Multilocal *Sclerotinia sclerotiorum* Resistance Tests

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Abstract

Three control inbred lines and 151 F3 or F4 families from a cross between 2 lines, SD and PAC1, with different types of *Sclerotinia sclerotiorum* resistance, were subjected to ascospore tests measuring reaction to capitulum attack, in 6 locations in 1994 and 1 location in 1995. The mean percentage attack on the controls in 1994 varied from 50 to 100%. In locations with intermediate levels of attack, there was a clear difference between resistant and susceptible controls, but when mean attack was above 80% there was little distinction. In contrast, although the mean delay in symptom appearance also varied (for the resistant control, SD, from 24 to 51 days), the ratio of the results for resistant and susceptible lines was quite stable, with latency indices for SD varying only between 1.19 and 1.29 and for the susceptible control, GU, from 0.71 to 0.80. For the F3/F4 families, analyses involving different numbers of locations always showed significant differences between families. All locations gave results significantly correlated with the mean, for percentage attack and latency index. However, generally there were closer correlations between the results in locations with similar mean attack levels. It is concluded that resistance tests of different intensities distinguish different types or origins of resistance.The consequences for breeding programmes for *S.sclerotiorum* resistance are discussed.

Introduction

Sclerotinia sclerotiorum (Lib) de Bary can attack most parts of the sunflower plant : roots (Huang and Hoes, 1980), leaves (Cuk, 1978), capitula (Putt, 1958) and terminal buds (Peres et al., 1989). Attacks on capitula are among the most economically damaging. When weather conditions during flowering are favorable, there can be yield losses of at least 50%, and no satisfactory chemical treatments are available. No complete resistance is known in sunflower, but considerable differences in susceptibility occur. Castaño et al. (1993) showed that two main types of resistance exist, firstly resistance to penetration of the fungus into the plant, secondly resistance to extension of mycelium in the plant tissues. To analyse the genetics of these two systems, ascospore tests (Tourvieille and Vear, 1984) were made on F3 and F4 families grown in the field in several locations. Environmental effects are known to be important in the development of *Sclerotinia* symptoms (Lamarque, 1983), so these trials were used to determine the repeatability of results in different conditions and when different numbers of plants per genotype were available. This paper reports preliminary analyses of the results.

Materials and methods

Sunflower genotypes : Three inbred lines with known reactions to *Sclerotinia* capitulum attack (Castaño et al, 1993) were used as controls : SD : good resistance to mycelium extension, little resistance to fungal penetration ; PAC1 : good resistance to fungal penetration, less resistance to

mycelium extension and GU : susceptible to all stages of *Sclerotinia* attack. The segregating families were 151 F3 and F4 progenies from a cross between SD and PAC1.

Fungal isolate : A mixture of ascospores produced from sclerotia collected from sunflower capitula was used for the artificial infections. The sclerotia were induced for ascospore production by placing them during three winter months in damp perlite out of doors, then replacing them in damp soil-less compost in an unheated greenhouse at 12-18°C (Tourvieille et al., 1978).

Artificial Infection Methods : Infections were carried out at the beginning of flowering of each plant by spraying the open florets with 5ml of a suspension containing 5 ascospores/mm3 (Vear and Tourvieille, 1988). To maintain sufficient humidity on the capitula, these were covered with grease-proof paper bags immediatly after infection. Infections were carried out two or three times a week to cover all plants at the correct stage, and each time, plants of the inbred lines, sown at different dates to give staggered flowering, were infected. Sprinkler irrigation was provided after each infection, in 1994 and once a week in 1995.

Measurements of resistance : Two criteria were used. Firstly the final percentage attack on each inbred line or F3/F4 family, which is considered to measure resistance to fungal penetration. Secondly, the mean delay in symptom appearance, which takes into account the rate of extension of *Sclerotinia* mycelium from the florets to the dorsal surface of the capitulum. This was measured by observing the first disease spots on each plant. Since weather conditions (rainfall, temperature) affect rate of *Sclerotinia* growth, a latency index was calculated from the ratio :

$$\frac{\text{Nb. days between infection and symptom appearance for genotype}}{\text{Nb. days infection-symptoms of controls (SD + GU) infected at same date}}$$

Experimental design : The trials were made at six locations in 1994, from central to southern France (with department number in brackets) : Boissay (28), St Florent/Cher (18), Clermont Ferrand (63), Rivières (81), Mondonville (31), Lectoure (32). Total numbers of control plants infected in each location are given in Table 1. For the F3/F4 families, plots of at least 20 plants were infected at each location, with 2 replications at Clermont-Ferrand. However, lodging caused by storms reduced the number of observable plants in several locations, so this factor was taken into account in analysis of results. In 1995, two replications were grown at Clermont-Ferrand only. Only partial data are so far available from this last trial.

Results

Percentage infection The results are presented in Table 1.

1. Inbred lines : They show that in locations with generally high final levels of attack, Clermont-Ferrand (1994), Boissay, Lectoure, and Rivières, there were no, or only small differences between SD and GU. In contrast, at St Florent/Cher and Mondonville, where there were lower mean levels of attack, SD was much less attacked than GU. PAC1 was included in the trials as being resistant to penetration, but in the locations with high levels of attack, most plants showed symtoms. In contrast, this line was by far the least attacked at Clermont-Ferrand (1995) and at St Florent/Cher, Rivières and Mondonville in 1994.

2. F3/F4 families : Mean attack levels showed the same range of variation as that of the inbred lines, with the highest mean of 93% at Boissay. This location showed little difference between families, whereas in the other locations, minimum attack did not exceed 30% whilst maximum attack was 100%. The lowest levels of attack occured at St Florent/Cher and Mondonville, where, at the same time, the numbers of observable plants was very low (8 or 9 per plot). An analysis of variance on the two replications at Clermont-Ferrand showed that genotype effects were highly significant (1994 : F = 3.89**, lsd(5%) = 22.9%; 1995 : F = 2.90, lsd(5%) = 17.9%). However, whereas at this location about 10% of families (14/151 in 1994, 19/148 in 1995) were significantly different from the mean, an overall analysis on 4 locations in 1994 showed only 5/115 significantly divergent families (4%).

Latency indices Table 2 presents the mean delay in symptom appearance for the inbred lines and F3/F4 families.

1. Inbred lines : The mean delay in symptom appearance for the inbred lines showed a range between St Florent/Cher (SD : 24 days) and Clermont-Ferrand (1995) (SD : 54 days). However, SD and GU always followed the same pattern, and the latency index calculated from the delays of these two lines are very stable (SD 1.20 to 1.30, GU 0.70 to 0.80). PAC1 showed values intermediate between SD and GU except at Rivières, where it was highly resistant. At Clermont-Ferrand, this line showed a lower latency index in 1995 when percentage infection was lower than in 1994. It should be noted that smaller numbers of plants of PAC1 were infected than for SD and GU, and when percentage infection was low, the numbers of results available to calulate the mean delay in symptom appearance was quite small (10 at St Florent/Cher and Mondonville, 20 at Lectoure, 30 at Rivières).

2. F3/F4 families : As for the inbred lines, the means are quite stable (0.90 to 1.12), except for St Florent/Cher, where the symptoms of SD appeared very quickly, so that the dividing factor for calculating the latency index was always small. Similarly, the range of latency indices was smallest for the locations with the longest delays in symptom appearance, giving the largest dividing factors (Boissay, Mondonville).

Correlations between the results of F3/F4 families in the different locations : Pearson phenotypic correlations are given in Table 3.

1. Percentage infection : For Clermont-Ferrand the values are taken from the means of the two replications. In 1994, the correlation between the two replications was r = 0.59** and in 1995, r = 0.57**. Between years it was r=0.57**. Between locations, the values were lower, but significant when levels of attack were similar. Boissay, Clermont-Ferrand (1994 and 1995) and Rivières were always significantly correlated. At Lectoure, correlated only with Clermont-Ferrand and Boissay, smaller numbers of plants were observed. Mondonville and St Florent/Cher were significanlty correlated with each other , but with no, or only 2 other, locations respectively. However, the results in each locations were always significantly correlated with the overall mean for each family at very similar levels (r = 0.55 to 0.75).

2. Latency indices : The correlation between replications at Clermont-Ferrand (1994) was closer than for percentage infection (r = 0.77**) but between locations coefficients were of a similar order to those for the first resistance factor (mean correlation coefficient r=0.274 compared with r=0.265 for % infection; table 3). As for percentage infection, Clermont-Ferrand was significantly correlated

Table 1 : Percentages of plants showing *Sclerotinia sclerotiorum* symptoms after articial ascospore infections of sunflower capitula of inbred lines and F3/F4 families

Locations	Number plants infected			% attack			Nb	Mean plant	% attack		
	Inbreds	SD	GU	PAC1	SD	GU	PAC1	families	nb/family	mean	mini	maxi
Clermont-fd 1994		409	400	128	100.0	100.0	89.8	151 F3	2 x 22	81.1	28.9	100.0
Clermont-fd 1995		410	415	136	93.8	95.8	64.0					
St Florent		120	120	60	35.8	99.2	15.0	112 F4	8	48.4	0.0	100.0
Boissay		204	204	88	96.6	100.0	100.0	71 F4	21	93.0	52.6	100.0
Rivières		199	161	142	78.9	89.4	26.1	73 F4	19	84.5	27.7	100.0
Mondonville		102	102	31	43.1	71.7	32.2	120 F4	9	33.7	0.0	100.0
Lectour		77	103	31	87.0	80.6	83.8	72 F4	13	84.6	25.0	100.0

Table 2 :Latency indices for sunflower inbred lines and F3/F4 families subjected to artificial capitula infection with *Sclerotinia sclerotiorum* ascospores.

Locations	mean delay (days) :			Latency indices :			Nb families	Latency indices :		
	Inbreds	SD	GU	PAC1	SD	GU	PAC1		mean	mini	maxi
Clermont fd 1994		37.0	20.6	33.3	1.29	0.71	1.18	151 F3	1.09	0.40	1.90
Clermont fd 1995		54.4	29.5	38.3	1.30	0.70	0.91				
St Florent		24.1	14.6	21.5	1.24	0.76	1.18	112 F4	1.58	0.56	2.60
Boissay		41.9	27.6	35.0	1.21	0.79	1.03	71 F4	1.12	0.85	1.54
Rivière		30.3	20.4	33.0	1.20	0.80	1.30	73 F4	1.12	0.80	1.41
Mondonville		51.3	29.4	34.5	1.27	0.73	0.88	120 F4	1.09	0.59	1.57
Lectour		49.3	32.1	34.8	1.22	0.78	0.87	72 F4	0.90	0.59	1.23

with all locations except Mondonville. For this character, St Florent/Cher and Mondonville were not correlated, and these two locations showed rather poor relations with the others. Frequently the data from these locations were calculated from only 1 to 5 plants (mean number of plants per plot 8 or 9 and 33-48% attack), which suggests that the indices were not very precise. However, as for percentage infection, all locations were significantly correlated with the mean (r=0.516 - 0.748).

Table 3 :Pearson correlations between the results of ascospores tests in different locations on F3/F4 families from cross SD x PAC1.

Percentage infection ,

	Clermont-Fd	Lectour	Mondonville	Rivières	Boissay	St Florent
Mean	**0.556 ****	**0.530 ****	**0.598 ****	**0.553 ****	**0.549 ****	**0.750 ****
Clermont-Fd		**0.426 ****	0.162 ns	**0.272 ***	**0.365 ****	**0.338 ****
Lectour			0.206 ns	0.058 ns	**0.483 ****	0.114 ns
Mondonville				0.069 ns	0.111 ns	**0.246 ****
Rivières					**0.497 ****	**0.392 ****
Boissay						0.241 ns

Latency index ,

	Clermont-Fd	Lectour	Mondonville	Rivières	Boissay	St Florent
Mean	**0.630 ****	**0.516 ****	**0.530 ****	**0.591 ****	**0.476 ****	**0.748 ****
Clermont-Fd		**0.336 ****	0.078 ns	**0.524 ****	**0.261 ***	**0.258 ****
Lectour			0.255 ns	**0.573 ****	**0.459 ****	**0.041 ns**
Mondonville				0.117 ns	**0.311 ***	0.163 ns
Rivières					0.219 ns	0.184 ns
Boissay						**0.325 ***

* : $P < 0.05$
** : $P < 0.01$
ns : non significant

Discussion

Resistance of sunflower capitula is determined by several characters under polygenic control. Tourvieille and Vear (1984) demonstrated the usefullness of artificial infections using ascospores. The results of these tests were shown to be correlated with observations of natural or semi-natural attacks in the field (Vear and Tourvieille, 1987). However, tests of plants in the field remain dependant on environmental conditions, temperature, rainfall and irrigation. The trials reported here illustrate this effect, with between 15 and 100% of plants of the inbred line PAC1 attacked in different locations. In addition, the period of delay in symptom appearance of, for example, SD at Mondonville was more than double that at St Florent/Cher. Vear and Tourvieille (1987) showed an effect of year, but which did not change the ranking of sunflower genotypes. These observations justify the use of an index which takes into account infection date and environmental conditions.
Of the inbred lines, GU showed the high level of susceptibility to all stages of disease that had previosly been reported. SD generally showed the relatively long latency period but high level of attack, as expected, except when there was a low infection pressure (Mondonville, St Florent/Cher) and some plants remained without symptoms at maturity. The greater variation of PAC1 may come from the fact that this line is branched, so that usually it dries quite quickly with many plants that

are healthy at maturity, whereas, if disease pressure is very high and irrigation maintained during maturation, the plants do not dry and some show more, and later, development of symptoms.
Some of the F3/F4 families showed greater resistance than the parental lines (Boissay : minimum 50% attack when the parents were both 100% attacked), suggesting that they contained a combination of the resistance genes from the two parents. Low numbers of plants in certain locations and very different disease pressures probably account for different behaviours of some families in different environments. It may be suggested that, to obtain the best measurement of percentage attack, mean attack should not exceed 70% and that at least 20 plants per family are necessary to assure accurate estimates of *Sclerotinia* reaction.
Nevertheless, all the trials show some significant relations with the others. The overall mean for each familly does appear to be a representitive value of the different factors of resistance to *Sclerotinia* capitulum attack, which are expressed to a varying extent according to disease pressure and environmental conditions. This preliminary analysis confirms the interest of multi-local trials to measure *Sclerotinia* resistance, and at the same time, the need to develop methods, such as use of molecular markers of resistance, which are less dependant on environmental factors and which could replace some of the tests necessary during breeding programmes.

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