REACTION OF SUNFLOWER LINES TO A SERIES OF *SCLEROTINIA SCLEROTIORUM* ISOLATES

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Abstract

Sclerotinia sclerotiorum isolates show differences in in vitro growth rate and sclerotia production. Ascospore tests on 16 sunflower lines with 10 isolates that produced sclerotia and ascospores showed that the latter differ in aggressiveness, as measured by mean percentage of capitulum attack (23 to 45%) and delay in symptom appearance (latency indices from 1.16 to 1.40). The sunflower lines showed greater differences than the isolates with mean levels of attack from 2% to 92% and latency indices (for the lines with at least 25% attack) of 0.93 to 1.74. However, factorial analyses of these tests indicated that while there were highly significant isolate and genotype effects, there was no significant interaction between sunflower genotype and *Sclerotinia sclerotiorum* isolates. Thus it can be concluded that the resistance presently available in cultivated sunflower is horizontal and should be durable. Progress in breeding for improved resistance may be rather slow but it should remain useful in the long term.

Introduction

Resistance of sunflower capitula to attack by *Sclerotinia sclerotiorum* (Lib.) de Bary is partial and polygenic. Very often, quantitative resistance is not considered to show interactions between host and pathogen genotypes; to be "horizontal" according to the definition of Van der Planck (1963). However, host/pathogen interactions are known for some partial resistances (Flier et al., 2003) and studies made on another pathogen of sunflower, *Diaporthe/Phomopsis helianthi* Munt-Cvet. et al. where quantitative resistance is also the rule, showed that there were small but significant interactions and thus that care was needed when choosing Phomopsis isolates for disease resistance tests (Viguié et al., 1999). Thuault and Tourvieille (1988) made a preliminary study of the relation between sunflower genotype and *Sclerotinia* spp. and concluded that there were no significant interactions, but before widespread use of resistance QTLs, and perhaps increased selection pressures applied on the pathogen, it appeared useful to make a more detailed study of the reactions of the range of cultivated sunflower genotypes now available to a wide range of *S. sclerotiorum* isolates. To obtain reactions closest to those following natural attack, infections were made at flowering with ascospores (Tourvieille and Vear, 1984).

Sclerotinia sclerotiorum isolates are known to show differences in in vitro characteristics such as rate of mycelial growth and sclerotia production (Ziman et al., 1998). A primary study of 27 isolates available in the collection at INRA, Clermont-Ferrand showed that the isolates from sunflower collected in France, and retained for use in sunflower resistance tests generally showed most active growth and sclerotia production However, analysis of DNA

polymorphism indicated that these isolates showed variation, with some isolates having the same profiles as those obtained from other host species (Tourvieille et al., 2004).

Materials and Methods

Sclerotinia sclerotiorum Isolates. Ten isolates were chosen from the collection available at INRA, Clermont-Ferrand, according to their diversity in mycelial growth and sclerotia production, and the production of a sufficient number of apothecia to collect the ascospores necessary for the interaction studies. Isolate 3 was collected in the USA in 1981 on a sunflower capitulum. The others were collected in France from 1990 to 2002 on sunflower cotyledons (2), leaves (5), stems (4, 6 and 10), terminal buds (1, 9) and capitula (7, 8).

Sunflower Genotypes. Sixteen inbred lines, all bred by INRA, were chosen to represent the known range of reactions to *S. sclerotiorum* capitulum attack. They are listed in Table 2. Lines 1 to 9 are cytoplasmic sterility maintainers, 10 to 16 are fertility restorers. The lines SD, VLQ, PAC1 and PSC8 are or have been used as resistant checks and CP73, GB, GU and PR56 as susceptible checks.

In Vitro Observations. For each of the 10 isolates, Petri dishes containing 1% malt and 1.5% agar were inoculated with 5 mm explants. Four dishes were incubated at each temperature, and then two measurements of diameter at right angles to the colonies were taken after three days before the mycelium reached the edge of the dish. Results are the mean of these diameters in mm/day. One of the dishes grown at 24C was retained for four months, when all growth had ceased. The sclerotia that had been produced were counted, dried and weighed.

Ascospore Test. This test was described by Vear and Tourvieille (1988). Two replications of the 160 treatments (16 genotypes x 10 isolates) were grown in the field, with 25 plants per plot. Ascospore suspensions (10,000 spores/ml) of each isolate were sprayed on the florets of sunflower capitula at the beginning of flowering (1/7 to 21/7/2003), at the same time as control varieties, for which staggered sowing dates gave plants flowering over the whole period. From the beginning of flowering to the end of observations, irrigation provided at least 40 mm water during the weeks when there was no rainfall. Observations were continued until 16 September when several of the sunflower lines were completely dry and very few plants showed new *S. sclerotiorum* symptoms. From two weeks after infection, observations of symptom appearance on the dorsal surface of capitula were made twice a week. The results obtained gave the total percentage attack per plot and a latency index, which is the mean ratio of the number of days between infection and symptom appearance for each plant and that of the control plants infected the same day.

Results

Table 1 presents the in vitro data concerning the *S. sclerotiorum* isolates used for the ascospore test. Growth rates varied from 4.73 to 5.69 mm/day at 12C and 7.44 to 10.96 mm/day at 24C. Isolate 3 always grew most slowly and isolate 4 most quickly. Sclerotia production varied from 11 (isolate 3) to 41 (isolate 1). Isolate 3 had the largest sclerotia, isolate 1 the smallest.

Table 1. In vitro characteristics of 10 *Sclerotinia sclerotiorum* isolates used for the sunflower ascospore test.

Isolate	Growth rate (mm/day)		Sclerotia production after 4 months at 24°C	
	12°C	24°C	Number	Mean weight 1 sclerotium (mg)
1	4.93	7.81	41	0.89
2	2.56	10.10	13	3.61
3	5.69	10.96	11	4.10
4	4.73	7.44	27	1.15
5	5.44	8.29	19	2.96
6	5.42	8.98	19	2.96
7	5.27	9.00	26	1.46
8	5.08	9.56	20	1.22
9	5.10	9.06	19	2.67
10	5.58	10.38	21	1.89
Mean	**5.27**	**9.16**	**23.2**	**2.12**
F isolates	**8.55****	**58.13****		
lsd	**0.63**	**0.84**		
cv	**4.21**	**3.22**		

Percentage infection observed after the ascospore test is presented in Table 2. The different treatments showed percentages from 0 to 100 with a mean of 32%. Sunflower genotype means varied from 2 to 92%, whereas those of *S. sclerotiorum* isolates only varied from 23 to 45%. A factorial analysis carried out on the complete data showed significant treatment genotype and isolate effects but no significant genotype/isolate interaction (Table 3). Since some genotypes showed very low levels of attack, which could reduce apparent differences between isolates, a second analysis was carried out, excluding the five genotypes for which some isolates gave 0% attack (VHQ, VLQ, PSC8, PST5 and PSU7). The F values, shown below in Table 3, are very similar in the two analyses, with preponderance of the effect of sunflower genotypes, significant isolate effects and no interaction between the two.

Table 2. Percentage attack of 16 sunflower lines infected with ascospores of 10 *Sclerotinia sclerotiorum* isolates.

Isolates / Inbred lines	1	2	3	4	5	6	7	8	9	10	Mean
1 CP73	38.3	85.6	66.6	90.0	38.4	61.6	58.0	56.0	80.4	61.1	**63.6**
2 FN	6.2	8.5	9.6	4.0	6.5	4.0	12.0	4.0	7.8	14.6	**7.7**
3 FU	15.3	49.6	23.7	28.4	28.0	29.9	24.0	22.0	10.7	26.8	**25.8**
4 GB	84.2	94.2	90.1	91.8	87.3	98.0	90.5	90.3	87.6	100.0	**92.4**
5 GH	33.3	80.0	48.6	64.4	29.9	67.3	36.9	34.5	55.1	26.1	**47.6**
6 GU	70.5	91.7	92.3	90.1	92.2	84.0	94.0	82.9	94.2	93.2	**88.5**
7 SD	36.7	86.3	57.8	70.8	34.0	56.9	48.0	36.9	51.4	36.7	**51.6**
8 VHQ	0.0	15.6	3.8	14.5	9.9	12.3	8.4	4.2	7.8	16.7	**9.3**
9 VLQ	0.0	4.0	1.9	5.8	4.2	2.1	2.0	2.0	4.0	8.0	**3.4**
10 PAC1	4.0	34.9	15.8	27.6	10.0	16.0	13.7	7.6	12.0	1.9	**14.4**
11 PAZ2	18.9	43.2	27.0	25.4	21.4	36.3	16.3	17.9	42.7	35.1	**28.4**
12 PR56	43.1	82.4	54.9	69.0	60.9	37.5	51.8	45.2	65.6	50.4	**56.1**
13 PSC8	0.0	0.0	3.9	7.8	0.0	0.0	0.0	0.0	2.0	6.0	**2.0**
14 PST5	4.0	11.8	0.0	9.6	2.1	4.0	3.9	0.0	2.0	2.2	**4.0**
15 PSU7	2.0	18.0	7.9	16.7	11.9	19.3	13.6	0.0	5.9	1.9	**9.7**
16 PSS2	11.9	14.1	3.8	6.3	5.9	7.9	10.0	2.0	11.9	3.9	**7.8**
Mean	**23.0**	**45.0**	**31.7**	**38.9**	**27.7**	**33.6**	**30.2**	**25.5**	**33.8**	**30.3**	**32.0**

Table 3. F-values for percentage of Sclerotinia attack.

	Mean % attack	F treatment	F genotype	F isolate	F interaction
16 sunflower lines	32.0	20.89**	200.03**	14.19**	1.43 ns
11 sunflower lines	44.0	15.23**	143.46**	11.73**	1.33 ns

The latency indices for the eight sunflower lines which showed some infection with all isolates and a mean of at least 20% attack are presented in Table 4. The data for the most resistant lines are not presented in this table either because it was impossible to calculate a latency index because there were no plants with symptoms or because the index would depend on the dates of symptom appearance on less than five plants/plot.

Table 4. Latency index for 16 sunflower lines infected with ascospores of 10 *Sclerotinia sclerotiorum* isolates.

Isolates Inbred lines	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
1 CP73	1.31	1.05	1.11	1.02	1.11	1.09	1.09	1.26	1.02	0.90	**1.10**
3 FU	1.41	1.20	1.48	1.47	1.48	1.52	1.49	1.49	1.30	1.63	**1.45**
4 GB	1.16	0.79	0.96	0.82	0.96	0.91	0.93	1.04	0.87	0.87	**0.93**
5 GH	1.41	1.18	1.32	1.24	1.20	1.26	1.25	1.26	1.28	1.35	**1.28**
6 GU	1.08	0.75	0.89	0.83	0.93	0.92	1.11	1.07	0.87	1.01	**0.95**
7 SD	1.68	1.55	1.58	1.59	1.63	1.60	1.61	1.64	1.50	1.69	**1.61**
11 PAZ2	1.72	1.71	1.64	1.81	1.82	1.77	1.80	1.65	1.74	1.73	**1.74**
12 PR56	1.45	1.06	1.08	1.11	1.19	1.07	1.28	1.42	1.16	1.22	**1.20**
Mean	**1.40**	**1.16**	**1.26**	**1.24**	**1.29**	**1.27**	**1.32**	**1.35**	**1.22**	**1.30**	**1.28**

This rather reduced the range of indices since, when calculated on the plants that did show symptoms, the line PSU7 had a mean latency index of 1.87. However, the second longest mean index was for PAZ2 (1.74) which was retained since 28% of its plants showed symptoms. For the eight lines with at least 25% attack, a factorial analysis of latency indices gave the same results as that for percent attack: highly significant genotype and isolate effects and no sunflower genotype/ *S. sclerotiorum* isolate interaction (Table 5).

Table 5. F-values for eight lines with at least 25% attack.

	Mean % attack	F treatment	F genotype	F isolate	F interaction
8 sunflower lines	56.8	15.63**	156.93**	6.70**	1.20 ns

There was no significant correlation between mycelium growth rate of the 10 isolates and their mean % attack. Isolate 4, which showed rapid growth was one of the most aggressive isolates on sunflower capitula (mean 38.9%, second in order after isolate 2), but isolate 3 which showed the slowest in vitro growth was intermediate in % attack (31.7%, fifth in order). The mean latency indices of the isolates, based on the 8 sunflower lines presented were not significantly correlated with mycelium growth rate but there was some relation with sclerotia production (latency index - number of sclerotia: $r = 0.60$, $p = 0.07$; latency index - weight of 1 sclerotia: $r = -0.63$, $p = 0.05$). It would appear that the isolates which produce the largest sclerotia are the more aggressive.

Discussion

The mean level of attack by ascospores was lower than that observed in recent years at Clermont-Ferrand (Bert et al., 1992, 1993). In 2003, temperatures in August were very high, up to 25C at night and 40C by day, which is not very favourable for *S. sclerotiorum* growth. However, the irrigation provided meant that the plants did not mature early and the results were extremely similar to those obtained by Castaño et al. (1993) with the same level of attack, the mean for the 5 common lines being 34.6% in 1991 and 35.8% in 2003. The data for the 5 lines showed a correlation coefficient of r = 0.966 between the results of the two years, the most susceptible line being CP73, 51.9% in 1991, 63.6% in 2003, and the most resistant being PSC8 0% in 1991, 2.0% in 2003.

The main conclusion of this study is that the reaction of sunflower genotypes does not vary according to *S. sclerotiorum* isolate, there being no genotype/isolate interaction effect for percentage attack (16 inbred lines) and/or for latency index (8 intermediate or rather susceptible lines). Thus, breeding and disease resistance tests with any isolate or population of *S. sclerotiorum* should be valid for all areas and many years. There are differences of aggressiveness between isolates, but since when ascospores are produced for the ascospore test, it is not possible to know whether the weather conditions during the incubation period will be favourable, this factor cannot be used to decide which isolate to use. A mixture of spores from different origins is probably always the safest to ensure useable results; especially since *S. sclerotiorum* ascospores can be kept at -20C for several years (Tourvieille de Labrouhe, 1988).

The absence of interactions but the existence of considerable host genotype and pathogen isolate effects means that resistance of sunflower to *S. sclerotiorum* can truly be classed as "horizontal" and "race non-specific." The problem at present is that progress in resistance breeding is very slow and, under conditions favourable for the pathogen, there can be economic losses due to the disease for many commercial varieties (Masirevic and Gulya, 1992). Many breeders are now looking for large increases in level of resistance by transferring genes from other *Helianthus* species or even from other crops (Scelonge et al., 2000). However, if a comparison is made with other host/pathogen combinations where both rather low levels of quantitative resistance and higher levels of more qualitative resistance are available (for example *Leptosphaeria maculans*/rapeseed [Brun et al., 2001]), the use of the latter, although easier, has not proved durable. To obtain durable resistance effective throughout the regions of the world where sunflowers are grown, a better solution might be to concentrate on combining the many resistance QTLs already identified, by marker assisted selection, and on completing these by addition of other similar factors probably present in cultivated sunflower genotypes not studied so far in detail (Bert et al., 2003). It is not yet possible to say what the maximum level of horizontal resistance of sunflower to *S. sclerotiorum* could be.

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