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EFFECT OF INFECTION BY *Sclerotinia* spp. ON THE PHENOLIC METABOLISM OF SUNFLOWER CAPITULA AND LEAVES

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SUMMARY

Sclerotinia spp. can attack most parts of the sunflower plant, including capitula and leaves. The different levels of partial resistance shown by a range of hybrids were determined by the rate of growth of *Sclerotinia* mycelium on plant parts. Even though *Sclerotinia* isolates varied greatly in agressivity, a similar ranking of the sunflower genotypes according to their resistance to mycelial growth was obtained with all isolates. HPLC analysis of soluble phenolic compounds present in healthy or infected capitula and leaves showed the presence of 25 different compounds in capitula and 19 in leaves. Total phenol content in leaves was much greater than that in capitula; both varied considerably between genotypes. In the two tissues, *Sclerotinia* infection stimulated the accumulation of existing phenolic compounds to different extents according to genotype. Relations between resistance level and phenol accumulation were tissue specific. In capitula, total phenol content was correlated with resistance, whereas in leaves, the best marker of resistance was the amount of compound 9. It is suggested that phenolic compounds in healthy sunflower plants could be used as markers of *Sclerotinia* resistance.

Key words: ***Helianthus*, *Sclerotinia*, phenols, resistance, HPLC**

INTRODUCTION

Under favorable climatic conditions, sunflower (*Helianthus annuus* L.) yields may be considerably reduced following attacks by *Sclerotinia* spp. (head or stalk rot). It has been suggested that phenolic compounds are involved in resistance to *Sclerotinia* spp. Accumulation of phenolic polymers such as melanin (Bhaskaran and Kandaswamy, 1978; Bazzalo *et al.*,1987) or lignin (Bazzalo *et al.*, 1987) rein-

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force plant cell walls, limiting fungal penetration. In addition, impregnation of cell walls by esterified phenolic compounds increases resistance by modifying the cell wall properties, which is no longer recognized by the depolymerizing enzymes produced by the fungus (Bazzalo *et al.*, 1985).

Sunflowers produce soluble phenolic compounds when the stem base (Bazzalo *et al.*, 1985,1987), stems, leaves, petioles or hypocotyls (Avila, 1984; Yang, 1986) are infected by *S. sclerotiorum* (Lib.) de Bary. Some of these compounds are strongly fungistatic. Bazzalo *et al.* (1985) showed that isochlorogenic acid is particularly active in limiting fungal extension. Avila (1984) and Yang (1986) demonstrated the fungistatic activity of compounds produced by sunflowers after infection with *S. sclerotiorum* which was shown on spores of *Cladosporium herbarum* Link. and of *C. cucumerinum* Ellis & Arth.

The two interesting features of phenolic compounds are that they are produced in greater quantities by resistant sunflower genotypes (Bazzalo *et al.*, 1987) and that they have the ability to limit *S. sclerotiorum* growth (Bazzalo *et al.*, 1985). Thus, they could be considered as potential markers of resistance in sunflower breeding programs.

This paper is devoted to a study of phenolic compounds in sunflower capitula and leaves before and after infection by *Sclerotinia* spp. The aim was to determine whether specific phenolic compounds could be used as markers of resistance to different forms of *Sclerotinia* attack. Capitulum attack was studied because it can cause most severe yield losses and leaf attack because there has recently been a considerable increase in this form of the disease in France.

MATERIALS AND METHODS

Fungal isolates

Five isolates were used : *S. sclerotiorum*: SS1, SS10, SS20; *S. minor* Jagger: (SMR) and *S. trifoliorum* Eriksson: ST61.01 (non-pathogenic on sunflowers). SS1 and SS10 were isolated from sunflowers in 1969 and 1984, respectively, SS20 from carnations in 1982, SMR from lettuce in 1983 (P.Davet, INRA, Montpellier, France) and ST61.01 from red clover in 1976 (G.Raynal, INRA, Grignon, France). These isolates are maintained in a mycological collection at INRA, Clermont-Ferrand at 12°C. Fungal growth started again 5 or 6 days after inoculation of fresh medium. The fungus was then maintained in a regular growth phase by transfer to fresh medium (1% malt agar) in Petri dishes every 3 days at 22±1°C.

Sunflower genotypes

Five sunflower genotypes, displaying large differences in *S. sclerotiorum* resistance level, were studied: two are experimental INRA hybrids (Clermont-Ferrand):

SD*PAC1 and GH*RHA266; three are old commercial varieties: AIRELLE, (nuclear male sterility), BOLERO and NSH15 (cytoplasmic male sterility).

Sclerotinia infection methods

a) **Capitula** (Vear and Guillaumin, 1977). Capitula with 30 cm of stem were harvested at the beginning of maturity (capitular bracts brown, capitulum yellow). Three mycelial explants 10 mm in diameter taken from the edge of a 3-day old culture were placed on the dorsal surface of the capitulum, and fixed in place with adhesive tape. The capitulum stems were soaked in a water bath. Humidity was maintained at 100% and temperature at 18°C. The lesions which developed on the dorsal surface of capitula around the mycelial explants were measured 3 days after the infection. Six capitula were tested for each genotype/isolate combination.

b) **Leaves.** The infection method was adapted from the leaf test described by Bertrand and Tourvieille (1987) to evaluate sunflower resistance to phomopsis (*Diaporthe helianthi*). It was carried out at the beginning of flowering. Mycelial explants 5mm in diameter cut from the edge of a 3-day-old culture were placed at the extremity of the main veins of young, but fully grown leaves, and covered with aluminium foil to prevent drying. After 15 days, the lengths of lesions along the main veins were measured. This test was carried out on one leaf for each of 15 plants for each genotype/isolate combination.

Sample harvest

Three days after infection, the infected tissues of capitula were removed under running water. An apparently healthy cylinder of tissue from around the lesion (about 2 cm in diameter) was cut out with a scalpel, deep frozen in liquid nitrogen and kept at -18°C until extraction. In addition to the 6 capitula infected for each host-pathogen combination, six healthy capitula of each sunflower genotype were sampled. Each series of 6 capitula was pooled and then divided into 2 sub-samples permitting two types of biochemical analyses for each treatment.

Infected leaves were sampled after measurement of lesion length; the petioles and lesions were eliminated, leaving only the apparently healthy laminas, which were frozen in liquid nitrogen, ground and then kept at -18°C until extraction. Samples were made up of one leaf from each of 3 healthy plants of each genotype or of 5 leaves chosen at random among the 15 infected for each host-pathogen combination.

Biochemical analyses

The dry matter content was determined for each sample. The samples were finely ground in liquid nitrogen. An alcoholic extraction (methanol/ethanol, 1/1, v/v) at 70°C for twice 30 min was carried out in the presence of an antioxidant (2% sodium metabisulphite in water) (Biolley *et al.*, 1994). Double extraction in cold and hot solutions was made to confirm the stability of phenolic compounds after

extraction at a high temperature. The extract obtained after filtration was evaporated to dryness under vacuum at 40°C. The residue was dissolved in 5 ml methanol and stored at -18°C.

The extracts were analyzed by HPLC using a reverse phase C18 nucleosil column. Phenolic compounds were eluted by a 70 min linear gradient consisting of 80% acetonitrile in water, in the presence of 2% acetic acid. Detection was at 280 nm since phenolic acids have an absorbance peak in this spectrum region, and because of the low content of the extracts in flavonoids (maximum absorbance at 340 nm). To determine the relative content of each of the phenolic compounds, a known quantity (0. 879 g) of gallic acid was added to each sample (Andersen and Pedersen, 1983); the gallic acid is used as a standard, for it does not interfere with the phenolic content of leaves or capitula, and it is the first peak which appears on the chromatogram.

Each chromatogram was compared with a sample made up of a mixture of the same quantities of all the samples studied. Each peak identified in this chromatogram was numbered according to its retention time. By comparison, the peaks in each sample were also identified. Each peak may represent one compound or a series of related compounds; it will be considered here that each peak represented one type of phenolic molecule, as determined with Diode Array Detection (scanned between 240 and 400 nm). Estimates of the content of each compound were obtained from peak height. A correction factor according to dilution, extract dry matter content and the estimated weight of gallic acid in the extract was applied in order to obtain data expressed in equivalents of μg gallic acid g^{-1} of dry matter.

Principal Component Analysis (PCA)

This multivariate method of analysis essentially descriptive using quantitative variables (Philippeau, 1986), was used to determine the range of compounds (variables) associated to the different hots-pathogen combinations. The software was STAT-ITCF.

RESULTS

Morphological responses of sunflower tissues to *Sclerotinia* infections

All the *Sclerotinia* infections induced lesions. The results are shown in Table 1. For both the capitula and leaf tests, SD*PAC1 and BOLERO displayed the smallest lesions, whereas GH*RHA266 always showed the largest. The reaction of AIRELLE varied according to test. This genotype showed a relatively high degree of resistance

to the leaf test but was very susceptible to capitulum infection. NSH15 was subjected only to the leaf test, to which it showed considerable susceptibility.

Table 1: Reaction of sunflower genotypes to leaf and capitulum infections with *Sclerotinia* mycelium

Hybrid	SD*PAC1	BOLERO	AIRELLE	NSH15	GH*RHA266	LSD
Leaves (L)	3.09	> 3.88	~ 4.09	> 4.77	> 5.89	0.62
Capitula (C)	2.57	~2.43	> 3.72	ND	3.68	0.36

(L): mean length of foliar lesions in cm, calculated from 15 plants for each of five host/pathogen combinations per genotype.
(C): mean diameter of capitula lesions in cm, calculated from 6 plants, with 3 measurements per plant for each of five host/pathogen combinations per genotype.
>: genotypes significantly different. P = 0.05
~: genotypes non-significantly different. P = 0.05
ND: resistance level not determined.

Table 2 shows that for both types of infection, *Sclerotinia* spp. isolates differ in aggressivity. However, the ranking of sunflower hybrids according to their resistance level was not significantly affected by the difference in aggressivity of the isolates. The isolates SS20 and ST61.01 (non-pathogenic) were only weakly aggressive on capitula and SMR grew only very slowly on leaves. In contrast, the most aggressive isolate on capitula was SS10 and on leaves both SS1 and ST61.01 grew quite rapidly.

Table 2: Mean lesion size in cm resulting from mycelial infections of *Sclerotinia* spp. isolates on the capitula (mean lesion diameter of 6 plants) and leaves (mean lesion diameter of 6 plants) and leaves (mean lesion length of 15 plants) of 5 sunflower hybrids

	CAPITULA					LEAVES				
Isolate / Hybrid	SS1	SS10	SS20	SMR	ST61.01	SS1	SS10	SS20	SMR	ST61.01
SD*PAC1	2.90	4.20	1.15	3.10	1.50	4.13	3.00	2.53	2.63	3.20
BOLERO	2.60	3.25	1.60	3.10	1.60	4.93	3.13	4.17	3.39	3.77
AIRELLE	4.80	5.60	2.00	4.10	2.10	4.97	4.11	4.60	2.83	3.93
GH*RHA266	4.90	5.30	2.50	3.50	2.20	8.10	5.50	4.63	3.93	7.29
NSH15	ND	ND	ND	ND	ND	5.93	5.37	5.57	2.63	4.33
	LSD = 0.83 P = 0.05					LSD = 1.39 P = 0.05				

ND = resistance level not determined.

Qualitative and quantitative variation of phenolic content in healthy or infected sunflowers

The quantitative data of 25 phenolic compounds (coded A to Y) in capitula and of 19 compounds (coded 1 to 19) in leaves were measured before and after *Sclerotinia* infections. No correspondance can be established between the phenolic compounds in capitula and in leaves. Table 3 shows the relative amount of the 25 compounds in the capitula of the four genotypes studied, before and after infection.

Table 4 shows equivalent data for the 19 compounds detected in leaves of the five genotypes studied.

Table 3: Mean concentrations (in equivalents of µg gallic acid g^{-1} dry matter) of 25 phenolic compounds (A to Y) in healthy (H) sunflower capitula and in capitula artificially infected with *Sclerotinia* spp. (I)

Hybrid	SD*PAC1		BOLERO		AIRELLE		GH*RHA266	
	H	I*	H*	I**	H*	I**	H*	I**
Peak								
A	0.00	0.10	0.00	0.24	0.00	0.04	0.00	0.06
B	0.12	0.09	0.20	0.55	0.09	0.40	0.11	0.09
C	0.09	0.19	0.02	0.13	0.03	0.27	0.00	0.05
D	0.06	0.07	0.02	0.12	0.04	0.23	0.04	0.06
E	0.09	0.29	0.07	0.55	0.01	0.03	0.00	0.04
F	0.01	0.08	0.01	0.16	0.02	0.26	0.03	0.07
G	0.03	0.10	0.05	0.41	0.00	0.26	0.02	0.06
H	0.02	0.11	0.00	0.27	0.00	0.14	0.00	0.05
I	0.11	0.12	0.05	0.13	0.00	0.06	0.07	0.11
J	0.16	0.93	0.03	1.35	0.00	0.20	0.00	0.11
K	0.08	0.23	0.00	0.31	0.00	0.37	0.00	0.06
L	0.08	0.24	0.04	0.42	0.00	0.11	0.03	0.09
M	0.00	0.26	0.00	0.19	0.00	0.08	0.00	0.01
N	0.00	0.25	0.00	0.51	0.00	0.32	0.00	0.05
O	0.00	0.04	0.00	0.39	0.00	0.12	0.00	0.08
P	0.00	0.06	0.00	0.10	0.00	0.03	0.00	0.00
Q	0.10	0.23	0.00	0.46	0.00	0.04	0.00	0.08
R	0.03	0.11	0.04	0.39	0.00	0.17	0.00	0.07
S	0.06	0.08	0.03	0.52	0.00	0.01	0.00	0.02
T	0.08	0.17	0.00	0.30	0.06	0.09	0.04	0.06
U	0.04	0.92	0.00	0.83	0.00	0.45	0.00	0.20
V	0.00	0.12	0.00	0.32	0.00	0.04	0.00	0.04
W	0.73	1.20	0.05	1.51	0.12	0.70	0.00	0.38
X	0.09	0.31	0.02	0.71	0.00	0.17	0.00	0.10
Y	0.45	0.90	0.03	0.63	0.00	0.34	0.00	0.20
Total content ***	2.43	7.20	0.66	11.50	0.37	4.93	0.34	2.14

* : mean calculated from biochemical analyses on 2 replicates from a pool of 6 healthy capitula per genotype.

** : mean calculated from biochemical analyses on 2 replicates from a pool of 6 capitula per host/pathogen combination.

*** : total content being the sum of the average values of each compound.

In healthy plants

The total phenol content of healthy capitula was very low, whereas in leaves it was 10 (SD*PAC1) to 50 fold (GH*RHA266) higher. Analysis of individual compounds showed a qualitative genotypic specificity both in capitula and in leaves. The compounds B, D, F, G, I, L and T were detected in GH*RHA266, B, C, D, F, T and W in AIRELLE, and a larger number of compounds in BOLERO (14) and SD*PAC1 (18).

In leaves, compounds 4, 6, 7, 8, 9 and 13 distinguished SD*PAC1 from the four other hybrids. Except for compound 8, all other compounds were present in SD*PAC1 in greater amount (>2x) in comparison with the other genotypes. The content of compounds 7, 8 and 9 distinguished BOLERO and AIRELLE from GH*RHA266 and NSH15. The genotypes can, as a result, be divided into 3 groups:

(a) SD*PAC1, with high concentrations of compounds 7, 8 and 9; (b) BOLERO and AIRELLE, with intermediate concentrations of these compounds and (c) GH*RHA266 and NSH15, in which compound 8 is largely predominant.

Table 4: Mean concentrations (in equivalents of µg gallic acid g^{-1} dry matter) of 19 phenolic compounds (1 to 19) in healthy (H) sunflower leaves and in leaves artificially infected with *Sclerotinia* spp. (I)

Hybrid	SD*PAC1		BOLERO		AIRELLE		GH*RHA266		NSH15	
	H*	I*	H*	I**	H*	I**	H*	I**	H*	I**
Peak										
1	0.48	0.66	0.40	0.57	0.43	0.54	0.54	0.48	0.51	0.71
2	0.46	0.37	0.23	0.63	0.27	0.29	0.34	0.59	0.38	0.47
3	0.42	1.04	0.32	0.60	0.42	0.47	0.21	0.28	0.36	0.45
4	0.80	1.69	0.42	1.04	0.52	0.98	0.19	0.34	0.28	0.69
5	0.32	0.46	0.18	0.50	0.22	0.36	0.19	0.30	0.26	0.42
6	0.82	1.20	0.39	1.01	0.43	0.70	0.22	0.28	0.26	0.39
7	2.53	1.27	1.03	2.37	1.12	1.15	1.97	2.87	1.81	1.63
8	2.64	3.50	2.06	3.10	1.23	2.02	3.67	4.56	4.47	7.18
9	3.83	5.34	1.16	3.43	1.55	3.46	0.28	0.42	0.42	1.08
10	1.07	1.93	1.03	2.50	0.95	1.69	1.05	1.58	1.85	2.85
11	0.66	0.79	0.97	1.74	0.92	0.95	0.93	0.98	2.43	1.49
12	0.76	0.79	0.55	1.36	0.50	0.64	0.73	0.83	0.90	1.07
13	1.78	1.22	0.35	2.29	0.42	0.85	0.74	2.15	1.03	2.00
14	0.72	0.62	0.80	1.34	0.50	0.63	0.73	0.83	0.49	0.82
15	0.03	0.39	0.14	0.38	0.03	0.18	0.15	0.32	0.18	0.68
16	0.80	2.36	0.89	2.07	0.44	1.44	0.90	1.23	0.41	1.89
17	0.18	0.56	0.28	0.74	0.20	0.51	0.19	0.37	0.24	0.55
18	0.07	0.33	0.07	0.25	0.10	0.41	0.22	0.46	0.13	0.67
19	0.44	1.10	0.73	2.19	0.51	1.74	0.26	0.70	0.41	1.76
Total content ***	18.81	25.62	12.00	28.14	10.76	19.01	13.51	19.57	16.82	26.80

* : mean calculated from biochemical analyses on 3 replicates per genotype.
** : mean calculated from biochemical analyses on 5 replicates per host/pathogen combination.
*** : total content being the sum of the average values of each compound.

In *Sclerotinia* infected plants

Infection strongly stimulated phenolic metabolism, in both capitula and leaves. The 25 compounds identified in capitula could be detected in all the genotypes after infection. The total phenol contents of capitula were highest for BOLERO and SD*PAC1; although they increased considerably, they remained much lower for AIRELLE and GH*RHA266. In all genotypes, the compounds most notably stimulated were U, W, and Y SD*PAC1 and BOLERO could be distinguished from the two other genotypes by their strong stimulation of a fourth compound, coded J.

The total amount of phenolic compounds in infected leaves of SD*PAC1, BOLERO and NSH15 was higher than in leaves of GH*RHA266 and AIRELLE. Genotypic specificity appeared for the content of compounds 8 and 9. There was a larger amount of compound 8 in NSH15 (7.18 µg) and GH*RHA266 (4.56 µg), then in SD*PAC1 (3.50 µg), BOLERO (3.10 µg) and AIRELLE (2,02 µg). The relative level of compound 9 was in contrast greater for SD*PAC1 (5.34 µg) than for AIRELLE

(3.46 µg) and BOLERO (3.43 µg) and the lowest for NSH15 (1.08 µg) and GH*RHA266 (0.42 µg).

Thus, the sunflower genotypes most resistant to *Sclerotinia* infection of capitula, SD*PAC1 and BOLERO, were also those which showed the largest total phenol content after infection. The more susceptible genotypes, GH*RHA266 and AIRELLE, were characterized by small post-infection phenol production.

Table 5: Ranking of sunflower genotypes according to their reactions to leaf infections with *Sclerotinia* spp. and to their phenolic compound contents

	Leaf infection		Phenolic compound content	
Genotype	Lesion length (cm)	Homogeneous group	Compound content (µg/g DW)	Homogeneous group
SD*PAC1	3.10	A	5.35	A
BOLERO	3.88	B	3.34	B
AIRELLE	4.09	B	3.44	B
NSH15	4.77	C	1.10	C
GH*RHA 266	5.89	D	0.42	C
		LSD = 0.62 P = 0.05		LSD = 0.76 P = 0.05

In contrast with these results obtained on capitula, total amounts of phenolic compounds in leaves could not be related to levels of resistance. NSH15 (susceptible) showed a level similar to SD*PAC1 and BOLERO (resistant). In this case, the level of resistance appeared to be more closely related to the quantity of compound 9 after infection, this compound accumulating to its greatest quantity in the most resistant genotypes (Table 5).

Table 6: Total phenolic compound contents in the capitula of 4 sunflower hybrids after artificial infection by 5 *Scleronia* spp. isolates. The contents are expressed as equivalent of µg gallic acid g^{-1} DW

Hybrid	SD*PAC1	BOLERO	AIRELLE	GH*RHA266
Isolate				
SS1	6.05	14.22	6.02	1.16
SS1	7.78	16.33	5.69	1.17
SS10	5.28	12.32	1.48	1.24
SS10	6.15	13.49	3.57	1.21
SS20	8.92	3.13	8.85	0.93
SS20	ND	3.61	2.80	1.12
SMR	8.17	17.82	4.05	3.01
SMR	9.38	17.40	6.69	3.89
ST61.01	5.42	5.21	5.91	3.04
ST61.01	7.05	ND	ND	4.18

ND : not determined.

Table 6 presents the total phenol content in capitula infected with the different *Sclerotinia* spp. isolates. Phenol accumulation in SD*PAC1, AIRELLE and GH*RHA266 did not differ according to *Sclerotinia* isolate. In contrast, BOLERO showed considerable variations. There was a large production when SS1, SS10 or

SMR were used, and much less in the case of SS20 and ST61.01, with levels as low as those seen in the susceptible hybrids, AIRELLE and GH*RHA266. These observations can be related to those of isolate aggressivity, since SS1, SS10 and SMR are much more aggressive on capitula than SS20 and ST61.01.

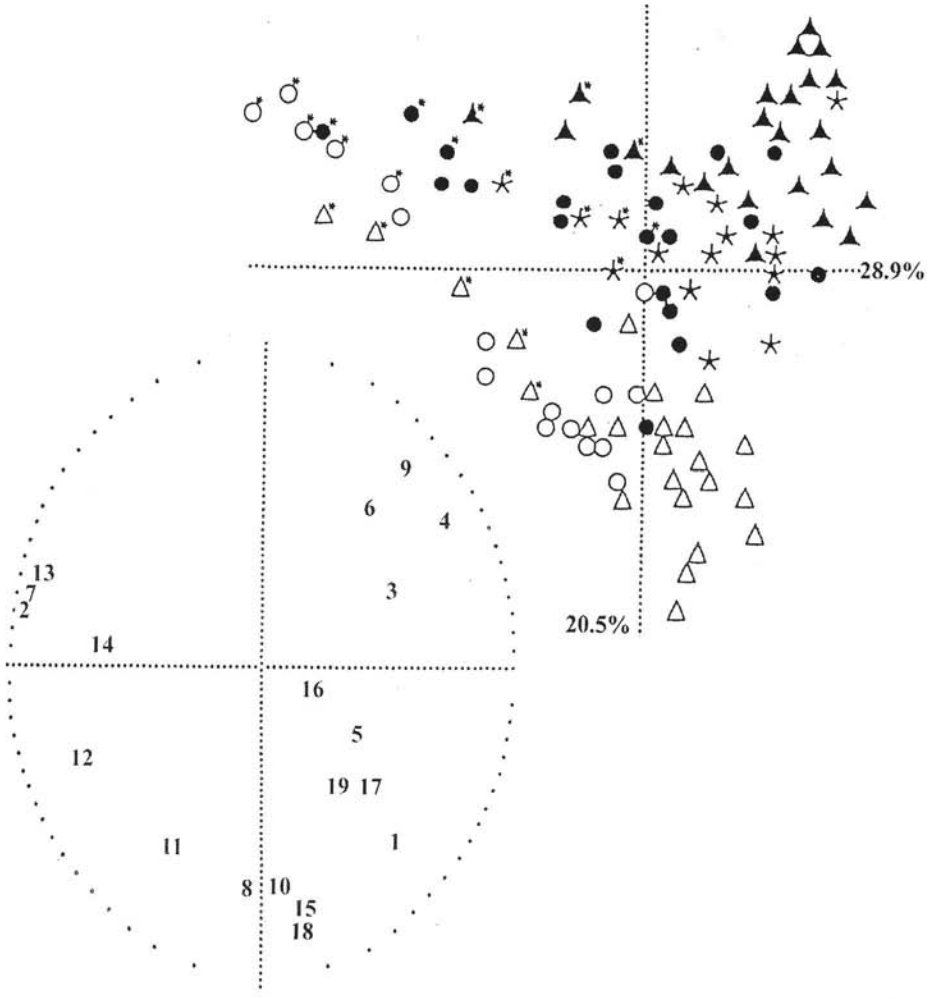

Figure 1: Circle of correlations and factorial plane 1-2 of the P.C.A. carried out on the relative content of phenolic compounds of 5 sunflower genotypes following artificial infections of leaves by Sclerotinia spp.
*O: GH*RHA266, △: NSH15, ●: BOLERO, ▲: SD*PAC1, ★ : AIRELLE, ✱: SS1*

Principal Component Analysis

Figure 1 presents the circle of correlations and the factorial plane 1-2 of the PCA carried out on the relative phenol contents of leaves of the sunflower genotypes infected by the different *Sclerotinia* spp. isolates. The contents in the leaves of each genotype are distinguished but only SS1 is indicated separately by asterisks to avoid overcrowding of the diagram of the isolates used.

The position of the five genotypes on the figure appears to be determined by their compound 9 content, with SD*PAC1, BOLERO and AIRELLE towards the top and GH*RHA266 and NSH15 towards the bottom of the graph. When the genotypes were infected with SS1, the points representing these host/parasite combinations are found towards the lefthand side, and if only these points are considered, it is not possible to distinguish BOLERO from GH*RHA266 or NSH15. Comparison between the factorial plane 1-2 and the circle of correlations shows that compounds 2, 7 and 13 are most important when infections were made with SS1. These observations may be related to the strong aggressivity of SS1 on sunflower leaves.

DISCUSSION

Two of the problems that occur in the breeding of sunflowers for resistance to *Sclerotinia* are that the resistance tests usually employed measure reactions that are specific to each plant part and that it is necessary to choose the best isolate for a particular test. For example, AIRELLE is susceptible to capitulum attack, but reasonably resistant to leaf infection; ST61.01 grows strongly on leaves but very weakly on capitula. Genotypic effects may be dependent on the plant parts which are colonized, while aggressivity is known to vary with duration and conditions of maintenance of isolates in mycological collections. Castaño *et al.* (1992) found that when infected with the isolate SS29, GH*RHA266 was relatively less susceptible than in the present study, and that for all genotypes, SS29 gave average lengths of lesions after 5 days similar to those obtained here after 15 days.

There are a large number of different phenolic compounds in sunflower leaves (19) and capitula (25). Their molecular structures are not known, but detection at 280nm is representative of the absorbance of phenolic acids. It may be suggested that the much higher concentrations of these compounds in leaves compared with capitula may be due to the much more active phenolic metabolism of young but fully grown leaves compared with that of capitula at the beginning of maturation, where there is more oxidation and breakdown of phenolic compounds.

In both leaves and capitula, infection by *Sclerotinia* spp. stimulated the production of inhibitin type phenolic molecules (compounds also present in healthy plants). Avila (1984) and Martinson *et al.* (1988) also described the production of this type of compound in sunflower hypocotyls infected with *S. sclerotiorum*. How-

ever, in contrast with the results of Avila (1984) and Yang (1986), the present study showed no evidence for production of phytoalexins, infection-induced compounds.

In capitula, total phenol content after infection by *Sclerotinia* spp. was higher than in the most resistant genotypes. These results are in good agreement with those of Bazzalo *et al.* (1985) who found a higher phenolic content in the stems of more resistant varieties. In contrast, total phenolic content in leaves does not appear to be related to resistance level. In particular, AIRELLE, resistant to leaf attack, produces a quantity of phenol compounds similar to that of GH*RHA266, which is more susceptible. Thuault and Tourvieille (1988) made the same observation.

The factor related to leaf resistance appears to be the content of compound 9, which appears to be some form of a phenolic acid either of benzoic or cinnamic type. This variable makes it possible to form the same groups as those obtained by resistance tests. However, it is the final content which is important and not the relative increase in compound 9 following infection, for SD*PAC1 shows an increase of 138%, AIRELLE of 328% and GH*RHA266 of 270%. It may be noted that, in infected capitula, the contents of certain individual compounds (J, U, W, Y) discriminate between resistant and susceptible genotypes, but their low concentrations make it necessary to treat these results with care. It is possible that one of these compounds is the same as compound 9 in leaves, although the different behavior of AIRELLE leaves and capitula would be in disagreement with this hypothesis.

Changes in the ratio between levels of compounds 8 and 9 may be involved in the resistance process in leaves, resistant genotypes producing compound 9 and susceptible genotypes, compound 8.

The use of several *Sclerotinia* spp. isolates showed that the biochemical resistance reaction depends not only on the host genotype, but also on the aggressivity of the parasite. However, no different phenolic compounds were stimulated by the different *Sclerotinia* isolates; their aggressivity only had a quantitative effect on phenolic accumulation in plant tissues. This effect was most noticeable for capitula infections and for the hybrid BOLERO, which reacted more to strongly aggressive isolates than to those with weak aggressivity. In leaves, the only clear effect was for SS1, which particularly stimulated the production of compounds 2, 7 and 13. These results indicate that when biochemical measurements are made to determine resistance level, an aggressive isolate must be used, so that it will be sure to stimulate reaction by the plant.

It may be noted that defense reactions involving phenolic compounds have been frequently reported in plants. This synthesis has been shown to be involved in the development of systemic acquired resistance (SAR) (Lamb and Dixon, 1990; Enyedi *et al.*, 1992). In their search for early markers of resistance, Franqueville *et al.* (1990), in particular, showed that phenolic metabolism activation by oil palm clones resistant to *Fusarium* spp. was greater than that in susceptible clones.

CONCLUSION

In conclusion, these results suggest that it may be possible to use phenolic compounds as markers of *Sclerotinia* resistance in sunflower leaves and capitula. Their involvement in resistance mechanisms appears specific according to the plant part which is infected; total content is highest in capitula, compound 9 content in leaves. Taking the hypothesis that compound 9 is an inhibitor of *Sclerotinia* growth, it may be suggested that a low level of resistance is due to a low level of compound 9 in both healthy and infected plants, a moderate resistance results from a high post-infection compound 9 content only, and a high level of resistance can be explained by high levels of both pre- and post-infection compound 9 content.

Changes in the ratio between compounds 8 and 9 may also be important. Compound 8 could be a precursor of compound 9. The susceptible genotypes would lack the enzymes necessary to transform this precursor 8 into the resistance molecule 9. Small-scale measurements of the contents of compound 9 in healthy plants, in comparison with resistance levels, so far show a good correlation between the two factors. Further studies would be of interest, since such measurements would make infections with the pathogen unnecessary.

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REFERENCES

Andersen, J.M., Pedersen, W.B., 1983. Analysis of plant phenolics by high-performance liquid chromatography. J. Chromatogr. 259: 131-139.

Avila, F.J., (1984) The role of phenolic compounds in the resistance of sunflower to *Sclerotinia sclerotiorum*. Creative Component, Univ. Iowa: 108 pp.

Bazzalo, M.E., Heber, E.M., Delpero Martinez, M.A., Caso, O.H., 1985. Phenolic compounds in stems of sunflower plants inoculated with *Sclerotinia sclerotiorum* and their inhibitory effects on this fungus. Phytopathol. Z. 112: 322-332.

Bazzalo, M.E., Heber, E.M., Caso, O.H., 1987. Factores fisicos y localizacion anatomica de compuestos fenolicos en relacion con la tolerencia del tallo del girasol (*Helianthus annuus*) frente a *Sclerotinia sclerotiorum*, causal de la podedumbre basal. Bol. Soc. Bot. Arg. 25: 197-212.

Bertrand, F., Tourvieille, D., 1987. Phomopsis du tournesol : tests de sélection. Inf. Tech. CETIOM 98: 12-18.

Bhaskaran, R., Kandaswamy, T.K., 1978. Phenol metabolism of sunflower leaves as influenced by *Alternaria helianthi*. Z. Pflanzenkr. Pflanzenschutz. 85: 108-112.

Biolley, J.P., Jay, M., Forkmann, G., 1994. Pigmentation patterns of modern rose mutants throw light on the flavonoid pathway in Rosa x hybrida. Phytochem. 36: 1189-1196.

Castaño, F., Hémery-Tardin, M.C., Tourvieille, D., Vear, F., 1992. The inheritance and biochemistry of resistance to *Sclerotinia sclerotiorum* leaf infections in sunflower (*Helianthus annuus*). Euphytica 58: 209-219.

Enyedi, A.J., Yalpani, N., Silverman, P., Raskin. I., 1992. Signal molecules in systemic plant resistance to pathogens and pests. Cell 70: 879-886.
Franqueville, H., Diabates, S., Renard, J.L., 1990. Evaluation du comportement des clones de palmiers à huile vis à vis de la Fusariose vasculaire; Premiers résultats. Soc. Franc. Phytopathol., 2ème Congrès, Montpellier 28-30/11/1992.
Lamb, C.J., Dixon, R.A., 1990. Molecular communication in interactions between plants and microbial pathogens. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41: 439-463.
Martinson, C.A., Avila, F.J., Yang, Z.B., Daugherty, C.J., 1988. Phytoalexin type response of sunflower stems to *Sclerotinia sclerotiorum*. Proc. XII Int. Sunflower Conf. Novi Sad, Yugoslavia, 25-29/7/1988: 582-586.
Philippeau, G., 1986. Comment interpréter les résultats d'une analyse en composants principales. I.T.C.F., Paris.
Thuault, M.C., Tourvieille, D., 1988. A comparative study of the pathogenicity of *Sclerotinia sclerotiorum*, *S.minor* and *S.trifoliorum* on sunflowers. Sclerotinia newsletter 1, 13-14
Vear, F., Guillaumin, J.J., 1977. Etude de méthodes d'inoculation du tournesol par *Sclerotinia sclerotiorum* et application à la sélection. Ann. Amélior. Plantes 27: 523-537.
Yang, Z.B., (1986) Induced antifungal compounds in *Helianthus annuus* L. Master of Science thesis, Univ.Iowa, 75 pp.

REMARKS

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LOS EFECTOS DE LA INFECCIÓN POR *Sclerotinia* spp. EN EL METABÓLISMO FENÓLICO DE LAS HOJAS Y DEL CAPÍTULO DEL GIRASOL

RESUMEN

Sclerotinia spp. puede dañar todos los órganos del girasol (*Helianthus annuus)*, en particular el capítulo y las hojas. Los niveles de resistancia parcial de los híbridos son determinados por pruebas que evaluan el crecimiento del micelium en los órganos concernidos. El poder patógeno de las diferentes cepas de *Sclerotinia spp.* varia pero no modifica el orden de los genótipos del girasol según su nivel de tolerancia frente al patógeno. El análisis en CLAP (HPLC analysis) de los compuestos fenólicos presentes en los órganos sanos e infectados muestra la presencia de 25 compuestos diferentes en los capítulos y 19 en las hojas. La cantidad total de compuestos fenólicos en las hojas es claramente más importante que en los capítulos. En ambos casos, dicha cantidad fluctua considerablemente según los genótipos. En ambos tejidos, la infección por el parásito estímula la producción de los compuestos preexistantes pero de manera variable según los genótipos. Las relaciones entre el nivel de resistancia y la importancia de la producción de compuestos fenólicos son específicas de los tejidos. En el capítulo, la cantidad de compuestos fenólicos totales

está correlacionada positivamente con la resistancia, mientras que en las hojas, no. El mejor marcador de resistancia es la cantidad del compuesto 9 antes de la infección. Se propone que los compuestos fenólicos en las plantas sanas puedan estar usados como marcadores de la resistancia del girasol al *Sclerotinia*.

LES EFFETS DE L'INFECTION PAR *Sclerotinia* spp. SUR LE MÉTABOLISME PHÉNOLIQUE DES FEUILLES ET DU CAPITULE DE TOURNESOL

RÉSUMÉ

Sclerotinia spp. peut attaquer tous les organes du tournesol (*Helianthus annuus*), en particulier le capitule et les feuilles. Les niveaux de résistance partielle des hybrides sont déterminés par des tests mesurant la croissance du mycélium dans les organes concernés. Le pouvoir pathogène des différentes souches de *Sclerotinia* spp. varie, mais ne modifie pas le classement des génotypes de tournesol en fonction de leur degré de tolérance vis-à-vis du pathogène. L'analyse en CLHP des composés phénoliques présents dans les organes sains et infectés, montre la présence de 25 composés différents dans les capitules et 19 dans les feuilles. La quantité totale de composés phénoliques dans les feuilles est nettement plus importante que dans les capitules. Dans les deux cas, cette quantité fluctue considérablement selon les génotypes. Dans les deux tissus, l'infection par le parasite stimule la production des composés préexistants mais de façon variable selon les génotypes. Les relations entre le niveau de résistance et l'importance de la production de composés phénoliques sont spécifiques des tissus. Dans le capitule, la quantité de composés phénoliques totaux est corrélée positivement avec la résistance, contrairement aux feuilles. Le meilleur marqueur de résistance est la quantité du composé 9 avant infection. Il est proposé que des composés phénoliques chez les plantes saines puissent être utilisés comme marqueur de la résistance du tournesol au *Sclerotinia*.