T1985PHYT06

RESISTANCE TO SCLEROTINIA SCLEROTIORUM IN SUNFLOWERS

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Summary

This paper describes tests which can be used to determine the reaction of sunflower hybrids, inbred lines and segregating progenies to Sclerotinia sclerotiorum. The methods are based on artificial infections either with mycelium on the dorsal surface of the capitulum, followed by measurements of the rate of Sclerotinia extension or with ascospore suspensions on the floral surface of the capitula followed by measurement of the length of delay before symptom appearance. Results are compared in various climatic conditions. There are significant correlations between results obtained in different years. The ascospore test is successful at temperatures up to at least 30°C. These tests have been used in a recurrent selection programme for selection for Sclerotinia resistance. For the first two cycles, only the mycelium test was used, but for the third to the fifth cycle, both tests were employed. There was rapid improvement in reaction to the mycelium test at first, then there were three generations of stability, before the fifth generation once again showed improvement. Over the last three cycles in which the ascospore test was used, a gradual increase in the average length of the latent period was observed.

Résumé

Cet article décrit deux tests qui sont employés afin de déterminer le comportement du tournesol (variétés ou lignées) vis à vis du Sclerotinia sclerotiorum. Les techniques utilisées font intervenir des inoculations, soit à partir de mycélium sur la face stérile des capitules, avec mesure de l'extension de la tâche de pourriture, soit à partir d'ascospores, sur la face fertile des capitules, avec mesure du délai qui s'écoule entre l'inoculation et l'apparition des premiers symptômes sur la face stérile. Les résultats obtenus dans des conditions climatiques différentes sur plusieurs années sont comparables. Le test "ascospores" est réalisable même si les températures estivales dépassent 30°C (sous abris). Ces tests ont été utilisés dans un programme de sélection récurrente pour la résistance au Sclerotinia. Pour les deux premiers cycles, seul le test "mycélium" a été mis en oeuvre, alors que pour les trois suivants les deux test ont été employés. Pour le test "mycélium", la population a montré pour le premier cycle une très nette amélioration, puis il faut attendre le cinquième cycle pour retrouver un gain de résistance. Pour les trois cycles testés par ascospores, la population a montré une augmentation progressive de la durée moyenne de la période de latence.

Introduction

White rot of the capitulum, caused by Sclerotinia sclerotiorum (Lib) De By, is one of the most important diseases of sunflowers in France. Infection of the capitulum by ascospores occurs exclusively through the tubular florets (Tourvieille et al, 1978). The mycelium resulting from ascospore germination colonizes the superficial tissues and then, after a latent period of 15 to 40 days, invades the parenchymatous tissue, resulting in rotting of the head and loss of seed.

Observations of diverse sunflower genotypes under natural infection show almost continuous variation. Resistance appears to be partial and polygenic. Previous studies (Tourvieille and Vear, 1984) have shown that the resistance mechanisms

which may be involved during the first phase of infection, between pollution of the capitulum by spores and appearance of the first rot symptoms on the back of the capitulum, are not the same as those which determine the rate of extension of the disease (destruction of the parenchymatous tissue of the capitulum).

To try to combine different genes which may play a role in resistance to both phases of the Sclerotinia infection cycle, a recurrent selection programme was started in 1978. Five cycleshave been completed, the levels of resistance for each cycle being measured by the use of two complementary tests : the "ascospore" test for determining reaction to the first phase of infection and the "mycelium" test for the second phase.
This paper describes these tests and the results obtained with them.

Materials and Methods

A. The sunflower population The population was constitued by interpollination in an isolated plot of 30 restorer genotypes which had shown interesting levels of resistance to Sclerotinia either in the field or with the mycelium test.

B. The recurrent selection programme Each cycle is made up of two generations. In the first, 20 seeds from each of the best 30 individuals of the preceding cycle are sown at random in an isolated plot. Harvest is in bulk and a random cycle of 300 to 400 seeds are taken for the next generation. These are sown in a nursery, the plants are selfed and the two Sclerotinia tests are applied. To facilitate application of the two tests, the main stem of each plant is cut above the first pair of true leaves, so that two axillary stems develop, each producing a capitulum with about the same flowering date. One is inoculated with ascospores in the field, while the other is harvested and inoculated with mycelium in a growth chamber.

C. The resistance tests
1. The mycelium test in capitula in a growth chamber This test is a modified form of that described by Vear and Guillaumin (1976). The Sclerotinia mycelium is grown in Petridishes on medium containing 12 g agar and 10 g malt extract per litre, at a temperature of 23°C± 1° 10mm diameter pastilles are cut from the edge of 3 day old colonies and are placed on the back of the capitulum and maintained in place with adhesive tape. There are three repetitions per capitulum and for inbred lines and hybrids (controls), six capitula per genotype. The infections are made 4 to 6 weeks after flowering on capitula whose stems soak in water in a growth chamber (19°C±1°, humidity 100%) in the dark. After threedays the areas covered by Sclerotinia rot are measured.

2. The ascospore test on capitula in the field The methods for obtaining apothecia and collecting ascospores were described by Tourvieille et al, 1978. The inoculations are made at the beginning to one third of flowering, 5 ml of a suspension containing 5 spores/mm^3 are sprayed on to the floral surface of the capitulum using a hand sprayer. The capitulum is then recovered with the sulphurised paper bag used for selfing. This bag maintains sufficient humidity for the infection to occur. In the case of inbred lines and hybrids 50 plants per genotype are inoculated. After two weeks, each capitulum is observed twice a week, and the date of first symptoms on the back of the capitulum is noted. This makes it possible to calculate the length of latent period for each genotype.

Results

A. Preliminary studies on the test 1. Mycelium test Table 1 gives the results over 6 years for a series of hybrids. It shows that although the mean area of rotted spot may vary for a given genotype, the comparison between genotypes is repeatable from year to year. All the results in this paper are therefore given as percentages

of the diseased area on the control Rémil inoculated at the same time.

2. Ascospore test Studies made since 1980 (Tourvieille and Vear, 1984) show that according to weather conditions, the percentage of diseased plants and the length of latent period vary between years. However Table 2 shows that the classification of genotypes for both these factors remains constant from year to year. As for the mycelium test, control varieties must be used for each inoculation date to permit comparison between different dates and years. Lengths of latent period are given in number of days for a given infection date.

B. The recurrent selection programme For the first two cycles only the mycelium test was used. For the third to the fifth cycle both tests were employed.

1. Selection for resistance to the mycelium test Table 3 gives the results of the mycelium test over five cycles. From the first to the second cycle there was considerable improvement (the mean was reduced from 200% of Rémil to 104%), but then, from the second to the fourth cycle there was no significant change and it was only in the fifth generation that there was again a reduction in the mean rate of Sclerotinia extension on the population, to 79% of that on Rémil. At first the improvement came in the proportion of very susceptible plants, those on which the pathogen grew more than three times as fast as on Rémil (24,7% in the first two cycles, 7,8% in the third and fourth cycle) in contrast, from the fourth to the fifth generation, the improvement came from the increased proportion of plants with less Sclerotinia than on Rémil (4th cycle 51,8%, 5 th cycle 71,5%).

2. Selection for resistance to the ascospore test Table 4 shows the results of the ascospore test over three generations (ISCMR3 to ISCMR5). In 1982, and 1984, symptoms appeared at the same rate from three of the four inoculation dates (Vear and Tourvieille, 1984), so it was necessary to correct only a small part of the results through control varieties. In 1983, there was a significant regression between inoculation date and length of latent period, so these results were corrected to the date when most plants were inoculated. To determine progress made in selection for resistance from year to year, the results are compared with two control varieties : Rémil (relatively resistant) and CR2 (susceptible). From the first to the second generation, the mean delay did not increase significantly when compared with the mean of Rémil and CR2 (0.83 for ISCMR3, 0.85 for ISCMR4). However, it may be noted that compared with CR2 only, there is an improvement. For the third generation there was a highly significant improvement in the population mean (0.85 to 0.96). As with the mycelium test, the first improvement appeared as a reduction in the proportion of very susceptible plants (with a shorter latent period than that of CR2). So far, the proportion of plants with a greater degree of resistance than Rémil has not been increased.

DISCUSSION

Selection for resistance to Sclerotinia sclerotiorum using the ascospore and mycelium tests is possible every year at temperatures up to at least 35-40°C. The results of the recurrent selection programme indicate that most of the genes giving extreme susceptibility to Sclerotinia can be eliminated quite rapidly from a sunflower population but that it is more difficult and longer term to obtain genotypes with greater resistance than the best types known at present. After three generations of selection using the mycelium test, when there appeared to be no further improvement in the population, two hypotheses were considered (Vear and Tourvieille, 1984) : one was that there were no gene combinations for increased resistance in the population. The second was that selection pressure was too high and useful resistance genes which were either linked to susceptibility genes or

Table 1. Results of mycelium tests. (as % of the control hybrid Rémil).
N = number of inoculations (each of 6 capitula).

Year	area of rot on Rémil mm^2	Genotypes							
		GP4	N	4701	N	Airelle	N	CR2	N
1979	443	-		102	5	362	10	398	8
1980	701	-		100	1	211	11	-	
1981	1078	41	1	-		244	12	220	1
1982	936	68	1	125	1	157	1	196	1
1983	1220	46	6	-		-		-	
1984	1073	56	6	-		-		204	1

Table 2. Results of ascospore tests
T = % of plants with symptoms D = length of latent period in days.

Years	Genotypes							
	Rémil		H9P3		Airelle		CR2	
	T	D	T	D	T	D	T	D
1980	44	52	62	45	66	41	92	31
1981	85	49	94	35	96	34	100	37
1982	65	41	75	40	87	33	93	31
1983	45	52	-	-	86	47	97	31
1984	71	49	-	-	-	-	94	32

Table 3. Results of the mycelium test over 5 generations of recurrent selection

	ISCM	ISCMR2	ISCMR3	ISCMR4	ISCMR5
Year	1979	1981	1982	1983	1984
Population plant number	457	312	387	222	302
Population mean (% of Rémil)	200.4	104.2	108.3	122.4	79.0
% of plants with more than 3 times extension on Rémil	24.7	4.8	1.0	6.7	1.7
% of plants with less extension than on Rémil	43.1	54.2	52.7	51.8	71.5
Mean of plants taken for next generation (% of Rémil	14	28	47	78	44

Table 4. Results of ascospore tests over 3 generations of recurrent selection expressed as the delay between inoculation and symptom appearance. Means are corrected to the nearest whole day.

	ISCMR3	ISCMR4	ISCMR5
Year	1982	1983	1984
Population plant number	352	224	258
Population mean (days)	30	35	39
Rémil (days)	41	52	49
CR2 (days)	31	31	32
Population mean / (CR2 + Rémil)/2	0.83	0.85	0.96
% of plants with a longer latent period than Rémil	11.8	0	8.1
% of plants with a shorter latent period than CR2	61.1	12.1	13.9

showed no effect alone, were being eliminated from the population because there was insufficient chance for recombination. With the introduction of the ascospore test, more closely correlated with natural attacks by Sclerotinia (Tourvieille and Vear, 1984), less weight was put on the mycelium test and the selection pressure was thus reduced. The results of the fourth and fifth generations suggest that the second hypothesis may be correct since, after three generations of stability there was again an increase in resistance compared with Rémil. As with many long term selection programmes (Leng, 1961), it may be that improvement will be irregular, according to chance recombinations.

Concerning the ascospore test, for which the selection pressure has never been so severe as for the mycelium test, a gradual increase in the mean level of resistance appears over three generations. However, if the proportion of plants with a better level of resistance than Rémil is considered, once again the problem appears of the presence of the necessary genes in the population. Only further generations will show whether it is possible to surpass Rémil in the present context, or whether it will depend on the introduction of new material, from interspecific crosses for example.

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