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Recurrent selection for resistance to *Sclerotinia sclerotiorum* in sunflowers using artificial infections

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SUMMARY

Three generations of recurrent selection for *Sclerotinia sclerotiorum* resistance have been carried out on 2 populations of sunflower. For the first 2 cycles a test measuring the extension rate of *S. sclerotiorum* mycelium on the back of the capitulum was used. For the 3rd cycle this test was employed together with one based on inoculation of the floral surface of the capitulum with an ascospore suspension, after which the length of the latent period until symptom appearance was measured.

From the 1st to the 2nd cycle, both populations showed considerable improvement. The mean of mycelium extension on the restorer population was reduced from 200 % of that on control variety 'Remil' to 104 % and on the maintainer population from 281 % to 91 %. At the same time the variance was considerably reduced. However the results of the 3rd cycle did not show any improvement. The restorer population had the same mean, 106 % as in the second cycle, although with a further reduced variance and the maintainer population was slightly poorer than in the 2nd cycle (with a mean of 113 %).

In the 3rd cycle there was no correlation between results of the mycelium and ascospore tests. The results of the latter showed wide variation, with the population mean between those of 'Airelle' and 'Remil', so it should be possible to breed for resistance to ascospore installation. Since this test is similar to the natural cycle of *S. sclerotiorum* most weight should be given to its results.

The possible reasons for the lack of improvement with the mycelium test are discussed. It is proposed to continue the programme with a weaker selection pressure for both characters during several generations in order to determine whether real increases in *S. sclerotiorum* resistance can be obtained.

Additional key words : *Test, mycelium, ascospores.*

RÉSUMÉ

Sélection récurrente pour la résistance à Sclerotinia sclerotiorum chez le tournesol avec emploi d'infections artificielles.

Trois générations de sélection récurrente pour la résistance à *Sclerotinia sclerotiorum* ont été menées sur 2 populations de tournesol. Pour les 2 premiers cycles, un seul test a été appliqué, mesurant la vitesse d'extension du mycélium de *Sclerotinia* sur la face stérile des capitules. Pour le 3^e cycle, on a employé à la fois ce test et un autre, consistant en l'infection des faces fleurées des capitules avec une suspension d'ascospores, la variable mesurée étant le délai jusqu'à l'apparition des symptômes.

Du 1^{er} au 2^e cycle, les 2 populations ont montré une amélioration très nette. La moyenne de la croissance mycélienne sur la population restauratrice est passée de 200 % à 104 % par rapport au témoin « Remil » et, sur la population mainteneuse, de 281 % à 91 %. En même temps, la variance a été considérablement réduite. Cependant, au troisième cycle, il n'y a pas eu d'amélioration ; la population restauratrice avait la même moyenne qu'au deuxième cycle, bien qu'avec une variance encore réduite, et la sensibilité moyenne de la population mainteneuse était légèrement supérieure à celle calculée pour le 2^e cycle.

Au 3^e cycle, il n'y avait pas de corrélation entre les résultats fournis par le test mycélium et par le test ascospores. Les résultats de ce dernier test montraient une variabilité importante par rapport au témoin. Il devrait donc être possible de poursuivre la sélection en utilisant ce test. Puisqu'il est plus proche du cycle naturel de *Sclerotinia*, il semble raisonnable de lui attribuer un poids plus élevé. Les raisons possibles des limites de l'utilisation du test mycélium sont discutées. Il sera nécessaire de poursuivre la sélection avec une pression moins élevée pour 2 caractères pendant quelques générations, afin de déterminer si de réels progrès en résistance à *Sclerotinia* sont possibles.

Mots clés additionnels : *Test, mycélium, ascospores.*

I. INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is probably the most important pathogen of sunflowers not controlled chemically or by complete genetic resistance, in the world as a whole, and in France in particular. However, observations of the frequency of natural attack have shown significant differences in susceptibility between genotypes (LECLERCQ, 1973; TOURVIELLE & VEAR, 1984). In different countries, different forms of attack are observed: in Canada, Romania and the U.S.A., most damage is caused by root and basal stem attack (HOES & HUANG, 1980; ILIESCU: pers. comm.; ZIMMER & FICK, 1974); in Yugoslavia, leaves and flower buds are attacked (CUK, 1976); in France and Hungary *Sclerotinia* disease takes the form of capitulum rot after flowering (REGNAULT *et al.*, 1976; KURNIK *et al.*, 1978). The relative resistance or susceptibility of sunflower genotypes to these 3 forms of attack does not appear closely related (TOURVIELLE & VEAR, 1984), or necessarily of the same type (CUK, 1980). Breeding for resistance to each form may thus be considered separately, although local conditions may require combined breeding programmes. The present paper deals only with capitulum rot, since this is at present the economically most damaging form in France.

In addition to significant differences between sunflower hybrids, observations of natural attack have shown significant heritable effects of parental lines (VEAR, internal report). In this case, resistance to *S. sclerotiorum* appeared additive for there were no significant effects of interactions between parental lines in hybrid combinations. Thus it appears possible to select for partial resistance in sunflowers.

However, in order to carry out breeding programmes, it has been necessary to develop tests useable each year, regardless of climatic conditions, to select between individual plants and families. VEAR & GUILLAUMIN (1977) described a test using mycelium, which measured the rate of extension of *S. sclerotiorum* on sunflower capitula. Results were heritable and significantly correlated with field observations. More recently, tests using ascospores, repeating much more closely the natural cycle of *S. sclerotiorum* have been developed. First results were described by TOURVIELLE *et al.* (1978) and LAMARQUE (1980) and further developed by TOURVIELLE & VEAR (1984).

This paper discusses the 1st cycles of recurrent selection in sunflower populations using these mycelial and ascospore tests.

II. MATERIAL AND METHODS

A. Sunflower populations

Two populations, one destined to give fertility restorer « male » lines and the other male sterility maintainer « female » lines were obtained by intercrossing in isolated plots about 30 mostly unrelated genotypes in each case. These genotypes had been chosen because they, or their hybrids, showed satisfactory results in field trials or in mycelium tests. They were of Russian,

American, Canadian, Romanian and French origin. In the female population some lines with translocations from *Helianthus tuberosus* were included and in the male population the male parent of the control hybrid: 'Remil' was used.

B. Control varieties

These were 'Remil' the most *S. sclerotiorum* resistant hybrid so far commercialized, and 'Airelle', a rather susceptible hybrid. Results are given as percentages of those of 'Remil', whose reaction is taken as the base level for selection. 'Airelle' was used simply to verify the pathogenic activity of the inoculum.

C. Inoculum

The *Sclerotinia sclerotiorum* mycelium and ascospores are prepared according to the methods described by TOURVIELLE & VEAR (1984).

D. Breeding programme

A recurrent selection programme for *S. sclerotiorum* resistance was carried out, with alternate generations of interpollination between chosen genotypes in isolated plots without selection (when possible in a winter nursery) and self pollination and testing of a sample of about 400 plants in the summer at Clermont-Ferrand. For the interpollinations, equal quantities of seed of the chosen genotypes were mixed and sown at random in the isolated plot, at 2 dates to favorise crosses between early and late genotypes. The harvest was in bulk and the 400 plants tested constituted a random sample. Selection therefore depended entirely on individual performance. Self pollination was obtained by covering the capitulum with a sulphurised paper bag just before flowering. The tests were applied during and after flowering and the best genotypes were retained for the following generation. Although reaction to *S. sclerotiorum* was the main selection criterion, excessively tall, late or self-sterile plants were eliminated.

For the 1st and 2nd cycles, only the mycelium test was used. In the 3rd cycle, an ascospore test was introduced, with comparison of 2 systems:

for the restorer population, the main stem of each plant was cut above the 1st pair of true leaves, so that 2 axillary stems developed, each producing a capitulum with about the same flowering date. One was inoculated with ascospores in the field, while the other was harvested after flowering and inoculated with mycelium in a growth chamber (see diagram in figure 1);

the other method, used on the maintainer population, was to inoculate all the plants with ascospores and then, when about 70 % of the plants showed symptoms, the remaining 30 % were harvested and the mycelium test applied.

E. Mycelium test

This test is described in detail by VEAR & GUILLAUMIN (1977) and TOURVIELLE & VEAR (1984). Three to

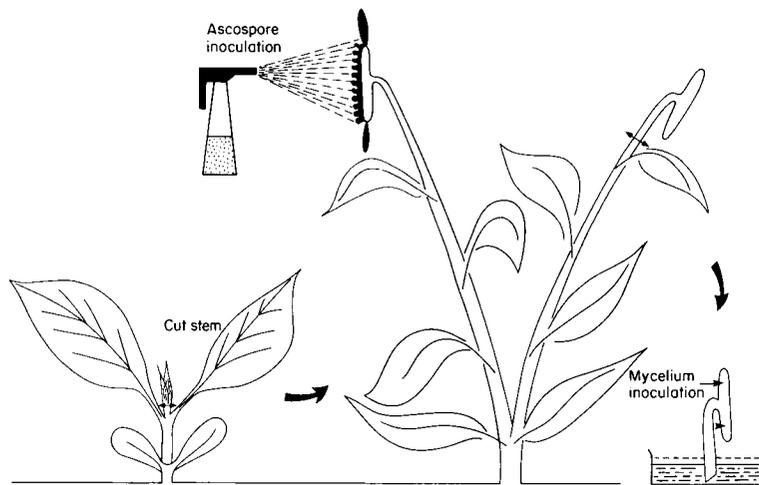


Figure 1

Diagram of inoculation of the 3rd selection cycle of the restorer population.

Schéma de l'inoculation des plantes du 3^e cycle de la population restauratrice.

6 weeks after flowering, the sunflower capitula are harvested and placed in a growth chamber with their stems soaking in water at a relative humidity of 100 % and a temperature of 18 °C in darkness. Three agar explants containing young *S. sclerotiorum* mycelium are placed on the back of each capitulum and the areas attacked by the fungus after 3 days are measured. The mean area of diseased spot per plant is then expressed as a percentage of the mean on the 6 control plants infected the same day.

F. Ascospore test

This test is described in detail by TOURVIELLE & VEAR (1984).

The floral surfaces of the capitula are sprayed, during the period of anther dehiscence, with 5 ml of a suspension of ascospores in water (concentration: 5 spores/mm³). The capitula are then covered with paper bags. The plants are examined for symptoms twice a week. Resistance is measured as the number of days until disease symptoms appear on the sterile side of the capitula.

III. RESULTS

A. Selection for resistance by the mycelium test

Figure 2 shows histograms of the evolution over 3 cycles of the restorer population and the control variety. In table 1 it may be noted that the variance of the control was reduced from cycle to cycle. This was probably due to improved techniques and the use of more exactly controlled conditions.

Although the genotypes used to constitute the population had been chosen for their useful reaction to *Sclerotinia*, there was considerable variation among the plants of the 1st cycle of selection with extremes of 0 and 940 % around a mean of 200.3 %. The 46 genotypes chosen for interpollination to give the 2nd cycle had only 13.5 % of the growth of *S. sclerotiorum* on 'Remil'.

There was considerable improvement from cycle 1 to cycle 2 : the mean diseased area on the plants tested was reduced from 200 % of that on 'Remil' to 104 % (table 1a). The variance was also reduced, much more than for 'Remil' and whereas in the 1st cycle on

TABLE 1

Evolution of sunflower populations selected for resistance to extension of *S. sclerotiorum* mycelium.
Evolution de 2 populations de tournesol sélectionnées pour la résistance à l'extension du mycélium de *Sclerotinia*.

	(a) Restorer population (ISCM)			(b) Maintainer population (ISCF)		
	ISCM	ISCMR 2	ISCMR 3	ISCF	ISCFR 2	ISCFR 3
Plant number	457	312	387	443	357	186
Mean % of 'Remil'	200.3	104.2	106.8	281.5	91.4	112.9
Variance	39 966.6	9 154.3	4 002.9	123 660.6	3 605.5	6 283.5
% of plants with more than 3 times extension on 'Remil'	24.7	4.8	1.0	17.0	0.28	2.7
Mean of best 50 plants	13.5	27.7	32.7	24.2	28.3	43.9
Variance among control plants	1 447.6	740.4	311.4	1 447.6	740.4	311.4

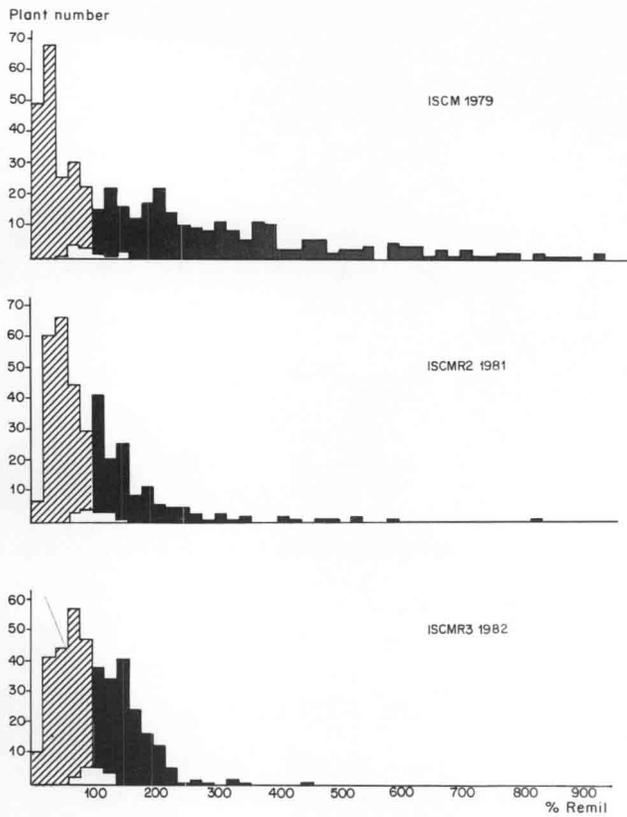


Figure 2

Evolution of a restorer population during 3 cycles of recurrent selection with the mycelium test. In white, control plants.

Evolution d'une population restauratrice pendant 3 cycles de sélection récurrente avec le test mycélium. L'histogramme blanc représente les variations du témoin.

24.7 % of the plants the disease spread at least 3 times as rapidly as on 'Remil', in the 2nd cycle this was the case for only 4.8 % of the plants. However, the reduced variance was also visible among the more resistant genotypes, the 50 chosen to make up the 3rd cycle showed more *S. sclerotiorum* growth (27.7 % of that on 'Remil') than those selected from the 1st cycle (13.5 %).

The 3rd cycle did not show any improvement in the average level of resistance to *S. sclerotiorum* extension, the mean (106 %) not being significantly different from that of the 2nd cycle. The variance was reduced only to the same extent as that of the control but it may be noted that the mean of the best 50 plants increased to 32.7 % of 'Remil'.

Figure 3 and table 1b give the results for the maintainer population. From the first to the second cycle the improvement was even more spectacular than for the restorer population. Mean spread of the fungus dropped from 281 % of that on 'Remil' to 91 %. The variance was reduced by 97 % and the average extension on the 50 plants chosen for interpollination increased from 24.2 % of 'Remil' in the 1st cycle to 28.3 % for the 2nd cycle.

The 3rd cycle showed not only no improvement but a slight reverse in the evolution of the population, the mean being 113 % of 'Remil'. However, it is not entirely comparable with the other cycles since an ascospore test was applied and only the 186 plants which showed no symptoms after 30 days were tested with mycelium.

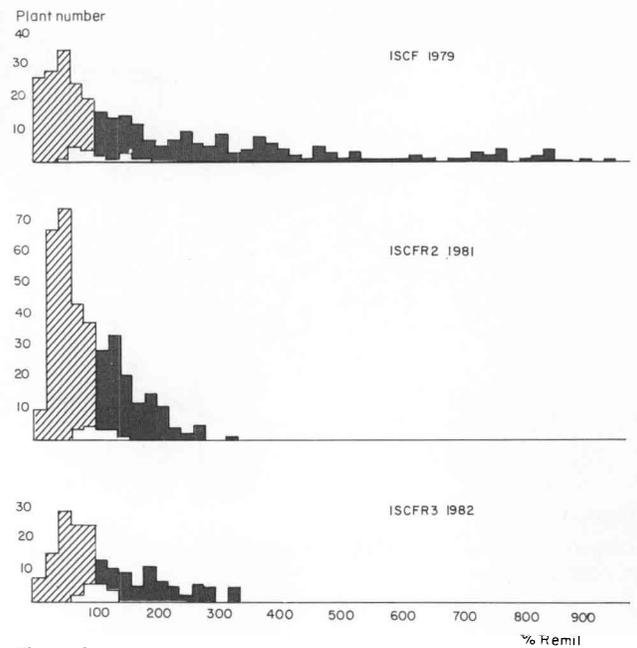


Figure 3

Evolution of maintainer population during 3 cycles of recurrent selection with the mycelium test. In white, control plants.

Evolution d'une population mainteneuse pendant 3 cycles de sélection récurrente avec le test mycélium. L'histogramme blanc représente les variations du témoin.

B. Selection for resistance to ascospore inoculation

The plants of the 3rd cycle of the restorer population were inoculated with ascospores at 1 of 4 dates (July 6, 9, 13 and 16, 1982), according to their date of flowering. The delay in symptom appearance for each inoculation date is shown in figure 4. For the

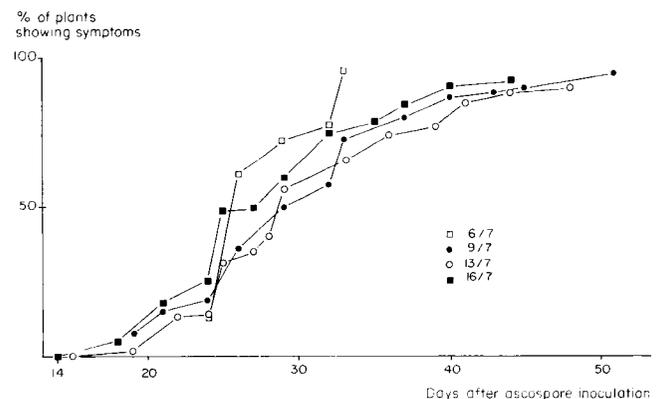


Figure 4

Appearance of *Sclerotinia* symptoms on the restorer population after inoculation with ascospores at 4 different dates.

Délais d'apparition des symptômes de *Sclerotinia* sur la population restauratrice après infection par ascospores à 4 dates différentes.

small number (18) of plants inoculated on July 6th, disease symptoms appeared more rapidly than on the plants inoculated later. This is in agreement with other results (TOURVIEILLE & VEAR, 1984) which suggest that the increased rate of symptom appearance is related to higher temperatures before July 10th. The closest relation between number of days after inoculation and proportion of plants showing symptoms was second

degree ($r = 0.99$) but with a linear relation there was a very good correlation ($r = 0.96-0.98$). Comparison of the linear regressions for the 3 dates shows that they are not significantly different. The results for these

3 days were therefore pooled and symptom appearance is given as number of days after inoculation. The histogram of figure 5 shows the variation from 13 to more than 50 days (after which the dry capitula were

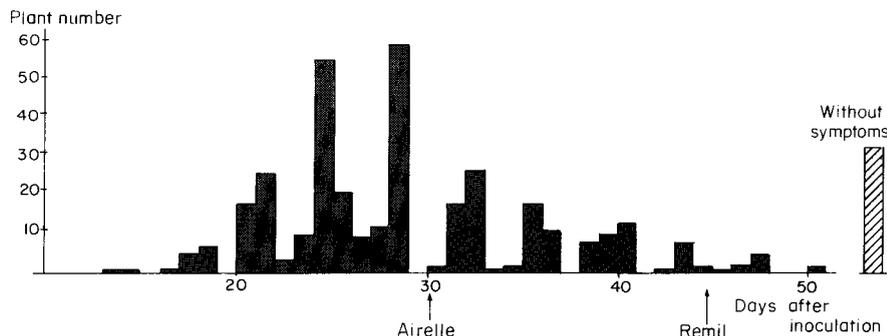


Figure 5
Appearance of symptoms on the restorer population after inoculation with ascospores in comparison with the control varieties.

Délais d'apparition des symptômes sur la population restauratrice après infection par ascospores en comparaison avec les variétés témoins.

harvested). The mean was 31 days above the level of 'Airelle' (28 days) but well below that of 'Remil' (42 days).

There was no significant correlation between the results of this test and those of the mycelium test applied on the pairs of capitula of each plant ($r = 0.04$). This could be due to an absence of real variation for reaction to the mycelium test. Earlier studies (TOURVIEILLE & VEAR, 1984) showed a significant correlation between the 2 tests.

The appearance of symptoms on the plants of the maintainer population followed a similar pattern (fig. 6). The slightly earlier appearance of symptoms may be genetic, but could also be due to the different inoculation dates (13, 16, 20, 23 July, 1982). When 70 % of the plants inoculated at a given date showed symptoms the remaining 30 % were harvested and the mycelium test was applied. The 3 days in very humid conditions in the growth chamber led to symptoms from the ascospore inoculation in only a few cases. For the 4th cycle the plants were thus chosen from among the 30 % most resistant to ascospores and with 43.9 % of the mycelial growth on 'Remil'.

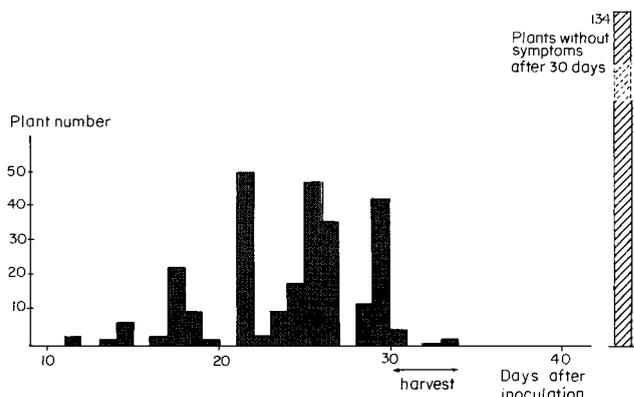


Figure 6
Appearance of symptoms on the maintainer population after inoculation with ascospores.

Apparition des symptômes sur la population mainteneuse après infection par ascospores.

IV. DISCUSSION

The results reported by TOURVIEILLE & VEAR (1984) indicate that it is possible to use the mycelium and ascospore tests each year. Thus, it should be possible to base a resistance breeding programme on them. The absence of correlation between the results of the 2 tests suggests that the characters studied may be independant so that the best programme would appear to be one using both tests concurrently in order to combine the genes necessary for each character. Since in both cases resistance is partial, addition of genes for each character should give a better total level of resistance. This idea is comparable with that of LECOQ *et al.* (1982) in breeding for resistance to viruses of melons, peppers and tomatoes.

However, examination of the results obtained so far suggests that it is necessary to vary the weight given and the selection intensities applied for the 2 tests.

The mycelium test has been used since 1976. That year, there was a significant correlation for 25 hybrids between the results of the test and observations of natural attack (VEAR & GUILLAUMIN, 1977) but, in 1982, the results for 9 hybrids did not show any significant correlation (TOURVIEILLE & VEAR, 1984). Since the test repeats only part of the *S. sclerotiorum* infection cycle, it must be used in conjunction with other tests to obtain most useful results. Nevertheless, resistance to mycelial extension is important for if symptoms appear on any present-day varieties the whole capitulum is rotted within a week and yield is lost. With sufficient resistance for the disease to spread only slowly it would be possible to harvest before complete capitulum disintegration.

During the first cycle of recurrent selection, there was a considerable improvement but this was not maintained. This could be due to phenotypic variability : while individual generations show reverse tendencies improvement is nevertheless visible over a large number of generations of selection. Such a result was obtained by LENG (1961) when selecting maize for high and low oil protein contents. Alternatively the lack of continued improvement could be genotypic, genes

giving greater resistance to mycelium extension than those of 'Remil' either not present at all in the populations or present but linked to other less favourable genes such that their favourable effect on resistance is not discernible. The strong selection pressure applied may have resulted in too little recombination between generations of selection. This would be comparable with the problems found by MATHER & HARRIS (1949) in the mass selection for high bristle number in *Drosophila* where further increases above a certain level were obtained only after stopping selection for several generations. The continued reduction in variance without any change in mean provides evidence for this.

To distinguish between the hypotheses, it is planned to continue selection for a few generations but with a reduced pressure such that a wider range of genotypes for the control of mycelial extension are included in the interpollination. If genes giving better mycelial resistance are present it should be possible to combine them gradually and they should become apparent. The reduced weight placed on the mycelial test will make it simpler to select at the same time for resistance to the ascospore test.

As might be expected from the absence of correlation, the 2 generations of selection for mycelial resistance do not appear to have any effect on the population reaction to ascospores. There is a wide variation in the length of latency with the mean situated nearer to that of 'Airelle' than that of 'Remil' but with extremes surpassing both. Selection for this character should thus be possible although heritability needs to be confirmed. Since even with only 9 genotypes the results of ascospore tests were significantly correlated with field observations (TOURVIEILLE & VEAR, 1984) it seems reasonable to put the greatest weight on this test

and select for mycelial resistance only among plants which have good ascospore resistance.

Following the problems with the 1st generations of recurrent selection, for the 4th cycle the 50 plants chosen were among the 40 % best for the ascospore test. It remains to be seen whether a selection pressure of this type will give a slow but continued increase in resistance. It also remains to determine whether the plants that did not show symptoms escaped and give progeny which are no better than the average or are in fact highly resistant with such a delaying action on symptom appearance that they died before the disease appeared.

Comparison of the 2 inoculation techniques indicated that both were useful but for different purposes. The technique with 2 capitula per plant was very satisfactory for comparing reactions to the 2 tests, but retarded plant development made it impossible to measure plant height and seed production and the top heavy plants were susceptible to lodging. Therefore this technique will be continued mainly for genetic studies. The technique in which the ascospore test is applied first followed by the mycelium test on the same plant has the disadvantage that it is not possible to follow appearance of symptoms for ascospore inoculation on the more resistant plants. However for a breeding programme once the selection level has been decided, when a given proportion of plants shows symptoms, those remaining can be subjected to the mycelium test without any problems and the secondary selection criterion applied.

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