24	$\frac{1}{6^*} = 2,8\%$	$\frac{1}{24} = 0,2\%$	
n + 5	6:1	7	47:1	<b>48</b>	$\frac{1}{7^2} = 2\%$	$\frac{1}{48^3} = 0.04 \%$	
n + 6	7:1	8	95 : 1	96	$\frac{1}{8^4} = 1,5\%$	$\frac{1}{96^{3}} = 0.02 \%$	
'n+p	(p + 1) : 1	p + 2	(3.2¤-1-1):1	3.2p-1	$\frac{1}{(p+2)^s} \times 100$	$\frac{1}{(3.2p-1)^{3}} \times 100$	

After the derivations, already given, table 1 does not need further explanation. It is interesting to compare columns 2 and 4 and columns 6 and 7 derived from them, as well as to compare columns 9 and 11 and columns 13 and 14 computed from them by means of the zygotic ratio AA : Aa : aa (which is not given).

Most important are the data of columns 6 and 7 and those of columns 13 and 14 which have been rendered again in graphical form in figures 1 and 2.

238

[ 12 ]



[ 13 ]

## § 3.5. Methods with use of clones.

Essential to our problem is first of all the recognition of AA and Aa in the population, and the multiplication free from impurity of the identified AA's. By means of pedigree selection the first thing is possible as we have seen, but not the second. By using vegetative multiplication and by working with clones, however, this end can be achieved in various ways. I have elaborated 5 different methods that will be discussed at first separately, after which, through mutual comparison, their practical value will be investigated.

## § 3.5.1. Method 1. Test crossing and vegetative maintenance of the clones.

In all cases we shall take as starting point the mutual cross  $Aa \times Aa$  when in the last backcross population, composed of Aa and aa, the aa's have been eliminated. The cross  $Aa \times Aa$  then yields AA + 2Aa + aa and again the aa's are eliminated.

Now we multiply vegetatively the remaining plants, which are AA or Aa, and can subsequently in two ways determine by means of test crosses whether we deal with AA or with Aa. These two ways of test crossing may be denoted the individual and the mass test crossing.

In the individual test crossing a certain number of clones is crossed individually to an  $\alpha\alpha$  type, for instance a Petkus. Two cases may present themselves:

1°. The clone is AA, which is shown by the failure of susceptible segregates to result from the test cross  $AA \times aa \rightarrow Aa$ .

2°. The clone is Aa. Then the test cross gives  $Aa \times aa \rightarrow Aa + aa$ , a segregating offspring.

With mass test crossing a number of clones are sown in a mixture, so that they pollinate each other mutually. The descendants are individually examined. Now the gametic ratio in a population AA + 2Aa is 2A : a. An AA-clone consequently produces  $A(2A + a) \rightarrow 2AA + Aa$  which is a phenotypically homogeneous progeny. On the other hand an Aa-clone produces  $(A + a) (2A + a) \rightarrow 2AA + Aa$  which is a phenotypically homogeneous progeny. On the other hand an Aa-clone produces  $(A + a) (2A + a) \rightarrow 2AA + aa$ 

 $\rightarrow 2 AA + 3 Aa + aa$  which is a segregating descendance and that in the proportion 5 resistant to 1 susceptible.

Mass test crosses are much easier to execute than individual ones, because only one isolated field is needed. A drawback, however, is that the susceptibility investigation requires many more plants per family. For with individual test crossing the relation susceptible : resistant in the segregating families is 1:1, with mass test crossing 5:1. In the first case a total of 12 plants with the expectation  $6:6 \pm 1,7$  is certainly sufficient. In the second case at least 48 plants must be used; the expectation is  $40:8 \pm 2,6$ .

The test crosses still are conceivable without vegetative multiplication, although the mass test crossing becomes much more reliable, if say 25 plants per clone are used which are planted scattered over the field. The second phase of the method, the multiplication free from impurity of the AA's, is inconceivable without previous clone formation. To this end we keep a certain number of plants of all clones involved in the test crosses in a vegetative condition, until the result of the test crossing is known, so that we know with certainty which clones are AA. These last then are crossed among themselves and we have attained our aim. To emphasize the necessity of vegetative maintenance we point to the fact that the test crosses produce, also with AA, always impure families.

We have mentioned before (p. 231) that for practical purposes it is not yet possible to maintain plants of rye clones in a vegetative condition for more than

240

## [ 14 ]

## 241

2 years. To apply the method just outlined they should be kept during 3 years. This condition cannot yet be fulfilled at present, hence the method must be discarded. I only mentioned it, because it can be used in connection with other crop plants. I refer, for instance, to most grasses, furthermore to plants which bear fruit several times, with asparagus and Cyclamen as typical exemples, especially the first, because in Cyclamen self-fertilization is possible. Hence we are justified in following up the method in all its consequences, which will also be done for the other procedures. We still have to find out the number of plants and clones necessary with a reasonable chance to attain our end. Naturally no certainty can be reached in this respect and all we can do is to make an estimate.

If we start from 48 plants of the population AA + 2 Aa + aa which is obtained by mutual crossing of the Aa's, then the expectation resistant : susceptible is 3 : 1 or 36 :  $12 \pm 3,0$ . It follows that we are almost certain to obtain at least 24 plants AA or Aa. Of the 24 clones to be made from these plants 1 will be AA to 2 Aa or 8 :  $16 \pm 2,3$ . Hence there is a good chance to get at least 2 AA's. We may count on 50 plants per clone of which 25 are used for testcrossing and 25 for vegetative maintenance followed by sexual multiplication of the AA's. Finally, as was computed above in relation with the number of plants per family available in a susceptibility test, for individual test crosses 12 plants are enough, whereas mass test crosses require not less than 48. With an assumed number of 24 clones the total susceptibility investigation in the case of individual test crosses amounts consequently to  $24 \times 12 = 288$  plants, in the case of mass test crosses  $24 \times 48 = 1152$  plants.

We conclude by giving as summary of the method the following time scheme in which with "year" we mean a calendar year and not one generation, while the numbers are of course only approximate averages.

1st year: Crossing  $Aa \times Aa \rightarrow AA + 2Aa + aa$ .

2nd year: Susceptibility test for 48 plants in order to eliminate the aa's. Producing of clones of 50 plants each from 24 of the remaining plants.

3rd year: Test crossing with 25 plants per clone; keeping the remaining clone plants in a vegetative condition. Sowing of the test crosses in view of susceptibility investigation, 12 plants to the family after individual test crossing, 48 plants to the family after mass test crossing, that is respectively 288 and 1152 plants. Selection of the AA clones after the results of the susceptibility trials have become known.

4th year: Intercrossing of the AA clones, Harvest.

#### § 3.5.2. Method 2. Test crossing and pair crossing.

We start exactly like in method 1 and obtain consequently a number of clone of which, by virtue of the results of the test crosses, we know with certainty that they are AA. At the same time that the test crosses are run, other plants of the clones involved in these crossings are crossed two and two. There is a possibility that among these crosses in pairs occurs the combination  $AA \times AA$ , with which our aim is attained.

Schematically the procedure is this way. Suppose that we identify of the clones  $a, b, c, \dots, h$  subjected to test crossing the clones b, e, f and h as AA. If as crosses in pairs are made:  $a \times b$ ,  $c \times d$ ,  $e \times f$  and  $g \times h$ , then it appears that the combination  $e \times f$  is the desirable one.

This way of proceeding can be done in practice without difficulties, but the probability of success verges on certainty only if we work with a rather large number of clones. Because the population from which the clones are drawn is AA + 2Aa we have a probability equal to  $\frac{1}{3}$  to get a clone AA. The chance to get a pair  $AA \times AA$  then is  $\frac{1}{3} \times \frac{1}{3} = \frac{1}{9}$ . Hence the expectation with 9 pairs is that 1 pair will be  $AA \times AA$  and 8 pairs of an undesirable composition. A reasonably good chance to come upon at least 1 pair of  $AA \times AA$  exists when

242

the work is done with 36 pairs. The expectation is  $4:32 \pm 1.9$ . The chance is not very great, but because in plant breeding the element of luck is always present, we may content ourselves with it. It must be noted that in case of failure the method may be repeated a year later with other material, so that the uncertainty factor ultimately has no fatal consequences. However, chance may upset the results of the susceptibility tests due to a relatively small number of plants per family, so that we must make more exacting demands here.

For 36 pairs 72 clones are needed. As starting material a number of 120 plants of the population AA + 2 Aa + aa may be considered sufficient. The expectation of resistant (AA or Aa) to susceptible (aa) then is  $90 : 30 \pm 4.7$ . Twice 25 = 50 plants per clone are needed.

For the susceptibility investigation after test crossing the same numbers of plants per family hold good as in method 1.

Summarizing we may establish the following time scheme.

1st year: Crossing  $Aa \times Aa \rightarrow AA + 2Aa + aa$ .

2nd year: Susceptibility investigation of 120 plants to eliminate aa. Making clones of 50 individuals from 72 of the resistant plants.

3rd year: Test crosses with 25 plants per clone and pair crossings with 25 plants per clone. Autumn sowing of the descendants of the test crosses for susceptibility trials, 12 plants per family after individual test crossing, 48 plants per family after mass test crossing, i.e. respectively  $72 \times 12 = 864$  plants and  $72 \times 48 = 3456$  plants. Autumn sowing of the descendants of the crosses in pairs and selection of the pairs  $AA \times AA$  when the results of the susceptibility trials are known.

4th year: Isolated multiplication of a mixture of the pairs  $AA \times AA$ . Harvest.

#### § 3.5.3. Method 3. Pair crossings with generative testing.

In this method crosses in pairs are made in the same manner as in method 2, but the test crosses are replaced by a testing of the sexually obtained descendants of the crosses in pairs. Three combinations are possible in connection with the crosses in pairs:

1st  $AA \times AA \rightarrow AA$ .

2nd  $AA \times Aa \rightarrow AA + Aa$ .

3rd  $Aa \times Aa \rightarrow AA + 2 Aa + aa$ .

As we see, the descendants of the 3rd combination segregate directly susceptible aa's, so that this combination may be recognized immediately. This is not the case with the first and second combinations and the problem is how to distinguish the desirable first combination from the undesirable second combination. This can be done by means of a generative test, by which is meant a subsequent evaluation of the descendants. The first combination produces again solely AA, but the second combination forms gametes A and a in a proportion 3 : 1 and hence a zygotic ratio of  $(3 A + a)^2 = 9 AA + 6 Aa + aa$ , which means 15 resistant to 1 susceptible. The generative test can be essentially assimilated to test crosses.

The practical application of the method does not confront us with difficulties,

[ 16 ]

but it becomes fairly complicated, because the second susceptibility investigation must be done in two stages. Moreover, rather close inbreeding is performed, because the descendants of the crosses in pairs are sisters and brothers. This, however, may be prevented by using the reserve seed method.

243

As to the number of initial plants and clones, the same rules hold as in the second method. But now 25 plants per clone suffice. We consequently work with 36 crosses in pairs, from which it is easy to derive that the most probable proportion of the combinations  $AA \times AA$ ,  $AA \times Aa$  and  $Aa \times Aa$  is equal to 1:4:4. Hence, on the average we may expect 16 combinations  $Aa \times Aa$  which are detected in the second susceptibility investigation by means of the direct descendants of the crosses in pairs. Of course the progenies of all 36 crosses in pairs must be drawn into this susceptibility investigation. Because the expected segregation in the offspring of  $Aa \times Aa$  equals 3 resistant (AA or Aa) to 1 susceptible (aa) 40 plants per group are sufficient, for the expectation then is 30:  $10 \pm 2.8$ . Consequently, a total of  $36 \times 40 = 1440$  plants are necessary.

Let us assume that for the third susceptibility examination after discarding on the average 16 pairs of the type  $A\alpha \times A\alpha$  20 groups remain. So far as these segregate, the expectation is 15 resistant : 1 susceptible. Only with 160 plants per group it may be assumed with a sufficient degree of certainty that the segregation will manifest itself. The expectation namely is 150 :  $10 \pm 2.9$ . In all the third susceptibility investigation demands  $20 \times 160 = 3200$  plants.

The time scheme becomes:

1st year: Crossing  $Aa \times Aa \rightarrow AA + 2 Aa + aa$ .

2nd year : Susceptibility tests of 120 plants to eliminate  $\alpha a$ . Of 72 of the resistant plants clones of 25 plants are made.

3rd year: Making 36 pair crosses of clones. Autumn sowing of part of the offspring of all pair crosses to detect the undesirable combinations  $Aa \times Aa$ , amounting to 16 on an average, in a second susceptibility examination. To this end  $36 \times 40 = 1440$  plants are needed. Saving reserve seed of all combinations except of the recognized  $Aa \times Aa$ .

4th year: Of the remaining families of the crosses in pairs, 20 on an average, seed is won under isolation. Autumn sowing for susceptibility trials for which  $20 \times 160 = 3200$  plants are needed. Selection of  $AA \times AA$ -pairs, sowing of their reserve seed.

5th year: Isolated multiplication of a mixture of all  $AA \times AA$  pairs. Harvest. Because the number of plants per clone is relatively small, we could perhaps work with autumn clones, by means of which the period required for the execution of the method would be reduced to 4 years.

#### § 3.5.4. Method 4. Diallel crossing.

We understand under diallel crossings between a certain number of plants or clones the crosses two by two in all possible combinations. If we apply this to our case then it is possible to detect immediately the good combinations through an examination of the progenies. When it is kept in mind that the possible combinations are the same as those indicated in method 2, the following scheme becomes clear. We start with 4 clones designated as cl. a, cl. b, cl. c and cl. d and suppose the following results of the diallel crosses:

1. cl.  $a \times cl. b \rightarrow$  segregates, hence cl. a as well as cl. b are Aa and every other combination involving cl. a or cl. b is undesirable.

2. cl.  $a \times cl. c \rightarrow$  phenotypically alike; because cl. a is Aa, cl. c has to be AA.

244

3. cl.  $a \times cl. d \rightarrow$  phenotypically alike; because cl. a is Aa, cl. d has to be AA.

4. cl.  $b \times$  cl.  $c \rightarrow$  phenotypically alike; it is known already that the combination is  $A\alpha \times AA$ , i.e. undesirable.

5. cl.  $b \times cl. d \rightarrow$  phenotypically alike; but again  $Aa \times AA$  and consequently undesirable.

6. cl. c × cl. d → phenotypically alike; on the strength of previous results the combination can be diagnosed as AA × AA, that is to say the desired one. We can consider the crosses which do not represent the combination AA × AA as test crosses, so that the factor of test crosses appears here also.

This method, undoubtedly, is very elegant. It is practically feasible when the number of clones is not too large, because otherwise the number of combinations soon becomes unwieldy. Furthermore, because every clone is used in various crosses, many plants per clone are needed.

Among 12 clones the expectation is  $4 AA : 8 Aa \pm 1.6$  and with this number there is a reasonable chance to obtain 2 AA's. This probability, however, becomes much greater with 15 clones with an expectation  $5 : 10 \pm 1.8$ , and because it still is possible to handle such a number, we shall adhere to it.

To obtain 15 clones AA or Aa it is enough to start with 32 plants of the population AA + 2Aa + aa, for the expectation then is  $24:8 \pm 2.4$ .

Between 15 clones 105 diallel crosses are possible. Every clone is crossed with 14 other clones, so that the number of plants per clone is  $14 \times 25 = 350$  plants. This is much, but still feasible practically. To work with such large clones means to perform a selection, for many plants per clone are indicative of a strong tillering capacity.

For the susceptibility tests a number of 40 plants per cross is enough. The exceptation then is  $30:10 \pm 2.8$ . Hence the total number of plants of the susceptibility investigation becomes  $105 \times 40 = 4200$  plants.

We may summarize this method in the following time scheme.

1st year: Crossing  $A\alpha \times A\alpha \rightarrow AA + 2A\alpha + \alpha\alpha$ .

2nd year: Susceptibility examinations of 32 plants to eliminate  $\alpha a$ . Making clones of 350 plants from 15 of the remaining plants.

3rd year: Diallel crossing. Autumn sowing of the descendants of the diallel crosses for susceptibility trials, 40 plants to each cross, i.e.  $105 \times 40 = 4200$  plants. Selection of the pairs  $AA \times AA$ .

4th year: Isolated multiplication of a mixture of the pairs  $AA \times AA$ . Harvest.

#### §3.5.5. Method 5. Inbreeding by means of self-fertilization.

The most direct recognition of AA and  $A\alpha$  is done by means of self-fertilization, which we may consider, though somewhat forced, a special case of test crossing. For in this case AA gives a constant and  $A\alpha$  a segregating progeny. After artificial self-pollination seed setting takes place, though to a very limited extent. When the kernels are sown, the plants show a pronounced loss of vigor, but this can be offset completely by mutual crossing of two inbred lines. Of the extensive literature on this subject I only refer to the much too little known researches made by MAYER GMELIN (8, p. 750; 9).

After self-pollination of rye we cannot count on much more than 1 grain per plant. From this follows that the method does not offer any perspectives when we work with separate plants. But if vegetative multiplication is introduced, the situation changes. If we think of clones of 50 plants, then 50 grains could be 245

obtained and this is enough for the susceptibility investigation, for this procedure only calls for 40 plants, as many as in method 4.

Among the initial clones the relation is  $AA : A\alpha = 1 : 2$ . With 30 clones the expectation is  $10 : 20 \pm 2.6$  and the chances are good to meet at least two AA clones. It is enough to start from 56 plants, for the expectation then is  $(AA + A\alpha) : \alpha\alpha = 42 : 14 \pm 3.3$ .

If 50 isolations are performed with 30 clones, the total amounts to 1500 plants, which certainly is feasible though laborious.

The time scheme of the method is:

1st year: Crossing  $Aa \times Aa \rightarrow AA + 2Aa + aa$ .

2nd year: Susceptibility investigation of 56 plants to eliminate aa. Making clones of 50 plants each from 30 of the remaining plants.

3rd year: Artificial isolation of all clone plants. Autumn sowing of the progenies of the isolated plants in view of the susceptibility investigation, 40 plants to each line,  $30 \times 40 = 1200$  plants in all. Selection of the AA-lines.

4th year: Isolated multiplication of the mixed AA-lines.

#### § 3.5.6. Mutual comparison of the methods.

In the subjoined outline data are summarized concerning the estimated quantities of plant material involved in the 5 methods that have been discussed.

traethod	duration in ycars	initial plants for first susceptibility investigation	number of clones AA or As	number of plants per clone	total number of clone plants	aumber of planis in the second (or third) susceptibility investigation
1. Test crossing and vegative		10	~ ~		1000	0.1 10 1170
2. Test crossing and crossing	4	48	24 70	50	1200	$24 \times 48 = 1152$
3. Crossing in pairs with	4	120	14	50	3000	$12 \times 40 = 3400$
generative testing	5(4)	120	72	25	1800	$36 \times 40 + 20 \times 160 = 4640$
4. Diallel crosses	4	32	15	350	4250	$105 \times 40 = 4200$
fertilization	4	56	30	50	1500	$30 \times 40 = 1200$

With the aid first of these figures exclusively let us investigate whether a given method be preferable. To begin with we notice that the duration is the same in all cases except perhaps method 3 which may last a year longer. The number of initial plants for the first susceptibility examination, true enough, is different. However, even in the extreme cases, manipulation still is easy. In method 4 the number of clones is small, but because of the very large number of plants per clone the method is exceedingly time consuming. The other methods are not especially exigent. Finally, methods 1 and 5 require relatively few plants for the second susceptibility investigation, which undoubtedly brings them into prominence.  $\checkmark$ 

However, when we consider the practical feasibility, method 1 really cannot compare with the others, as we already saw, while method 5 requires much work because of the 1500 isolations.

Method 3 is rather complicated and lasts perhaps a year longer than necessary, whereas method 4 is exigent, because of the fairly large number of crosses.

Method 2, then, remains as the most practical. However, the reasons, virtually all negative, that have been advanced here must not be considered as definitely turning the scale. It would be recommendable to apply all methods alongside each other, in order to determine whether one precedure be more cumbersome than another. Various stages coincide which causes the total material required to be much smaller than the sum of all material involved in each method. The same initial material, for instance, can be used and the test crosses of method 1 can serve also for method 2.

The further development of this last point would, however, constitute a plan for experimental research and as such it is out of order now. It is enough to point out that 4 methods certainly are practical and that they lead to the goal with a good chance of success.

#### § 3.5.7. Supplementary remarks.

In discussing pedigree selection we came to the conclusion that after 4 generations of selection a population can be obtained in which 0.2 % of the plants is susceptible, while further selection encounters practical difficulties (cf. p. 237). We saw before that with the aid of vegetative multiplication a perfectly resistant group of plants can be obtained in a period of 4 years. In the last case the starting point was the cross  $Aa \times Aa$ ; in the pedigree selection, however, the population resulting from the cross  $Aa \times Aa$ . The methods drawing in clones consequently are a year shorter, except perhaps method 3. Much could be obtained meanwhile by applying first 1 or 2 years pedigree selection and to adopt afterwards a method using clones. The reason for this is that the proportion AA : Aa in the initial population determines the scale on which the work should be performed and hence the chance of success. Now we saw that this relation is in the beginning 1 : 2, but changes after 1 year pedigree selection into 4 : 2 and after 2 years pedigree selection into 10 : 2. Suffice it to point out the importance of the consequences resulting from this, without entering in details.

Finally a remark on the crossing in pairs and especially about the area needed for this. If the breeder is tied down by a certain field and uses space isolation; the possibilities are limited. It is, however, also possible to perform artificial isolation in which case the possibilities are much wider. KRISTENSEN and TROELSEN (4, see fig. 1 on p. 15) constructed glasshouses for crossing in pairs which seem to solve the question in a simple way.

#### IV. RYE BREEDING IN GENERAL

#### § 1. Extension from eelworm resistance to other characters

The great difficulty in producing a true breeding variety resistant to eelworm is caused by the dominance of resistance which renders it impossible to distinguish AA immediately from the undesirable Aa which possesses a hidden gene for susceptibility. We can also state the problem thus: the difficulty lies in the permanent elimination of susceptible segregates. And in this connection a few other characters may readily be cited which yield a similar difficulty and which must be treated by one of the methods, described for the selection of eelworm resistant rye.

In the first place, Ottersum rye may be mentioned which, though to a consi-

#### $246^{\circ}$

derable extent resistant to eelworm, still contains susceptible individuals. In all likelihood it will be possible to select an Ottersum rye that is 100 % resistant.

White or yellow seedlings occur as segregates in various varieties. Their elimination through selection constitutes a problem analogous to the elimination of resistance to eelworm, as well as the phenomenon of dwarfism which appeared in one of my otherwise very prominent selections as an undesirable characteristic.

Methods analogous to those used in breeding rye resistant to eelworm must be developed, if a given dominant character has to be incorporated in some variety by means of repeated backcrosses. Examples are resistance to orange leaf rust, to stem rust, to powdery mildew which according to MAINS (7) represent, each of them, separate dominant characteristics.

Before going any further, it may be useful to remark that the selection for recessive characteristics does nog entail difficulties. Also in the most complicated case, when the evaluation can only take place after flowering, it is enough to make a number of crosses in pairs, of which only those are kept for further work of which both members exhibit the desirable characteristic. These crosses are of the type  $aa \times aa$ .

Passing on to the selection for characters that after disease resistance are the most important, we notice high yield and good quality. These characters are almost always complex, depending on a number and often a large number of hereditary factors. Some of these will be dominant, others recessive. In general the breeder does not know this and he does not work according to a definite factorial scheme, but he can and should work along lines developed for cases in which it is possible to proceed in accordance with a factorial scheme.

By far the safest way in such a situation is to follow a scheme in which the selection is done for a dominant character. This undoubtedly is the right thing to do with regard to the dominant elements of the yield or quality complex, while it does not harm the recessive elements. If on the contrary a scheme of selection for a recessive character would be followed, this would be good for the recessive elements, but fatal for the dominant ones.

In such a way the methods developed in relation to resistance to eelworm find their application extended to breeding for high yield and good quality or in general for any character which is not exclusively recessive. If for convenience' sake we limit ourselves to high yield, it must be pointed out immediately that the use of inbreeding by means of self-fertilization cannot be considered, not because of inbreeding in itself, but because of the possibility that different degrees of loss of vigor due to inbreeding in different families make a comparison unreliable. Method 5, consequently, must be dropped immediately. And because in method 3, crossing in pairs with generative testing, inbreeding is applied in connection with the progeny test — the latter being a result of sister  $\times$  brother mating — this method too cannot be considered.

When we, moreover, bear in mind that due to the variation of the yield the work must be done on a much larger scale, also because we don't deal with a monofactorial character, method 4, the diallel crosses, certainly is not practicable.

Method 1 of course must be abandoned, because of the uncertainty to keep clones alive more than two years.

Method 2, the test crosses and crossing in pairs, then, is the only one that remains. This method can be built out entirely according to the demands made upon it, as we shall see in the next paragraph.

§ 2. The Scheme of breeding for high yield : test crossing and pair crossing The essential difference in the execution of the breeding methods, when selection is done for yield as compared to selection done for resistance to eelworm, is that the yield can only be determined after flowering. This difficulty can be solved by means of a modified reserve seed (remnant seed) method, as will be discussed presently.

To begin with, a certain number of clones are made, starting from the seed of the population which was chosen for selection. With these clones at the same time test crosses and crosses in pairs are made.

As procedure to be followed for the test crosses, mass test crossing is the way. The difficulty of individual test crossing consists in the virtual impossibility of obtaining the suitable partner for the cross. The latter should be an all-round recessive and such a type is not known. But mass test crosses produce the same final results as individual crosses and in an easier way.

Because the evaluation takes place only after flowering and undesirable types cannot be excluded from pollen production, other segregation ratios occur after backcrossing than we encountered in breeding for eelworm resistance. To illustrate this, let us assume again an initial population AA + 2Aa + aa. If the aa's are eliminated in good time, the gametic ratio 2A : a results, in consequence of which the progeny of the AA-plants is  $A(2A + a) \rightarrow 2AA + Aa$ , and the progeny of the Aa-plants  $(A + a) (2A + a) \rightarrow 2AA + 3Aa + aa$  which amounts to a visible segregation in the ratio 5 : 1.

If on the other hand it is not possible to eliminate the aa's before flowering, and this happens in the present case, then the gametic ratio is 4A: 4a = A: a. This means:

1st AA-plants give an offspring  $A(A + a) \rightarrow AA + Aa$ .

2nd Aa-plants give an offspring (A + a)  $(A + a) \rightarrow AA + 2Aa + aa$  which means a visible segregation in a ratio of 3 : 1.

3rd  $a\alpha$ -plants would, if they were not discarded after flowering, give an offspring  $a(A + \alpha) = A\alpha + \alpha\alpha$  or a visible segregation in a ratio 1 : 1.

The difference between the progenies of  $A\alpha$ -plants, when the  $\alpha\alpha$ 's have been timely eliminated or when this has not been done, is important. In the first case the segregation ratio in the progeny is 5:1, in the second case, however 3:1. Hence the recessive segregate is met with more frequently, to be precise 1/4:1/6 = 1.5 oftener.

Now in the case of yield we deal almost always with a multiple-factor segregation so that the above does not apply directly. However, the relative frequency of the occurrence of the recessive segregates will always, in case of a comparison between elimination before flowering and after flowering, turn out in favor of the former. This effects the minimum size of the families in the comparative trials of the clone families after the test crosses.

Because we don't deal with visible, sharp segregations, when selecting for yield, also on account of the fluctuation of this character, the evaluation will resolve itself in practice to a comparison of averages. We consequently plant all clones in a mixture for the test crosses, collect individual seed of these clones and sow these grains in a comparative yield trial, but we discard of course the progenies of the clones, which show themselves phenotypically inferior during the test crosses. For the sake of simplicity we can designate these as  $\alpha \alpha$ , by which we mean for the present the undesirable recessives. Naturally the clones which during the vegetative multiplication show undesirable characters, are immediately eliminated.

The essential point in the test crosses is, that ideal clones, that is to say homozygous ones, do not display visible segregation, in other words breed true. This ideal will be difficult to attain. When the ideal is approached, when most of the factors determining yield will have attained homozygosity, segregation will not be pronounced, consequently the average of the clone families concerned will be fairly high. In proportion as the homozygosity of the clones diminishes, segregation will increase and the average of the families will be brought down.

Thus the average yield of the clonal families reflects the degree of homozygosity of the parental clones. Except for chance variations, arranging the clonal families according to the productivity will also mean lining them up according to homozygosity. Together with the evaluation of other important characters, a criterion for selection is obtained. Often, in the case of a more or less gradually decreasing series of the mean yields, the dividing line between clones still to be selected and those just not, is fairly arbitrary. One can be guided in such circumstances by the number of clones desired. In some cases, however, the series of mean yields shows, after a few remarkably high values, a pronounced gap, after which the lower values come. In such fortunate cases the dividing line of the selection is easily drawn.

However this may be, the evaluation, especially in regard to the average yielding capacity of the clone families obtained through sowing the seed resulting from the test crosses, leads to a selection of clones. Now the seed of the best clone families has been partially contaminated by pollination with pollen of inferior plants and consequently is unfit for further work. The progenies of the paircrossings serve for this purpose.

These crosses in pairs yield their seed at the same time as the test crosses do. This seed, however, is not sown directly, but kept in reserve until the result of the test crosses is known. Then it is determined which crosses in pairs consist of two clones which both are qualified for selection and with these combinations work is continued.

I want to make a remark about the reserve or remnant seed method which has been introduced at this point. In the Petkus reserve seed method part of the seed produced by one plant — or bij one clone — is saved until judgment has been pronounced over the other part. In our present case, the seed of the crosses in pairs is kept in reserve, until the individual appreciation of the members of these crosses in twos with the material, secured from the mass test crosses, has taken place. The principle presents in both cases such a similarity that we may speak of reserve seed method in both events. But in order to make a distinction, it is good to refer to the procedure expounded here as the "modified reserve seed method".

To continue and conclude our discussion of the breeding scheme, we have to speak about the use of the seed of the selected crosses in pairs. Let us state, to begin with, that separate multiplication of the seed of each such cross would mean inbreeding and this must be avoided at this stage of the multiplication. We therefore mix the seed of all selected crosses in pairs. If the result of the selection is such that we may consider our end achieved, then the seed produced by the selected crosses in pairs is simply increased. If, on the contrary, the result of the selection is disappointing, an entirely new selection is started; from the seed of the selected crosses in pairs clones are made, which are used for new test crosses and new crosses in pairs.

As a rule, it will be safe to follow both procedures, to sow part of the seed for increase and to keep part of the seed for next spring as starting point for new clones by means of which the whole scheme is repeated on a higher level.

249

[23]

To determine the number of plants and clones necessary, a firm basis is lacking now that we deal with polyfactorial segregation and no longer, as was assumed in connection with eelworm resistance, with a monofactorial setup. But it is almost beyond doubt that the practically attainable thing is theoretically rarely sufficient. So the work must be executed with as much material as possible, first of all with as many initial clones as possible.

A restriction is possible in the number of plants per clone; 75 may be considered sufficient. That means 50 for the test crosses and 25 for the crosses in pairs which in that way will produce enough seed for further work. I would prefer, certainly in a first selection, to increase the number of clones and diminish later on the volume somewhat, than to perform more extensive trials with few clones.

As to the evaluation of clone families of the test crosses, according to calculations of FRANKE (3) it is desirable to have as many replications as possible. In order not to make the field needed too large and in order to use the limited quantity of seed per family to the maximum, the plots themselves are made as small as possible. I have worked myself with 10 replications of plots of 1 m<sup>2</sup> and found this system satisfactory. However, it may be well to draw attention to the opinion of DORST (2), who instead of working with replications receiving same treatment prefers to vary the exterior conditions, in order to find out the behavior of the selections under various circumstances. The ideal would be to combine both things, that is to say to vary the circumstances, but with replications. Because as a rule this is too exigent, a choice has to be made and in that case much can be said in favour of varying the external conditions.

In both cases a layout of the trial field according to the method of FISHER is the right procedure. Though it may seem strange at first sight, the computation of the reliability of the differences of the mean yields has not much sense, because it is not used. For if the clone families are arranged from high to low averages, it will happen only very rarely that the difference between 2 consecutive differences will be significant. Yet a dividing line has to be drawn somewhere which must be done rather arbitrarily. There may only be some point in ascertaining in general whether significant differences occur in the material. This is especially important, when in later years we must determine whether the material has become more homogeneous, that is to say, whether it exhibits less differences.

In connection with the size of the material, it may be well to recall the improvement in the composition of the initial population, which is achieved by pedigree selection during a few generations. This improvement consists in the more favourable proportion of the desirable AA's in relation to the undesirable Aa's. Though here, due to the impossibility of eliminating aa before flowering, the change in the ratio AA to Aa be different from the situation summarized in table 1, column 4 (p. 238), yet the principle remains valid. A drawback of applying pedigree selection during some generations before starting the method with the clones is that pedigree selection calls here for the reserve seed method, so that every generation takes 2 years instead of 1.

If our initial material is very impure, for instance the  $F_2$  of a cross, it will be useful to perform some purification by means of a few, say 2 generations of pedigree selection. If, on the contrary, the initial material is less impure, as may be expected, for instance, after some backcrossing to one given variety, it will be justified to start immediately with clonal selection.

If we sum up the whole course in a time scheme, we obtain the following, in

relation to which we refer to the schematic representation in fig. 3 on p. 253. 1st year: Making of clones from the initial material after spring sowing.

2nd year: After performing a first clonal selection using simultaneously the provisionally selected clones for mass test crosses and for crosses in pairs.

Performing a second clonal selection on the material of the test crosses and individual sowing of the seed of the selected clones in a comparative yield trial.

Harvesting the seed of the crosses in pairs and keeping it in reserve.

3rd year: Final selection of the clones on the basis of the results of the comparative yield trials. Mixed sowing for increase of part of the reserve seed of the crosses in pairs, in so far as the latter consist of 2 definitely selected clones.

4th year: Harvesting the increase field. Spring sowing of part of the seed of the selected crosses in pairs and production of new clones for the beginning of a new selection series.

This scheme, consequently, amounts to a 4 years program which is as long a duration as for method 2 on p. 242. But we started here one generation later so that in reality the method with selection for yield lasts 1 year longer. This follows immediately from the use of the modified reserve seed method.

In comparing with 4 generations of pedigree selection, it must be pointed out that to perform this by means of the reserve seed method 8 years are needed. The clonal method then, with its consequences, becomes much more attractive.

#### V. THE METHOD OF REPEATED BACKCROSSING

#### § 1. The method as such

In chapter 111 we already became acquainted with the method of repeated backcrossing in the discussion of the breeding of an eelworm resistant rye variety. In general the method must be considered, when in a variety that otherwise gives satisfaction one given undesirable characteristic must be replaced by a desirable opposite. A very typical instance is susceptibility to some harmful agent which has to be replaced by resistance to it. But the applicability of the method certainly goes much farther and embraces, for instance, also characteristics like factors of yield and quality.

The method originated already some time ago in the United States, but in my opinion not sufficient attention has been paid to it in the Netherlands. It seems that the possibility of application is restricted to those cases in which the characteristic to be incorporated is dominant and in which the plant is preferably selffertilizing. It is true that in this case the method is easily applied. But also when the characteristic to be incorporated is recessive, or when we deal with a crossfertilizing plant, we can use the method. According as circumstances may require, modifications must be introduced which, it is true, complicate the execution, but which do not lengthen the period in comparison with the time needed for incorporating a dominant characteristic in self-fertilizers.

Recently I investigated the various cases systematically (22) which, as far as I know, had not yet been done previously. Consequently, in the following it will be enough to indicate the essentials and refer to the publication cited for the schemes expounding the complete procedures for the various cases. All this relates to the execution of backcrosses in view of selection. The point which interests us here is the breeding of a homozygote for the desirable characteristic when backcrossing has been completed. This point was described in detail in the preceding chapter III, in relation to eelworm resistance in rye, but we must now consider

252

the problem in its generality. Again the cross-fertilizers confront us with the greatest difficulties.

#### § 2. The carrying out of the backcrosses with regard to the selection, in various cases

# § 2.1. Dominance or recessiveness of the desired characteristic in connection with self- or cross-fertilization.

When the characteristic to be transferred is dominant, the backcross parent may in general be denoted by aa — for this variety lacks the desirable dominant characteristic AA — and the backcrossing is of the type  $Aa \times aa \rightarrow Aa + aa$ . In the offspring of the cross consequently two types arise which can be readily distinguished from each other, so that the desired Aa's may be selected immediately for the next backcross. It does not matter in this case whether the crop be selfor cross-fertilizing. In § 2.2 I shall return to the complication which arises when the evaluation can only be done after flowering.

If the characteristic to be transferred is recessive, the backcross parent may be represented by AA and the backcrossing will be of the type  $Aa \times AA \rightarrow AA + Aa$ . We desire a variety aa, so that the selection in the backcross progeny is aimed at Aa, but this genotype can not be distinguished from AA. The "reaction" which we use to discriminate between AA and Aa differs for self-fertilizers and cross-fertilizers.

In self-fertilizers, we apply self-fertilization with other flowers of the plants than used for the backcrosses, simultaneously with the backcrosses. This selffertilization results into a constant or a segregating offspring, according to the initial plant was AA or Aa. In the case of cross-fertilizers, the genetical composition is revealed by the progenies of test crosses with aa, which are made simultaneously with the backcrosses with AA. In this way a selection a posteriori becomes possible, but no time is lost, for it is possible to continue with the backcrosses in each generation.

#### §2.2. Complete valuation and selection before or after flowering.

In the method mentioned in § 2.1 it has been tacitly assumed that a complete determination of the characteristic, for which selection is performed, is possible before flowering, so that selection occurs before that stage. But especially when the character in question concerns the fruit or seed, such an early selection is not possible. Here too we apply a selection a posteriori after having backcrossed a certain number of provisionally selected plants. After flowering it becomes apparent which backcrosses serve our end.

Let us take as example the simplest case, the backcross  $Aa \times aa \rightarrow Aa + aa$ , in which we want to select for Aa in view of the next backcross, but we cannot make this selection before flowering. We then cross back to aa a certain number of plants, of which we only know that they are either Aa or aa, ascertain after flowering which mother plants are Aa and continue only with the corresponding backcrosses.

§2.3. Grouping of the different cases.

A survey of all points, discussed in § 2.1 and § 2.2, leads to a classification of the various cases, each demanding a somewhat different method.

The first grouping is based on the dominance or recessiveness of the characteristic to be transferred. In the first case the nature of fertilization is immaterial.



Figure 3. Scheme of breeding for yield in rye, combining vegetative reproduction, mass test crossing, pair crossing and a modified reserve seed method. Further explanation in the text.

[27]

In the second case a dichotomic subdivision has to be made, so that a total of 3 groups is obtained.

Each of these 3 groups finally is subdivided according to the possibility or impossibility of evaluation before flowering. Ultimately 6 different groups are established.

As I mentioned before, the methods to be used for each of the 6 groups have been described in extenso and depicted in a previous publication (22, schemes on pp. 27, 28, 29), to which it suffices here to refer. Let us only reiterate that with all 6 methods we may proceed regularly with the backcrosses, without skipping a generation. Hence they all progress at the same pace.

#### § 3. The selection of homozygotes after backcrossing

### §3.1. The problem.

When the backcrosses have been made a sufficient number of times, there remains for final selection either a population Aa + aa from which AA must be obtained and selected, or a population AA + Aa from which aa must be obtained and selected. The methods to be followed in this work correspond in principle with the selection of certain homozygotes of a given type from an arbitrary population, of which problem they constitute a special case.

With self-fertilizers the answer is easily found and we shall discuss this group only for the sake of completeness. The selection in cross-fertilizers is more difficult, especially when a dominant characteristic is concerned that can only be evaluated after flowering.

After the extensive derivations given above, a summary discussion will be enough.

#### §3.2. The selection of AA from Aa + aa.

## § 3.2.1. Self-fertilizers.

From the population Aa + aa, before or after flowering aa is eliminated. Selffertilization of the remaining Aa's gives a population AA + 2Aa + aa, from which aa is again eliminated. The remaining AA's and Aa's are identified by growing again progenies obtained through self-fertilization. If these breed true, they are AA; if they segregate, we have progenies of undesirable Aa's.

#### § 3.2.2. Cross-fertilizers which can be evaluated before flowering.

This case has already been treated in detail, with eelworm resistance in rye as example. In this case vegetative maintenance of the mother plants for more than 2 years was not possible. We shall now develop a method for crops that can be maintained for at least 3 years.

Here too we begin with eliminating the *aa*'s from the initial population and cross the remaining Aa's among themselves, from which the population AA + 2Aa + aa originates. After discarding the *aa*'s, the problem becomes how to tell AA from Aa. This can be done in two ways.

First of all, the test cross of the AA's with aa yields a non segregating progeny, whereas a test cross of the Aa's with aa produces a segregating progeny.

Secondly, a mass test cross in the population AA + 2Aa, followed by pedigree selection, conducts to the goal. The gametic ratio in this population is 2A : a. Hence the AA's give a progeny  $A(2A+a) \rightarrow 2AA + Aa$ , whereas the Aa's give  $(A + a) (2A + a) \rightarrow 2AA + 3Aa + aa$ . In other words, the AA's produce a true breeding, the Aa's a segregating progeny. So it is fairly easy to distinguish the AA's from the Aa's in the next generation. When the mother plants are maintained vegetatively, in the third year the mother plants diagnosed as AA can be intercrossed for increase.

It must be pointed out that every year one generation has been accepted. With biannual plants the procedure, of course, takes more time.

#### § 3.2.3. Cross-fertilizers which cannot be evaluated before flowering.

This is the case of which an example, the selection of rye for yield, has been fully discussed. However, as in § 3.2.2, we shall examine the method to be adopted, if the crop can be maintained in a vegetative condition for more than 2 years.

In principle the method is the same as in the preceding case, but the zygotic ratio is different. For in the initial population, Aa + aa, it is impossible to recognize aa before flowering, so that the gametic ratio becomes A : 3a and the progeny of Aa will consist of (A + a)  $(A + 3a) \rightarrow AA + 4Aa + 3aa$ . Now in applying individual test crosses, a certain number of plants must be crossed with aa, while we don't know yet whether they are AA, Aa or aa. The latter are detected after flowering and the corresponding backcrosses are discarded.

Mass test crosses have in this case an advantage, because they can be executed much easier than the many individual testcrosses that would be needed. The gametic ratio in the population AA + 4 Aa + 3 aa is 3A : 5a, so that the AA's yield  $A (3A + 5a) \rightarrow 3AA + 5Aa$ , while the Aa's segregate into  $(A + a) (3A + 5a) \rightarrow 3AA + 8Aa + 5aa$ , or in 11 that are AA or Aa to 5aa, which ratio is favourable in the sense that relatively few plants are enough to demonstrate the segregation.

Anyhow we can recognize the AA's and cross the vegetatively maintained motherplants for increase.

#### §3.3. The selection of aa from AA + Aa.

## § 3.3.1. Self-fertilizers.

The procedure is very elementary. In the offspring of Aa we find the desired aa as segregate and can multiply it by means of self-fertilization.

#### § 3.3.2. Cross-fertilizers which can be evaluated before flowering.

Here too the method is simple. Increase of the initial population without selection namely yields:  $(3 A + a)^2 \rightarrow 9 AA + 6 Aa + aa$ . In this population some *aa*'s can be recognized before flowering, after which they are mutually crossed for increase.

#### § 3.3.3. Cross-fertilizers which cannot be evaluated before flowering.

In this case too the increase of the initial population without selection to 9AA + 6Aa + aa constitutes the starting-point. But the aa's cannot be recognized in time. The problem in this instance can be solved in two ways.

If vegetative maintenance is possible, in the second year the aa's are crossed mutually, so that only vegetative maintenance during 2 years is necessary.

If, however, such a maintenance is not possible, crossing in pairs offers a solution. But this requires that first the composition of the population has been made more appropriate through pedigree selection. With a gametic ratio of 3A : a in a population, aa gives a progeny  $a (3A + a) \rightarrow 3Aa + aa$ . If we continue pedigree selection for one generation, we obtain  $a (3A + 5a) \rightarrow 3Aa + 5aa$  and now the relative frequency of aa is very favourable. Crossing in pairs then produces as possible combinations:

[29]

#### 1st $Aa \times Aa \rightarrow AA + 2Aa + aa$ .

2nd  $Aa \times aa \rightarrow Aa + aa$ . 3rd  $aa \times aa \rightarrow aa$ .

The 3rd combination, which is the desired one, may consequently be recognized easily. Mixed increase of some of the desirable combinations prevents loss of vigor due to inbreeding. This can be done very efficiently by having recourse to the reserve seed method.

#### VI. DISCUSSION OF RESULTS: THE BREEDING OF CROSS-FERTILIZERS IN GENERAL

It remains for us to survey the whole of the foregoing results, to determine to which crops they apply and to make a few complementary remarks.

The process of growth has been the following. The starting-point was the method of breeding rye varieties resistant te eelworm, a special case of the method of repeated backcrossing, in which attention was particularly paid to the production of homozygosis after the last backcrossing. Subsequently a broadening in two directions was made. First, rye breeding was considered with special emphasis on yield. Secondly, the method of repeated backcrossing in general was discussed, in connection with which the plants were divided into six groups, according to more or less important differences in the procedure.

In studying the various cases, we started from as simple a situation as possible and developed all consequences arising from it on a purely mendelian basis. More complicated situations, such as are met with almost always in practice, can be treated on the analogy of the simpler ones, without the mendelian basis being known in all its details.

When selection is done for a recessive characteristic — see the 3 cases in V, § 3.3 on pp. 255-256 — self-fertilizers and cross-fertilizers which can be estimated before flowering don't produce any difficulty. In cross-fertilizers which can be estimated only after flowering, the end may be attained by means of crossing in pairs after pedigree selection, but vegetative maintenance simplifies things considerably. This group forms a transitional case.

When selection is made for a dominant characteristic — see the 3 cases in V, § 3.2 on pp. 254–255 — in self-fertilizers there is again no problem. But in crossfertilizers the solution becomes much more difficult and complete success in a relatively short time can only be obtained through methods other than pedigree selection alone, which I designated in the title of this study as "rational methods".

The case of the problem consists of two elements:

1st. Detection of AA and Aa, that is to say of the homozygotes and the heterozygotes. This can be done by means of test crosses in some form or other, but the method of mass test crosses deserves special attention in virtue of its ready applicability.

2nd. Multiplication true to type of the homozygotes. Here the following cases must be distinguished:

A. Crops bearing fruit more than once produce no difficulties.

B. When vegetative propagation as selection measure is possible — with plants usually sexually propagated — two cases may present themselves:

1) the vegetative propagation is permanent, in which case the plants concerned can be entirely assimilated to those of group A;

2) the vegetative propagation is temporary (rye); the problem is then solved through the modified reserve seed method after crossing of pairs.

C. When vegetative propagation is excluded, the situation becomes very difficult, but still not hopeless in all cases. Artificial crosses may be resorted to, crosses in pairs are of course easily executed this way, whereas for mass test crossing pollen mixtures can be used. But this method will only produce practically valuable results in crops that produce relatively much seed per fruit or per cross.

When we distil from the above the crop plants to which rational methods apply, it must be pointed out in advance that selection for a recessive or a dominant characteristic is not decisive, because both cases occur in all plants. I have stressed in these pages the desirability to work always according to a scheme for dominant characteristics, if the exact mendelian basis is unknown, because almost every character of practical importance is complex and certainly will consist partially of dominant components. The rational methods consequently apply to all cross-fertilizing crops, but not to all in the same degree. The necessity of test crossing holds universally. But the multiplication true to type of the homozygotes, after ascertaining the homozygous mother plants by means of the progenies of the test crosses is an easy matter in crops bearing fruit more than once. As examples I cite the coconut tree and oil palm, asparagus, salsify, Anthurium and Cyclamen. It is true that in a few of these crops self-fertilization may be practised, but we can put, though somewhat forced, self-fertilization on a level with a test cross.

Corresponding to the latter plants are the plants which as a selection measure can be multiplied permanently vegetatively, with various meadow grasses as example. One simply propagates on a large scale certain crosses between given parental clones.

In crops in which a permanent vegetative multiplication is not possible, the solution is found on the analogy of rye by making crosses in pairs, among which is selected only after test crosses have indicated which pairs are composed of combinations of 2 homozygotes. With some crop plants it will be possible to execute compulsory self-fertilization instead of crossing in pairs. Eventual injury from inbreeding is not troublesome, because in this case the progenies obtained through self-fertilization don't serve for valuation, but for mixed increase.

Now the simultaneous application of test crosses and crosses of pairs — or compulsory self-fertilization — becomes very difficult without the use of a vegetative reproduction, even though temporary. From a breeding point of view, a pressing need for vegetative multiplication exists in cross-fertilizers which fructify only once. The possibility or impossibility of estimating the merits before flowering has no essential consequences for the methods used. To cite a few examples besides rye, I point to crop plants such as all kinds of beets, chicory, maize, swedes and many vegetable crops, such as all cabbage species, gherkin, cucumber, melon, radish, onion, spinach, witloof, carrots and others.

For some of these plants it will be easy to develop a method of vegetative multiplication. For others it will be more difficult. It seems probable that a temporary vegetative multiplication as for rye is possible in practically all cases, but that a more permanent vegetative multiplication or vegetative maintenance often will encounter difficulties similar to those met with in rye. Methods for devernalization must then be elaborated which in general is not an easy thing. But as we have seen, a temporary vegetative multiplication would solve the breeding problem.

We conclude that the elaboration of methods for vegetative multiplication is of the utmost importance for cross-fertilizers fructifying only once. The reason

for this is that by working with clones the rational breeding methods, developed above, become applicable. A second reason, though it does not come into prominence in the compass of this study, is that valuation of clones for selection pur-

poses gives a much more reliable result than that of individual plants.

As new conceptions have been brought to the fore: the principle of mass test crossing and the modified reserve seed method. Together with the already known principle of pair crosses of Von SENGBUSH, they constitute the three main elements of my method which become practically feasible by introducing vegetative multiplication.

Especially the mass test crossing opens up perspectives for large scale application of the general principle of test crossing, so obvious from a genetical point of view.

I conclude with a few general remarks.

The breeding system which I have proposed for cross-fertilizers, fructifying only once, is meant in connection with the general line. Each crop plant has its own typical peculiarities and in developing a precise breeding plan for a given crop, these peculiarities must be taken into account. Far from forcing everything into one bodice, crop improvement preserves a large measure of individuality and consequently an attractiveness in relation to each individual crop.

In this study the homozygous form and the isohomozygous variety have been taken as an ideal. Often the desirability of this is called in question for crossfertilizers, as it is feared that the plasticity of the variety would thus be lost. In contradiction with this point of view is the purification of mixtures which is performed by all breeders of cross-fertilizers. I sometimes wonder whether the great difficulty of breeding homozygous cross-fertilizers be not the source of the above mentioned opinion, while one may also ask whether possible disappointments be not the consequence of a wrong selection in connection with certain cultural conditions. Why should the situation in self-fertilizers and especially in cross-fertilizers which are vegetatively propagated, where also one genotype is involved, be so entirely different? The solution is first to obtain an isohomozygous variety of a cross-fertilizer and then see what it is worth.

In the procedure which I have thought out, injury due to inbreeding has been avoided as much as possible. I don't want to suggest with this that passing from inbreeding to heterosis has no value. I am convinced of the contrary. But this way of breeding is entirely besides the method, developed here and was out of order. I wish, however, in connection with the preceding point, to state that the ideal heterosis cross is composed of one genotype.

The possibilities, offered by the course pursued in the foregoing, are by no means exhausted with the results described. The importance of working out methods for vegetative multiplication of cross-fertilizers, fructifying only once, has already been pointed out. This proposes as many problems as corresponding crops exist.

Furthermore, it would be interesting to extend the calculations which I made for cases, kept as simple as possible, to more complicated situations in order to arrive finally at general formulas for polygenic segregations of various aspects.

## 259 VII. SUMMARY

1. At the end of 1944 it was known that winter rye can be multiplied vegetatively by means of division after spring sowing, but that the clones could not be kept alive and serve practical purposes for more than two years. Two selection schemes with clones were drawn up, but as to the ultimate accomplishing of these schemes no clear picture existed. At present a basis has been worked out for a complete breeding system for rye, which has been extended to all cross-

fertilizers. 2.1. As starting point, the method of breeding a rye variety resistant to eelworm was developed as a special case of the method of repeated backcrossing. Attention was particularly drawn to obtaining the homozygote AA from the last backcross population Aa + aa, out of which arises the population AA ++ 2 Aa, after elimination of the susceptible aa, increase and another elimination of aa.

2.2. The change in the composition of the population after continuous mass selection or pedigree selection was calculated and general formulas were given, see table 1 on p. 238 and figures 1 and 2 on p. 239. After 4 generations of pedigree selection and after 20 generations of mass selection, a population is obtained which segregates 0.2 % susceptible  $a\alpha$ 's.

2.3. By using clones, a complete elimination of the heterozygotes can be obtained in less time. Five methods involving clones were described in detail.

Method 1. - Test crossing and reverting to the vegetatively maintained clones for increase, after the test crosses have revealed the desirable clones.

As modes of the test crossing, the individual and the mass one are distinguished. In the individual fest crossing all clones are crossed individually with an  $\alpha\alpha$ . In the mass test crossing all clones are crossed among themselves in bulk, after which the estimation of the individual offsprings enables to descriminate between the homozygotes and the heterozygotes.

Method 2. – Test crossing associated with crossing in pairs and continuing the work with those pair crosses for which the test crosses have shown that they consist of combinations of clones having the desired composition.

Method 3. - Crossing in pairs with generative progeny test, through which in the first progenies the undesirable combinations  $A\alpha \times A\alpha$  can be discerned, while in the second progenies the undesirable combinations  $AA \times A\alpha$  are told from the desirable  $AA \times AA$ . The progeny test may be considered as a series of test crosses.

Method 4. – Diallel crosses, in which crosses of all possible combinations 2 by 2 are made between the clones. From a comparison between the descendants of these crosses, the desirable combinations can be distinguished. Those crosses which do not belong to the desired combination, can be considered as test crosses.

Method 5. – Inbreeding through self-fertilization. The test cross takes place as self-fertilization, which tells in the most direct manner the homozygotes from the heterozygotes. Inbreeding must be followed by a heterosis cross.

2.4. On p. 245 a tabular view is given of the plant material that is needed. In connection with the practical realization, method 2 stands out, but a decisive answer on this point cannot yet be given.

2.5. All methods will produce quicker results, if first through 1 or 2 years of pedigree selection the ratio  $AA : A\alpha$  of the initial population has been made more advantageous for AA.

3.1. The results outlined above were extended to rye breeding in general. All characters of practical importance must be selected in principle according to one of the methods under 2.3., except the exclusively recessive ones, which, however, occur very seldom.

260

3.2. As method, applicable in all cases, only method 2 remains: test crosses and pair crosses, the test crosses being made as mass test crosses. For characteristics that can be estimated only after flowering, a reserve seed method must be introduced for the descendants of the crosses in pairs, method which, in order to distinguish it from the Petkus method, has been called the "modified reserve seed method".

3.3. The corresponding selection scheme, that was described in detail, is represented in fig. 3 on p. 253.

4.1. The method of repeated backcrossing — of which the above points 2 represent a special case — has been developed for all possible cases. To be able to proceed with the backcrosses in every generation without loss of time, certain devices must be introduced, varying with the circumstances.

4.2. When in the progeny of a backcross direct selection is impossible — the characteristic to be incorporated being recessive i.e. a cross of the type  $A\alpha \times AA \rightarrow AA + A\alpha$  — for the next backcross a certain number of arbitrarily chosen plants is backcrossed to AA, with simultaneous application of self-fertilization in autogamous plants, of individual test crosses with  $\alpha\alpha$  in allogamous plants. From this it becomes apparent in the next generation which backcrosses have been made with the desirable  $A\alpha$ -plants and with them work is continued.

4.3. When the estimation and consequently the selection is possible only after flowering, again a certain number of arbitrarily chosen plants are backcrossed and after flowering it is determined which backcrosses have been made with the desired plants.

4.4. With regard to small variations of method, all cases can be divided into six groups accordingly as we deal with:

1. a dominant characteristic, appraisable before flowering;

2. a dominant characteristic, not appraisable before flowering;

3. a recessive characteristic in self-fertilizers, appraisable before flowering;

4. a recessive characteristic in self-fertilizers, not appraisable before flowering;

5. a recessive characteristic in cross-fertilizers, appraisable before flowering;

6. a recessive characteristic in cross-fertilizers, not appraisable before flowering.

4.5. The selection of homozygotes after backcrossing either produces no difficulties at all, or these difficulties can be solved according to some of the methods under 2.3. or under 3.2 and 3.3, accordingly as we deal with a dominant characteristic which can be appraised before flowering or with a dominant characteristic which cannot be appraised before flowering.

5.1. When selecting in cross-fertilizers for a characteristic not exclusively recessive or for a characteristic genetically unknown — that is to say in most cases — the work must proceed according to rational methods under which, in the extreme form, is understood a combination of:

1st. mass test crosses,

2nd. crosses in pairs,

3rd. a modified reserve seed method for the progenies of the crosses in pairs, consisting in continuing the work with those pair crosses of which during test crossing has been demonstrated that they are made up of two genetically good clones,

261

which combination of procedures becomes only possible in the vast majority of cases by introducing a vegetative multiplication.

In some crops it will be feasible to replace the crosses in pairs by compulsory self-fertilization.

5.2. When the crop plants fructify several times or can be multiplied vegetatively permanently, for reproduction the original mother plants or mother clones can be used, and the crossing in pairs — or compulsory self-fertilization and the modified reserve seed method of 5.1. are dropped.

5.3. In order to apply rational methods to crops other than those meant in 5.2, the elaboration of methods for vegetative multiplication is of primary importance.

5.4. As new conceptions have been brought to the fore the mass test crossing and the modified reserve (remnant) seed method. The principle of crossing in pairs has been developed previously by VON SENGBUSCH. The desirability of vegetative multiplication has been pointed out by me before.

Wageningen, 1 February 1947.

Acknowledgement. — MR L. A. VAN MELLE, B. Sc., M. A., has given valuable assistance in translating the manuscript into English.

#### LITERATURE CITED

- (1) AUST, SOPHIA: Erhöhte Saatgutgewinning bei Roggen durch vegetative Vermehrung. (Züchter 13, 1941: 84–87.)
- (2) DORST, J. C.: Moet de kweker ter beoordeling van nieuwe rassen zijn kracht zoeken in variëren van omstandigheden of in herhalingen? (Landb. Tijdschr. 58, 1946: 457-464.)
- (3) FRANKE, A.: Veredelingsonderzoekingen met rogge 111: Blanco proeven. (Landb. Tijdschr. 58, 1946: 77-87.)
- (4) KRISTENSEN, REINHARDT, en TROELSEN, KR.: Avl af havefrø. København, Det Danske Forlag, 1943, 336 p.
- (5) LAUBE, (W.): Einige Fragen über die praktische Roggenzüchtung. (Beitr. Pflanzenzucht 8, 1925: 73-89.)
- (6) LAUBE, W.: Petkus. (Mitt. Landwirtschaft 43, 1936; 934-936.).
- (7) MAINS, E. B.: Rye resistant to leaf rust, stem rust, and powdery mildew. (J. agr. research 32, 1926: 201-221).
- (8) MAYER GMELIN, H.: Een en ander over de korrelkleur bij onze granen. (Landb. Tijdschr. 40, 1928: 742-760.)
- (9) MAYER GMELIN, H.: lets over inteelt-proefnemingen bij rogge. (Verslag Studiekring Plantenveredeling 30 Maart 1944: 15-18.)
- (10) MUNERATI, O.: Beitrag zum Studium der Selbstunempfänglichkeit des Roggens (Secale cereale L.). (Zsch. Pflanzenzücht. 9, 1924: 176–178.)
- (11) REINDERS, DIRKJE E.: Veredelingsonderzoekingen met rogge II: De invloed van bemesting op de uitstoeling. (Landb. Tijdschr. 56, 1944: 9–13.)
- (12) REINDERS, DIRKJE E.: Veredelingsonderzoekingen met rogge V: De invloed van de kiemingstemperatuur op het schieten bij voorjaarszaai. (Landb. Tijdschr. 58, 1946: 362-366.)

- (13) RIEBESEL, G.: Vegetative Vermehrung von Getreide-Bastarden. (Züchter 9, 1937: 24.)
- (14) SEINHORST, J. W.: Een laboratoriummethode voor de bepaling van de vatbaarheid van rogge voor aantasting door het stengelaaltje (Ditylenchus dipsaci (Kühn) Filipjev.). (Tijdschr. Plantenziekten 51, 1945: 1–13.)
- (15) SENGBUSCH, REINDHOLD VON: Theorie und Präxis der Pflanzenzüchtung. Frankfurt a. M., Societäts Verlag, 1939, 127 p.
- (16) SENGBUSCH, R. VON: Pärchenzüchtung, unter Ausschaltung von Inzuchtschäden. (Forschungsdienst 10, 1940: 545-549.)
- (17) SIRKS, M. J.: Die Verschiebung genotypischer Verhältniszahlen innerhalb Populationen laut mathematischer Berechnung und experimenteller Prüfung. (Med. Landbouwhoogeschool 26 (4), 1923: 40 p.)
- (18) WELLENSIEK, S. J.: Nieuwe methoden voor roggeselectie, met behulp van vegetatieve vermeerdering. (Zaaizaad en pootgoed 2, 1940, no 7: 9-11, no 8: 8-11.)
- (19) WELLENSIEK, S. J.: Vegetatieve vermeerdering en plantenveredeling, speciaal bij rogge. (Landb. Tijdschr. 54, 1942: 422-436.)
- (20) WELLENSIEK, S. J.: Grondslagen der algemeene plantenveredeling. Haarlem, Tjeenk Willink, 1943, XVI + 492 p.
- (21) WELLENSIEK, S. J.: Veredelingsonderzoekingen met rogge I: Werkprogramma. (Landb. Tijdschr. 56, 1944: 3-8.)
- (22) WELLENSIEK, S. J.: De methode der herhaalde terugkruisingen. (Instituut Veredeling Tuinbouwgewassen Meded. 4, 1947: 20-31.)