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The inheritance and biochemistry of resistance to *Sclerotinia sclerotiorum* leaf infections in sunflower (*Helianthus annuus* L.)

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Summary

Resistance of sunflower leaves to attack by *Sclerotinia sclerotiorum* was studied by infecting them, in the field, with agar disks containing *Sclerotinia* mycelium. Resistance levels were determined by the length of lesions after a given period. There were significant differences in reaction between both sunflower hybrids and inbred lines. Different *Sclerotinia* isolates gave the same classification of sunflower genotypes. The results of trials repeated in one year or different years were significantly correlated. The general combining ability variance/specific combining ability variance ratio was 1.35. Strict sense heritability was 0.61 ± 0.03. The midparent-offspring correlation coefficient was significant, but the relation between *per se* values of the male parents used with the values of their hybrids was much closer than that for the female parents. Mean heterosis for resistance was 28.9%, compared with the midparent. The results of the leaf resistance test are frequently correlated with levels of resistance to root attack by *Sclerotinia.* Possible use of this test in breeding both directly for leaf resistance and indirectly for root resistance are discussed. H.P.L. Chromatography studies of the phenols present in healthy and infected leaves distinguished 19 compounds, all of the inhibitin type. There was a large increase in phenol content in leaves infected by *Sclerotinia,* for all genotypes. However, more especially in uninfected leaves, the contents of 3 chromatogram peaks, numbered 4, 6 and 9, showed a close relation with levels of *Sclerotinia* resistance. It is proposed that these compounds could be used as markers of certain types of resistance.

Introduction

Attacks by *Sclerotinia sclerotiorum* on adult leaves of sunflower are often confused with those starting on terminal buds. They can be distinguished by the date of symptom appearance in that those resulting from adult leaf attack do not spread to the petioles and main stem before flowering. Although such attacks occur relatively late in the season, they can cause economic yield losses when environmental conditions are favourable for *Sclerotinia.* If the parasite is able to spread from the leaves to the stem, seed filling will be halted and lodging may occur.

There have been few studies on this form of attack. Péres et al. (1989) described the epidemiology and proposed some methods of cultural contol. Chemicals with curative effects are not available and although preventive measures are possible (A. Péres, personal communication), this type of control is not practical, since it requires specialized machinery to cover sunflowers at flowering and

methods of forecasting leaf attack have not been developed. Thus, it appeared of importance to determine both the genetic variability in resistance levels available among cultivated sunflowers and those heridity parameters which would help to define the most efficient resistance breeding methods.

Earlier studies of the resistance of sunflower organs other than leaves to *Sclerotinia sclerotiorum* attack (Robert et al., 1987; Vear & Tourvieille, 1988; Tourvieille & Vear, 1990) showed that this resistance is polygenic and predominantly under the control of genes with additive effects. The authors concluded that it was possible to select for resistance during early generations of breeding programmes.

The different forms of resistance generally appear independent (Tourvieille & Vear, 1990), but Castaño et al. (1989) found a significant correlation between levels of resistance to infection of roots and those of adult leaves. They also proposed that it might be possible to use a leaf resistance test on the same plants as, for example, a capitulum test. These observations suggested that more detailed studies of a leaf resistance test would be of practical interest.

In parallel with studies of the heredity of *Sclerotinia* resistance, research has been undertaken into its possible biochemical basis. Phenolic compounds have been implicated in processes determining sunflower resistance to attacks by *Sclerotinia sclerotiorum* on stem bases (Bazzalo et al., 1985, 1987), capitula (Hémery et al., 1987) and stems, leaves, petioles and hypocotyls of young plants (Avila, 1984; Yang, 1986). Hémery-Tardin & Tourvieille (1990) suggested that it might be possible to use phenolic compounds as markers in resistance breeding programmes.

This paper presents results concerning the heredity of sunflower resistance to *Sclerotinia* leaf infections, the variability in phenolic metabolism between different sunflower genotypes and the response of this metabolism to parasitic infection.

Materials and methods

Sunflower genotypes

These were chosen to represent as wide a range of reactions to *Sclerotinia* as possible, and were largely the same as those used in earlier studies by Robert et al. (1987), Vear & Tourvieille (1988), Castaño et al. (1989) and Tourvieille & Vear (1990).

(a) Heredity studies (1990):
Six female lines: B11A3, 62, SD, GH, GU, F10
Four male lines: RHA266, V135, PAC1, PRS5

Twenty-two out of the possible 24 hybrids from the factorial cross of these parental lines (B11A3 * V135 and 62 * V135 were not available).

(b) Test correlation studies (1989 and 1990):
Five of the above parental lines, together with additional inbred genotypes were tested over two years:

1989 – 14 inbred lines: 62, SD, GH, RHA266, PAC1 + PRS7, PRS2, PSC4, CX, PSC8, CC40, SN, CP73, SP.

1990 – 19 inbred lines: The 14 lines used in 1989, with the exception of CP73 + HA61, CANP3, CERN.5.1, RE, 2603, UD.

(c) Biochemical studies (1989):
Three hybrids, involving 6 of the same parental lines as above: SD * PAC1, GH * RHA266, F10 * V135 and two additional commercial hybrids: BOLERO, characterised by a high level of capitulum resistance to *Sclerotinia* and NSH15, characterised by its resistance to Phomopsis (*Diaporthe helianthi*).

Sclerotinia isolates

In order to determine differences in aggressivity between isolates and to check for possible host-parasite interactions, the hybrids used for biochemical studies were infected with 5 isolates of *Sclerotinia:*

S. sclerotiorum : SS1
SS10
SS20
S. minor : SMR
S. trifoliorum : ST61.01 (not parasitic on sunflower)

For the other studies, one *S. sclerotiorum* isolate, SS26, was used in 1989 and another, SS29 in 1990.

Leaf infection method

The *Sclerotinia* test on leaves was adapted from that described by Bertrand & Tourvieille (1987) for determining sunflower resistance to Phomopsis. On each plant, a young fully grown leaf was infected. A *Sclerotinia* explant, 0.5 cm in diameter, obtained from the edge of a mycelial culture on 1% malt-agar medium, was placed on the extremity of the main vein, with the mycelium in contact with the upper surface of the lamina. The explant and leaf extremity were enclosed in aluminium foil, stapled in place, in order to avoid drying of the inoculum. Sprinkler irrigation at the rate of 5 mm per day was provided until observation of symptoms. Plant reaction was measured by the length of the brown rotted zone along the main leaf vein, typical of natural *Sclerotinia* attacks.

For the heredity and test correlation studies, 8 plants of each genotype were infected when the flower bud measured between 2 and 5 cm. In the present study no control (malt agar and aluminium foil without fungus) was used because earlier studies with such controls had never shown any symptom development (Bertrand, 1985; M.C. Thuault, personal communication). Symptom appearance was rapid and lesion lengths were measured after 5 days. For the biochemical studies, 15 plants of each genotype were infected with each of the 5 *Sclerotinia* isolates at the beginning of flowering. Delay in symptom appearance was such that the measurements of lesions were made after 2 weeks.

Biochemical analyses

After measurement, the diseased areas on 5 leaves, chosen at random among those infected, were removed and the healthy parts of the lamina frozen in liquid nitrogen, ground and stored at − 18° C until extraction. Dry matter content was determined for each sample. An alcoholic extraction (MeOH/EtOH: 1/1) at 70° C for twice 30 minutes was carried out in the presence of an antioxidant. The extract obtained after filtration was evaporated to dryness under vacuum at 40° C. The residue was dissolved in 5 ml MeOH and stored at − 18° C.

The extracts were analysed by High Performance Liquid Chromatography (H.P.L.C.). Maximal separation and visualisation of a wide range of phenolic compounds were obtained using a reverse phase C18 nucleosil column with a 70 min gradient of acetonitrile in water in the presence of 2% acetic acid, and U.V. detection at 280 nm. Each chromatogram was compared with a control made up of a mixture of the same quantities of all the samples studied. The peaks identified, each corresponding to a single compound or series of related compounds, were numbered and their heights measured to obtain estimates of the contents of each compound or group of compounds in the leaf extracts. A correction factor according to dilution and extract dry matter content was applied in order to obtain data on which semi-quantitative analyses could be made.

Statistical analyses

One way and factorial analyses of variance were made to determine genotype, parental and interaction effects. The values for the two missing hybrids were obtained using the missing data formula proposed by Federer (1955). These values were used to calculate mean parental effects. The relative importance of additivity in genetic control was estimated from the ratio:

$$\frac{\text{general combining ability variance } (\sigma^2\text{G.C.A.})}{\text{specific combining ability variance } (\sigma^2\text{S.C.A.})}$$

This was calculated using the method of Robert et al. (1987), adapted from Falconer (1964).:

$$\frac{\sigma^2 G.C.A.}{\sigma^2 S.C.A.} = \left[\frac{(\text{female mean square} - \text{interaction mean square})}{\text{nb. rep.} \times \text{nb.males}} + \frac{(\text{male mean square} - \text{interaction mean square})}{\text{nb. rep.} \times \text{nb. females}}\right] / \frac{(\text{interaction mean square} - \text{error mean square})}{\text{nb. rep.}}$$

Narrow sense heritability was calculated from the offspring-midparent regression (Falconer, 1964):

$$h^2 = b = {}^1/_2\, \sigma_a^2 \,/\, {}^1/_2\, \sigma_p^2$$

where $\sigma^2 a$ is the additive variance and $\sigma^2 p$, the phenotypic variance measured on the parents. An estimation of the precision of the heritability coefficient was calculated by the formula of Falconer (1964):

$$\sigma^2 h^2 = \sigma^2 b = 1 + (n-1)t/nN$$

where t = the phenotypic correlation between the midparent and offspring results, n = number of plants per genotype and N = number of pairs used to calculate the regression.

Table 1. Mean lesion lengths on the leaves of 5 sunflower hybrids infected with 5 *Sclerotinia* isolates. Results are means of 15 plants (in cm)

Isolates	SS1	SS10	SS20	SMR	ST61.01	Mean
Hybrids						
SD * PAC1	4.13	3.00	2.53	2.63	3.20	3.09
BOLERO	4.93	3.13	4.17	3.39	3.77	3.88
F10 * V135	4.97	4.11	4.60	2.83	3.93	4.09
NSH15	5.93	5.37	5.57	2.63	4.33	4.77
GH * RHA266	8.10	5.50	4.63	3.93	5.89	5.89
Mean	5.61	4.22	4.30	3.08	4.50	4.34

F hybrids = 22.18** highly significant ($P < 0.01$)
F isolates = 16.32** highly significant ($P < 0.01$)
F hybrids*isolates = 2.41** highly significant ($P < 0.01$)
lsd = 1.39
SS = *Sclerotinia sclerotiorum*
SMR = *Sclerotinia minor*
ST = *Sclerotinia trifoliorum*

For each hybrid a centered value, Vc, was calculated from the difference between the observed value and the general mean (μ). The general combining abilities for male and female lines (G.C.A.m. and G.C.A.f) were obtained by subtracting μ from the mean value of the hybrids with the line as parent. The specific combining ability for each hybrid was obtained by the formula:

$$S.C.A.fm = Vc - G.C.A.f - G.C.A.m.$$

Heterosis of resistance was calculated for each hybrid compared with the *per se* mean parent and better parent values, using the formula of Hallauer & Miranda (1981).

Results

The level of successful infection with the leaf test exceeded 90%: the results presented involve only plants which showed *Sclerotinia* symptoms.

1. Genotypic differences in reaction to the leaf test

The results for the 5 hybrids used in biochemical studies, tested at flowering, are given in Table 1. They confirm that differences in aggressivity exist between *Sclerotinia* isolates, but that there are no significant inversions in the relative reactions of

Table 2. Hybrids and mean parental reactions to *Sclerotinia* leaf infections (mean in cm of 8 plants)

Females	1) B11A3	2) 62	3) SD	4) GH	5) GU	6) F10	Mean
Males							
1) RHA 266	3.67	5.50	1.86	4.13	8.17	5.00	4.72
2) V135	(3.29)	(3.49)	3.50	3.62	3.82	4.29	3.68
3) PAC1	2.25	2.57	2.43	2.57	2.38	2.83	2.51
4) PRS5	3.43	4.63	3.29	4.38	5.88	4.29	4.32
Mean	3.16	4.05	2.77	3.68	5.07	4.10	3.81

(. . .) = estimated values
F hybrids = 12.00** highly significant ($P < 0.01$)
lsd = 1.17
CV = 30.7%

sunflower genotypes to these isolates. The 3 *S. sclerotiorum* isolates and *S. trifoliorum* gave the same classification. *S. minor* grew weakly, with no significant difference between hybrids. The results and discussion following utilize data from all the isolates used. There are significant differences in the lengths of *Sclerotinia* lesions between genotypes: SD * PAC1 appears the most resistant, F10 * V135 and BOLERO intermediate and GH * RHA266 and NSH15 the most susceptible. There is thus no apparent relation between leaf reaction to *Sclerotinia* and Phomopsis.

Table 2 shows the results for the hybrids from the factorial cross and mean parental effects. The hybrids differ highly significantly, with SD * RHA266 the most resistant (mean lesion length 1.86 cm) and GU * RHA266 the most susceptible (8.17 cm). Lesion lengths measured after 5 days were similar to those measured 15 days after infection with the 5 different *Sclerotinia* isolates. The results of SD * PAC1 and F10 * V135 are comparable in the two tests but GH * RHA266 appears relatively less susceptible when infected with SS29.

The mean *per se* values for the parental inbred lines of the factorial cross are given in the left-hand column of Table 3, showing highly significant differences. The inbreds SD and PAC1 show the smallest lesions (2.25 and 2.63 cm respectively), F10 and RHA266 the longest (7.57 and 7.86 cm respectively). The *per se* values of individual inbred lines were, with the exception of SD and GU, always greater than those of the means of their hybrids, (Table 2) by an average of 1.58 cm.

Table 3 also presents the results of the additional series of inbred lines studied in 1989 and 1990. The mean lesion length was greater in 1989, probably due to the difference in *Sclerotinia* isolate. Correlations between the results of the different test series are the following:

5 genotypes:

heredity studies 1990 – inbreds 1990 $r = 0.90^*$

heredity studies 1990 – inbreds 1989 $r = 0.94^*$

inbreds 1989 – inbreds 1990 $r = 0.95^*$

13 genotypes:

inbreds 1989 – inbreds 1990 $r = 0.72^{**}$

(* = significant, $P < 0.05$; ** = highly significant, $P < 0.01$)

The relative reactions of sunflower genotypes to the leaf test thus show good repeatability within and between years, irrespective of *Sclerotinia* isolate.

2. *Heredity parameters*

2.1. Additivity. Because of the absence of 2 hybrids from the factorial cross, 2 factorial analyses were made (Table 4). Both show highly significant male,

Table 3. Reactions of inbred lines to *Sclerotinia* leaf (LI) and root infections (RI)

Infection-Year	LI-90	LI-89	LI-90'	RI-90
Genotypes				
62	6.57	11.20	5.17	81.3
SD	2.25	5.50	2.56	45.0
GH	6.00	11.80	7.25	67.6
RHA 266	7.86	11.30	6.78	67.8
PAC1	2.63	4.40	2.67	63.3
B11A3	4.13			
GU	4.50			
F10	7.57			
V135	5.38			
PRS5	7.00			
PRS7		10.00	3.80	90.0
PRS2		7.50	4.56	61.5
PSC4		6.60	3.25	52.9
PSC8		5.30	3.00	75.7
CX		12.30	8.00	90.0
CC40		9.90	2.88	67.5
SN		11.20	3.17	80.8
SP		7.60	4.60	81.3
CP73		14.00	–	90.0
HA61			3.40	57.5
CANP3			4.80	90.0
CERN51			2.30	55.3
RE			2.33	55.4
2603			8.88	90.0
UD			1.86	58.8

Heredity Studies

LI-90 = Lesion lengths in cm. Mean of 8 plants. lsd = 1.51

Test Correlation Studies

LI-89 = Lesion lengths in cm. Mean of 10 plants. lsd = 1.76 (Castaño et al. 1989)

LI-90' = Lesion lengths in cm. Mean of 10 plants. lsd = 1.55

RI-90 = Arcsinus transformed percentage of plants attacked at the base of the stem. Mean of 2 blocks. lsd = 22.9

female and interaction effects, but with male effects always greater than those of female parents. The mean G.C.A. variance/S.C.A. variance ratio, at 1.35, indicates that additive gene control is preponderant, but that dominance interactions are far from negligible.

2.2. Heritability. The parental variances are not different ($P < 0.001$) so it was possible to calculate a midparent-offspring regression. The regression coefficient, an estimate of narrow sense heritability, was 0.61 ± 0.03.

2.3. Prediction of hybrid behaviour from parental values. Taking all the parental lines, the regression of mean parental effects in hybrids on *per se* values was not significant, but the significant correlation coefficient ($r = 0.72$, $P < 0.05$) indicates that the two series of results are associated. When male and female parents are studied separately, the regression for the former is highly significant, with a correlation coefficient of $r = 0.99$ ($P < 0.001$). The classification of resistance level, with PAC1 the best and RHA266 the poorest, is exactly the same whether *per se* values or combining abilities are considered. In contrast, for the females, there is no significant relation between the two series of values. Although SD appears the most resistant, both as an inbred line and in its hybrids, 62, GH and F10 give hybrids with relatively better resistance than those of their *per se* values.

2.4. General and Specific combining Ability. The left hand part of Table 5 presents calculations of general and specific combining ability (G.C.A. and S.C.A.). It should be noted that negative values for G.C.A. indicate lesions smaller than the trial mean. For S.C.A., negative values indicate that the hybrid showed shorter necrotic spots than those predicted from the G.C.A. of parents.

The importance of additive effects is confirmed, for 9 of the best 12 hybrids have either SD or PAC1, the lines with the best G.C.A., as a parent, whereas the hybrids in the lower half of the table are combinations of the more susceptible parents.

Specific combining ability, the deviation of the results observed for a hybrid from those predicted from its parental G.C.A., varies from negligible (SD * PRS5 : 0.01) to 2.19 for GU * RHA266. The significant correlation ($r = 0.58$, $P < 0.05$) between centered hybrid values (Vc in Table 5) and the S.C.A. indicates that significant interactions between parental genotypes are involved in the determination of resistance levels of hybrids.

2.5 Heterosis. The right hand half of Table 5 presents calculations of heterosis, the difference between the observed value of a hybrid and the *per se* value of its parental inbred lines. Negative values indicate heterosis for resistance. For the 22 hybrids studied, mean heterosis is 28.9%, compared with the midparent and 4% compared with the better parent. The Spearman rank correlation ($r_s = 0.69$) between the two types of heterosis for each of the hybrids is highly significant, indicating that there is no notable difference according to whether one or both parents are taken into consideration.

However, according to hybrid genotype, there are considerable variations in levels of heterosis, from 63.2% more resistance (SD * RHA266) to 32.2% more susceptibility (GU * RHA266), compared with there midparent. There is a significant correlation ($r = 0.45$, $P < 0.05$) between level of resistance (Vc in Table 5) and level of heterosis,

Table 4. Factorial analysis of the hybrids reactions

Females+ (f)	males+ (m)	F (f)	F (m)	F (fm)	σ^2GCA/σ^2SCA
1 2 3 4 5 6	1 3 4	24.49**	65.38**	9.31**	1.86
3 4 5 6	1 2 3 4	29.32**	31.07**	12.00**	0.84
Mean					1.35

+ The identification of the female inbreds numbered 1 to 6, and of male inbreds, 1 to 4, is given in Table 2
** = Highly significant ($P < 0.01$)

such that the most resistant hybrids show the greatest levels of heterosis.

It may also be noted that the levels of specific combining ability and heterosis are significantly correlated (S.C.A. – midparent heterosis: $r = 0.67$; S.C.A. – better parent heterosis: $r = 0.48$, $P < 0.05$).

3. *Analysis of phenolic compounds*

Table 6 gives the concentrations of each of the 19 phenolic compounds or series of closely related compounds separated by H.P.L.C. for each of the 5 sunflower genotypes from both control and infected leaves. Many of the compounds vary little between genotypes, but a certain number of peaks permit clear distinction of certain hybrids.

3.1 Uninfected leaves. Peaks 4, 6, 7, 8, 9, and 13 distinguish SD * PAC1 from the 4 other hybrids. With the exception of peak 8, these compounds are present in SD * PAC1 at at least twice the concentration found in the other hybrids. F10 * V135 and BOLERO are distinguished from GH *RHA266 and NSH15 by their contents of peaks 7, 8 and 9. There thus appear to be 3 groups of sunflower genotypes: (1) SD * PAC1, (2) F10 * V135 and BOLERO, distinguished only by their peak 8 con-

Table 5. General and specific combining abilities, and heterosis for resistance to *Sclerotinia* leaf infections

Hybrids	Vc	GCAf	GCAm	SCA	Heterosis	
					MP(%)	BP(%)
SD * RHA266	− 1.95	− 1.04	0.91	− 1.82	− 63.2	− 17.3
B11A3 * PAC1	− 1.56	− 0.65	− 1.30	0.39	− 33.4	− 14.4
GU * PAC1	− 1.43	1.26	− 1.30	− 1.39	− 33.2	− 9.5
SD * PAC1	− 1.38	− 1.04	− 1.30	0.96	− 0.4	8.0
GH * PAC1	− 1.24	− 0.13	− 1.30	0.19	− 40.4	− 2.3
62 * PAC1	− 1.24	0.24	− 1.30	− 0.18	− 44.1	− 2.3
F10 * PAC1	− 0.98	0.29	− 1.30	0.03	− 44.5	7.6
SD * PRS5	− 0.52	− 1.04	0.51	0.01	− 28.9	46.2
B11A3 * PRS5	− 0.38	− 0.65	0.51	− 0.24	− 38.4	− 16.9
SD * V135	− 0.31	− 1.04	− 0.13	0.86	− 8.3	55.6
GH * V135	− 0.19	− 0.13	− 0.13	0.07	− 36.4	− 32.7
B11A3 * RHA266	− 0.14	− 0.65	0.91	− 0.40	− 38.8	− 11.1
GU * V135	0.05	1.26	− 0.13	− 1.08	− 21.9	− 14.2
GH * RHA266	0.32	− 0.13	0.91	− 0.46	− 40.4	− 31.2
F10 * V135	0.48	0.29	− 0.13	0.32	− 33.7	− 20.3
F10 * PRS5	0.48	0.29	0.51	− 0.32	− 41.1	− 38.7
GH * PRS5	0.57	− 0.13	0.51	0.19	− 32.6	− 27.0
62 * PRS5	0.82	0.24	0.51	0.07	− 31.8	− 29.5
F10 * RHA266	1.19	0.29	0.91	− 0.01	− 35.2	− 33.9
62 * RHA266	1.69	0.24	0.91	0.54	− 23.8	− 16.3
GU * PRS5	2.07	1.26	0.51	0.30	2.3	30.7
GU * RHA266	4.36	1.26	0.91	2.19	32.2	81.6
Mean					− 28.9	− 4.0

Vc = centered value = hybrid value – general mean
GCAf and GCAm = general combining abilities for females and males = mean parental value-general mean
SCA = specific combining ability = Vc – GCAf – GCAm
MP(%) = percent heterosis relative to mid parent = [(F1-MP)/MP]*100
BP(%) = percent heterosis relative to better parent = [(F1-BP)/BP]*100

Table 6. Concentrations (eq μg gallic acid) of each of the phenolic compounds from both control (C) and *Sclerotinia* infected (I) sunflower leaves

Hybrids	SD * PAC1		BOLERO		F10 * V135		NSH15		GH * RHA266	
	C	I	C	I	C	I	C	I	C	I
Peaks										
1	0.58	0.79	0.48	0.72	0.52	0.69	0.55	0.79	0.66	0.65
2	0.57	0.55	0.28	0.73	0.33	0.46	0.38	0.53	0.42	0.63
3	0.52	1.38	0.38	0.91	0.51	0.91	0.33	0.56	0.26	0.52
4	1.31	1.89	0.51	1.35	0.64	1.43	0.33	0.81	0.23	0.55
5	0.38	0.58	0.22	0.61	0.28	0.54	0.23	0.49	0.24	0.48
6	1.00	1.43	0.48	1.23	0.53	1.05	0.29	0.43	0.27	0.40
7	3.09	1.92	1.26	2.41	1.37	1.88	2.12	2.05	2.41	2.55
8	3.22	4.56	2.52	3.89	1.51	3.14	4.77	7.60	4.48	5.02
9	4.68	6.47	1.42	4.66	1.89	5.11	0.47	1.48	0.35	1.08
10	1.31	2.14	1.26	2.55	1.16	2.18	1.88	3.27	1.28	1.91
11	0.80	1.13	1.18	1.98	1.12	1.40	2.44	1.79	1.14	1.35
12	0.93	1.03	0.66	1.53	0.61	1.00	0.77	1.10	0.89	1.00
13	2.18	2.12	0.43	2.47	0.52	1.47	0.91	2.12	0.90	2.08
14	0.88	0.85	0.97	1.36	0.61	0.90	0.53	0.77	0.89	0.95
15	0.04	0.47	0.17	0.49	0.04	0.29	0.15	0.82	0.17	0.48
16	0.97	2.97	1.09	2.52	0.54	2.21	0.46	2.16	1.09	1.88
17	0.22	0.66	0.34	0.92	0.25	0.80	0.17	0.67	0.24	0.62
18	0.09	0.37	0.08	0.26	0.13	0.59	0.14	0.78	0.27	0.62
19	0.54	1.41	0.89	2.49	0.62	2.37	0.44	2.08	0.31	1.15

tents, and (3) GH * RHA266 and NSH15, distinguished only by peak 11.

Peaks 4, 6 and 9 show the closest relation with the 3 levels of *Sclerotinia* resistance found among the hybrids (Table 1), each having 3 concentration levels (Table 6). Peak 9 is generally the most important.

3.2 Infected leaves. Analyses made on leaves that had been infected with *Sclerotinia* show, in general, a considerable increase in phenolic content (Table 6), but new compounds of the phytoalexin type do not appear.

Of the compounds apparently correlated with *Sclerotinia* reaction, peak 9 was still present in greatest quantity. The amount in individual genotypes remained well correlated with *Sclerotinia* reaction (SD * PAC1 : 6.47 μg; F10 * V135 : 5.11 μg;

Table 7. Correlation between sunflower genotype reactions for the *Sclerotinia* leaf resistance test and those for tests on other organs

		Heredity Studies				Test Correlation Studies	
		10 inbreds	6 females	4 males	20 hybrids	14 inbreds(+)	19 inbreds
Capitulum	(*mycelium*)	0.08	−0.03	0.21	0.12	0.63*	0.64**
	(*ascospores*)	−0.53	−0.89*	0.32	−0.25	−0.23	−0.54
	(*semi-natural*)	0.45	0.65	0.87	0.32	–	–
Roots	(*sclerotia*)	−0.09	−0.61	0.77	0.27	0.81**	0.59**

(+) = Castaño et al. (1989)
* = Significant ($P < 0.05$)
** = Highly significant ($P < 0.01$)

BOLERO : 4.66 μg; NSH15 : 1.48 μg; GH *RHA266 : 1.08 μg). This type of compound could thus be a good marker of *Sclerotinia* resistance. It may be noted that the relative increase of this compound between control and infected plants is not directly related either to its content in control leaves or to the apparent level of *Sclerotinia* resistance: SD * PAC1 showed an increase of 138%, F10 * V135, 328% and GH * RHA266, 270%.

4. Relations between response to the leaf test and to other Sclerotinia *resistance tests*

Table 7 presents correlations between the results of the leaf test and other resistance tests applied previously on the genotypes studied in this article. The previously unpublished results of the root resistance test applied on the 1990 series of inbred lines are given in Table 3 (RI-90). Three series of reactions to the leaf test are correlated significantly with those of tests on capitula. Two of the closest correlations are those with the reactions to the root test on the two series of inbred lines.

Discussion

In comparison with other tests for resistance to *Sclerotinia* in sunflowers (Vear & Guillaumin, 1977; Tourvieille & Vear, 1984), the leaf test applied at the 2 cm flower bud stage appears relatively simple. The short latency period of 5 days and the high successful infection rate make it possible to obtain results in homogeneous environmental conditions on individuals or small numbers of plants. It appears that any reasonably aggressive *Sclerotinia* isolate can be used. The test is non-destructive and, since results are obtained before flowering, it can be used in breeding programmes where only the most resistant plants are retained for selfing or cross-pollination. Precision is moderate (coefficient of variation = 27–30%) but good distinction is obtained between genotypes and repeatability is generally satisfactory.

The sunflower genotypes used for this study were chosen to represent the known range of capitulum resistance to *Sclerotinia*. Nevertheless, the overall variability observed with the leaf test was similar to that for the other tests (Robert et al., 1987; Vear & Tourvieille, 1988; Tourvieille & Vear, 1990), with significant differences between inbred lines and between hybrids.

As with the other resistance tests, a factorial analysis showed significant parental and interaction effects. Control by additive genes appears preponderant, to the same extent as effect as for resistance to the root test and the mycelium test on capitula (σ^2G.C.A./σ^2S.C.A. between 1 and 2), but less so than the ascospore test on capitula (3.76). The general parent-progeny correlation ($r = 0.72$) is at the same level as that for the other tests. However, the special feature of the leaf test is the correlation coefficient of $r = 0.99$ for the male parents and the absence of any significant correlation for the female parents. The quite high level of heritability of resistance expressed by the leaf test, at 61%, may be explained by the wide range of genotypic reactions and the rapid response which means that environmental factors intervene very little.

The part of hybrid reactions not predictable from G.C.A. was calculated as specific combining ability and heterosis. Since the first uses parental effects in hybrids and the second *per se* values of inbred lines, the significant correlation between the two confirms the relation between the resistance of inbred lines and that of their hybrids.

There appeared a mean heterosis for resistance of 28.9%. This is specific to the leaf test for *Sclerotinia* resistance; with the root and mycelium on capitula tests, the inbred lines appear more resistant then their hybrids and with the ascospore test, the hybrids have values close to those of the midparent. However, this heterosis appears specific to certain genotypes, varying from − 63.2% for SD * RHA266 to + 32.2% for GU * RHA266. The same hybrids show large S.C.A. Whereas 3 of the 4 male parents show a maximum of 60% variation between their hybrids (Table 2), RHA266 is parent of hybrids with more than 120% variation. SD * RHA266 is the most resistant hybrid and GU * RHA266, the most susceptible. RHA266 appears to increase the effects of normally additive genes in

the inbred lines SD (highly resistant) and GU (highly susceptible). Inclusion in the factorial analysis of hybrids with RHA 266 as male parent certainly reduced the significance of female general combining abilities. Ladsous et al. (1991) found that RHA266 showed the greatest S.C.A. for *Botrytis cinerea* resistance and this line is also known for its phenotypic variability (branching, flowering date). Thus, it may be suggested that it contains genes whose effects depend on interactions with other genes or with the environment. This could be an explanation for the varying results of the hybrid GH * RHA266 between the two series of tests to which it was subjected.

With this exception, resistance to *Sclerotinia* appears sufficiently heritable to be selected in early generations and inbred lines. It is thus possible to breed for resistance to *Sclerotinia* attack on adult leaves. However, the special interest of this leaf resistance test resides in the fact that it can be applied simultaneously with another test on one plant (Castaño et al., 1989) and in the significant correlations found between its results and those of other tests. The studies carried out up to the present show a large degree of independence between the reactions of sunflower genotypes to the other resistance tests, especially mycelium and ascospore tests on capitula (Vear & Tourvieille, 1988) and root and capitulum tests (Tourvieille & Vear, 1990).

However, some genotypes appear either generally resistant (SD, PAC1) or generally susceptible (GH, GU, V135). The results reported here indicate a certain correspondence between capitulum and leaf resistances. Of even more interest is the close correlation, for two series of genotypes, with the results of the *Sclerotinia* test on roots (Table 7). The root test is destructive, requires a large number of plants and contaminates the soil with *Sclerotinia*. Its use is therefore rather limited and the possibility of indirect selection through the leaf test would be of great interest. Even if the latter takes into account only some root resistance factors, it could help to assemble them with those giving resistance to other forms of attack in sunflower genotypes.

The correlations between the results of the leaf test and those on other organs could be due to resistance mechanisms that are active against several forms of attack. The more or less rapid extension of *Sclerotinia* mycelium along a leaf being quite a simple character taking place on a fully active organ, adult leaves provide material very suitable for study of such resistance mechanisms. The results presented here show very clearly that part of the defence mechanisms of leaves to *Sclerotinia* infection is related to their phenolic metabolism, and that the compounds involved are of the inhibitin type (present before infection). In contrast to Avila (1984) and Yang (1986), no phytoalexin type compounds were observed.

These results are comparable with those of Hémery et al. (1987) on capitula: the defence mechanism of both organs against *Sclerotinia* infection involves stimulation of phenolic metabolism. The metabolic differences between sunflower genotypes resistant and susceptible to the leaf test are specific to certain compounds (peak 9) and are quantitative. These differences occur in uninfected plants and persist after infection although to a reduced extent. This molecule, or group of molecules, could act not only as a marker of resistance but also may be actively involved in the resistance process. It appears that it is its content in healthy plants which determines the level of resistance to *Sclerotinia*. Infection always results in a large increase in overall phenolic content, but only where the initial concentration of the active compound in the healthy leaf is high, does it reach a final post infection level sufficient to limit fungal growth.

In conclusion, measurements of sunflower resistance to *Sclerotinia* as expressed by the test involving mycelium infection of leaves appear of fundamental interest, permitting studies of defence mechanisms and being also of practical utility in breeding. Further studies are necessary to improve the inter-relation between these two aspects; in the long term, the aim should be to determine the relative importance of 'general' and 'specific' resistances to *Sclerotinia* attack of the different organs of the sunflower plant in order to use them most efficiently in breeding programmes.

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References

Avila, F.J., 1984. The role of phenolic compounds in the resistance of sunflower to *Sclerotinia sclerotiorum*. Ph.D. Thesis, Iowa State University, Ames, USA., 108 p.

Bazzalo, M.E., E.M. Heber, M.A. Del Pero & O.H. Caso, 1985. Phenolic compounds in stems of sunflower plants inoculated with *Sclerotinia sclerotiorum* and then inhibitory effects on the fungus. Phytopathol. Z. 112: 322–332.

Bazzalo, M.E., E.M. Heber & O.H. Caso, 1987. Factores físicos y localización anatómica de compuestos fenólicos en relación con la tolerencia de los tallos de girasol (*Helianthus annuus*) frente a *Sclerotinia sclerotiorum*, causal de la podredumbre basal. Bull Soc Argentina Bot. 25: 197–212.

Bertrand, F., 1986. Mise au point de méthodes de contamination artificielle pour sélectionner les tournesols tolérants à *Diaporthe helianthi*. Mémoire de Fin d'Etudes, DEA. Université de Paris XI, 84 p.

Bertrand, F. & D. Tourvieille, 1987. Phomopsis sur tournesol: tests de sélection. Inf. Tech. CETIOM 98: 12–18.

Castaño, F., F. Vear & D. Tourvieille, 1989. L'utilisation de plusieurs tests simultanés dans la sélection pour la résistance du tournesol vis-à-vis de *Sclerotinia sclerotiorum*. Inf. Tech. CETIOM 107: 14–20.

Federer, W., 1955. Experimental design. Theory and Application. NW, The Mac Millan Co., 594 p.

Falconer, D., 1964. Introduction to quantitative genetics. Edinburgh and London, Oliver and Boyd Ltd., 366 p.

Hallauer, A. & J. Miranda, 1981. Quantitative genetics in maize breeding. Iowa State University Press, Ames, USA., 470 p.

Hémery, M.C., D. Tourvieille, M. Jay & F. Vear, 1987. Recherche de marqueurs phénoliques impliqués dans la résistance du tournesol au *Sclerotinia*. Inf. Tech. CETIOM 101: 20–29.

Hémery-Tardin, M.C. & D. Tourvieille, 1990. Biochimie de la résistance d'*Helianthus annuus* à *Sclerotinia sclerotiorum*. Le marquage phénolique: outil de sélection? (résumé). 2ème Congrés de la S.F.P., Montpellier 28–29 novembre, Supplément Bull. Soc. Française Phytopathol.

Ladsous, O., F. Vear & D. Tourvieille, 1991. Heredity of resistance to *Botrytis cinerea* in sunflowers (*Helianthus annuus*) measured by artificial infections. Plant Breeding 107: 235–241.

Péres, A., Y. Regnault & L.M. Allard, 1989. *Sclerotinia sclerotiorum:* incidence de cinq facteurs agronomiques sur l'intensité des attaques sur tournesol. Inf. Tech. CETIOM 107: 7–13.

Robert, N., F. Vear & D. Tourvieille, 1987. L'héréditéde la résistance à *Sclerotinia sclerotiorum* (Lib) de Bary chez le tournesol. I. Etude des réactions à deux tests mycéliens. Agronomie 7: 423–429.

Tourvieille, D. & F. Vear, 1984. Comparaison de méthodes d'estimation de la résistance du tournesol à *Sclerotinia sclerotiorum* (Lib.) de Bary. Agronomie 4: 517–525.

Tourvieille, D. & F. Vear, 1990. Heredity of resistance to *Sclerotinia sclerotiorum* in sunflowers. III. Study of reactions to artificial infections of roots and cotyledons. Agronomie 10: 323–330.

Vear, F. & J.J. Guillaumin, 1977. Etude de méthodes d'inoculation du tournesol par *Sclerotinia sclerotiorum* et application à la sélection. Ann. Amélior. Plantes 27: 523–537.

Vear, F. & D. Tourvieille, 1988. Heredity of resistance to *Sclerotinia sclerotiorum* in sunflowers. II. Study of capitulum resistance to natural and artificial ascospore infections. Agronomie 8: 503–508.

Yang, Z., 1986. Induced antifungal compounds in *Helianthus annuus* L. Ph. D. Thesis, Iowa State University, Ames, USA., 75 p.