RESEARCH INTO PHENOLIC MARKERS FOR THE RESISTANCE OF SUNFLOWER TO *SCLEROTINIA SPP.*

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SUMMARY

Sclerotinia sclerotiorum is the causal agent of one of the most important cryptogamic diseases of sunflower in France. It is able to infect the capitula, leaves and stems.
The response of the phenolic metabolism of 4 hybrids to 5 isolates of *Sclerotinia* of different aggressivity was analysed by HPLC. This study shows:
1. the lack of response of GH x Rha266 to aggression by the parasite to be reflected by its extremely poor phenolic metabolism which is very similar to that of healthy controls.
2. the richness in both quantity and quality of secondary metabolism in the more resistant hybrids : BOLERO and SD x PAC1 react strongly to parasitic aggression. Phenolic compounds specific to SD x PAC1 appear.
3. the contradictory behaviour of the variety AIRELLE which is susceptible to *Sclerotinia* but has a good quantitative response to infection.
4. the specificity of the response of certain genotypes to different parasites. SD x PAC1 reacts homogenously whatever the isolate of *Sclerotinia* whereas BOLERO is capable of different reactions depending on the isolate.
It is to be stressed that the interpretation of secondary metabolism must be made with caution : the overall phenolic response to aggression is not an absolute marker of resistance ; qualitative aspects should be taken into account in development of this technique in breeding.

INTRODUCTION

In sunflower, resistance to many diseases, including those caused by *Sclerotinia sclerotiorum*, *Diaporthe helianthi*, *Botrytis cinerea* and *Macrophomina phaseoli*, is partial and polygenic (Thuault M.C and Tourvieille D., 1988). Breeding programs for this type of resistance at present require tests and observation methods which are costly both in time and labour. To determine whether it would be possible to simplify selection methods and to obtain a better understanding of resistance, studies of the metabolism directly involved in sunflower pathogen relations were undertaken.
The metabolism of phenols appeared of particular interest, for these compounds have long been known to intervene in plant-pathogen relations (Kosuge T., 1969). Recent works by Bazzalo *et al.* (1985), Avila (1984), Yang (1986), Berger *et al.* (1987), Tal and Robeson (1986), indicate that they are of importance in sunflowers : caffeic acid, the cinnamic derivative : isochlorogenic acid and the coumarins : ayapin and scopolin have been cited as metabolites produced by sunflowers infected with *Macrophomina*, *Sclerotinia* and *Helminthosporium*. The reports by Sanlaville *et al.* (1988), of the existence of more than 20 phenolic molecules in sunflower, related to the genetic diversity of this

species, make it appears reasonable to search for biochemical markers of resistance to various diseases.
This paper presents first results related to the resistance of sunflowers to *Sclerotinia* head rot concerning : 1. the phenolic response of sunflower to parasite infection, 2. biochemical variation between sunflower genotypes, 3. biochemical variation between responses to different *Sclerotinia* isolates.
The possibility for use of quantitative and qualitative aspects of such techniques in breeding programs are discussed.

MATERIAL AND METHODS

A. SUNFLOWER GENOTYPES

SD x PAC1 (SP1) ; BOLERO (BOL) ; AIRELLE (AIR) ; GH x Rha 266 (CR2)
These four hybrids were chosen for their different genetic origins, for their wide range of reaction to *Sclerotinia* and for the similarity of their vegetative cycles.

B. *SCLEROTINIA* ISOLATES

- *S. sclerotiorum* : SS1, SS10 (from sunflower), SS20 (from carnation).
- *S. trifoliorum* : ST61-01 (from clover).
- *S. minor* : SMR (from lettuce).

The two last species were included to compensate for the small variation in aggressivity among the isolates of *S. sclerotiorum* available.

C. RESISTANCE TESTS

1. Observations of natural attack under partly controlled conditions (irrigation during flowering) (Vear and Tourvieille, 1987)
2. Infection with ascospores during flowering : "ascospore test" (Tourvieille and Vear, 1984).
3. Infection of the dorsal surface of maturing capitula with mycelium: "mycelium test" (Vear and Tourvieille, 1985). This test served not only for the measurement of resistance but also to produce samples for biochemical analyses. The mycelial explants are placed on the dorsal side of harvested capitula maintained alive in a growth chamber at 18°C. After 3 days the diseased area is measured and a surrounding ring (2 cm wide) of tissue without symptoms is removed and immediatly fixed in liquid nitrogen. Each sunflower genotype was infected with each of the 5 *Sclerotinia* isolates.

D. BIOCHEMICAL METHODS

Analyses were carried out on uninfected capitula (healthy control), on capitula wounded with liquid nitrogen (physical wound control) and on the rings of symptomless tissue from the infected capitula. All these tissues were extracted with a hot ethanol-methanol mixture (1-1) for 30 mn in the presence of an anti-oxidant. The extract was concentrated under reduced pressure at 40°C and redissolved in methanol to make a total volume of 5 ml. This extract was analysed by HPLC in the following conditions : C_{18} nucleosil column ; solvant A : H_2O/AcOH 50/1 ; solvant B : H_2O/ACN/AcOH 4/40/1 ; gradient of 70 mn and detection at 282 nm.
The chromatographic profiles obtained are translated as peak number (as a fraction of retention time) and height (absorbance).
For each peak, data are expressed as p. cent dry matter (D.M).

RESULTS

A. RESISTANCE TESTS

The results of the 3 observations of *Sclerotinia* reaction are given

TABLE 1 : Reactions of 4 sunflower genotypes to 3 Sclerotinia resistance tests

Genotypes / Tests		SP1	BOL	AIR	CR2
Natural infection	1	9.42	14.48	47.62	51.91
Ascospore test	2	31.00	27.00	22.00	25.00
Mycelium test	3	25.64	24.27	49.49	46.03

1 : percentage of plants attacked
2 : number of days between infection and symptoms appearance
3 : diseased area (cm²) 3 days after inoculation

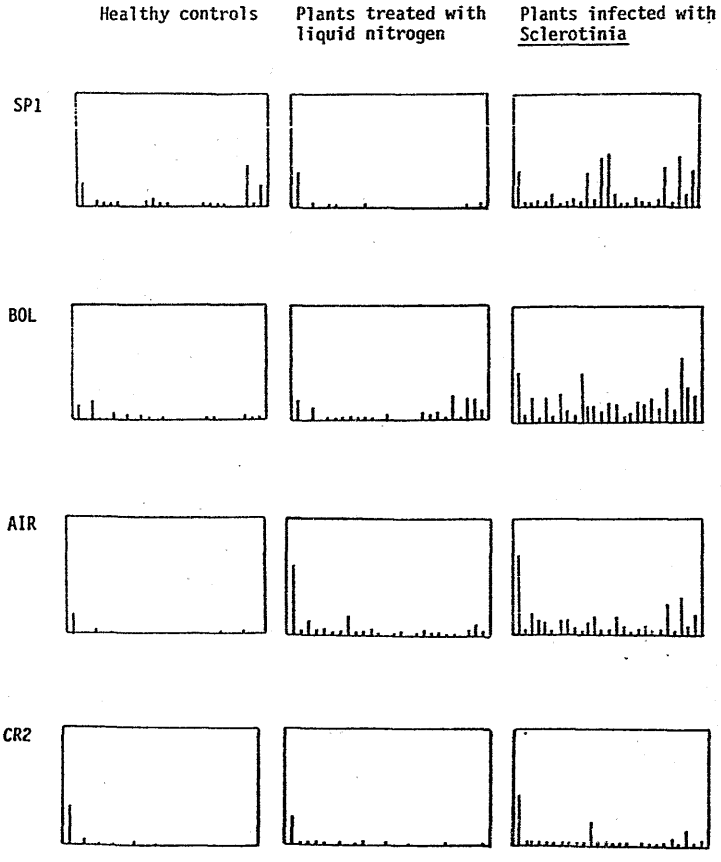

FIGURE 1 : Quantitative and qualitative aspects of phenolic metabolism in 4 sunflower genotypes in 3 experimental conditions

in table 1. They show that SD x PAC1 and BOLERO have higher levels of resistance than AIRELLE and GH x Rha266.

B. THE PHENOLIC RESPONSE OF SUNFLOWERS TO PATHOGEN INFECTION

Figure 1 displays the responses of the 4 genotypes in 3 average chromatograms:
1. control (sample of 6 plants) ; 2. physical wound control (sample of 6 plants) ; 3. infected plant (samples from 30 plants, in groups of 6 plants each infected with one of the 5 isolates)
The distribution and height of the chromatographic peaks attest to the intensity of response to physical wounding or mycelial infection.The healthy controls can be divided into 2 groups according to peak density: SD x PAC1 and BOLERO both contain at least 15 phenolic compounds, whereas AIRELLE and GH x Rha266 produce no more than 7. Responses to wounding with liquid nitrogen are weak, with the exception of BOLERO, in which there is a significant stimulation of certain compounds at the end of profile. Following mycelial infection, with the exception of GH x Rha266, all the genotypes show a dramatic change in their phenolic metabolism, concerning both the number of molecules and their concentrations.

C. PHENOLIC RESPONSES OF SUNFLOWER GENOTYPES TO *SCLEROTINIA* ISOLATES

Figure 2 provides the basis for comparisons : whatever the isolate, GH x Rha266 reacts very little, AIRELLE shows a moderate response to all the 5 isolates with the appearence of peaks spread over the whole chromatographic profile. SD x PAC1 develops a response generally similar to AIRELLE, but with a marked tendancy to production of phenolic compounds appearing at the end of profile. Finally, BOLERO shows the most distinct reaction of the 4 hybrids. It shows little response to SS20 and ST but in reverse a dramatic increase in phenolic metabolism in the presence of SS1, SS10 and SMR. In this case peaks are stimulated throughout the profile.

DISCUSSION

GH x Rha266 : The phenolic behaviour of GH x Rha266 is in complete agreement with its lack of resistance observed in classic test methods. This result is a confirmation of those obtained by Thuault (1986) during a quantitative study of total phenols where GH x Rha266 had a content of 0.11 p. cent, the lowest for the same 4 hybrids studied here (AIR : 0.17 p. cent, BOL : 0,30 p. cent, SP1 : 0.39 p. cent).
AIRELLE : in contrast with GH x Rha266, although AIRELLE shows the same susceptibility to tests and the same level of total phenols, it responds to mycelial infection : there is a strong reaction compared with phenolic metabolism in healthy plants, but it appears to have no effect on pathogen development. Two explanations may be suggested: either it is a question of kinetics, the phenol barrier being erected too slowly or a qualitative aspect is involved, stimulation not producing active antifungic compounds.
SD x PAC1 : this hybrid contains the greatest concentration and variety of phenolic compounds in healthy plants. Mycelial infection causes a moderate response in comparison with the healthy plant, phenols reaching a slightly higher level than that visible with AIRELLE. At present it is not possible to distinguish between quantitative and qualitative effects, but overall it can be said that the phenolic hybrid metabolism of SD x PAC1 is efficient since it is the hybrid with the best *Sclerotinia* resistance.

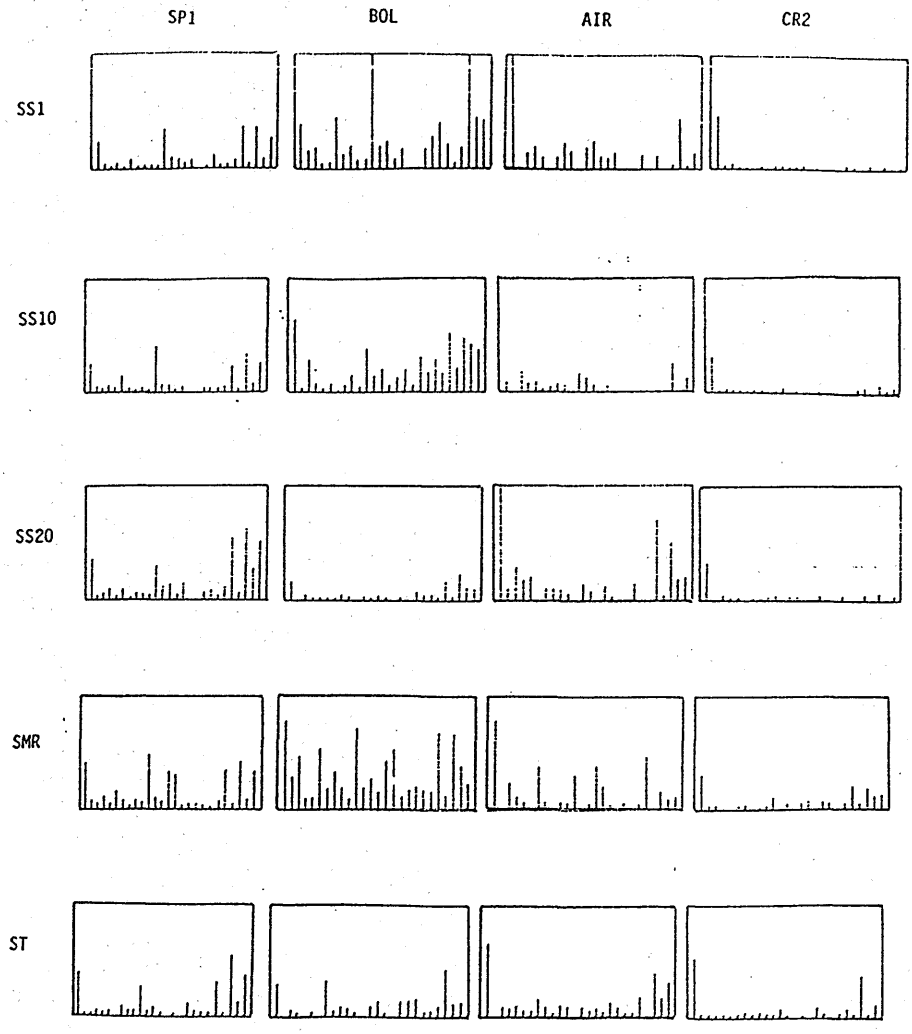

FIGURE 2 : Phenolic response of 4 sunflower genotypes to different Sclerotinia isolates

BOLERO : this hybrid, alone, reacted to physical wounding by liquid nitrogen. In addition, it shows important differences between its response to the isolates SS1, SS10 and SMR, which was dramatic and that to infection by SS20 and ST where there was little effect on its phenolic metabolism. Although at present unexplained, these results may be confronted with those of the mycelium test : the areas of diseased tissue due to SS1, SS10 and SMR were twice the size of those resulting from infections with SS20 and ST (Hemery M.C. *et al.*, 1988)
These preliminary analyses of phenolic compounds provide evidence that, through their phenolic metabolism, sunflowers are able to respond actively to both physical and biological factors in their environment. For each genotype, the response always concerns several molecules and the metabolic equilibrium can vary according to the situation (genotype/isolate, genotype/wound etc...). With further application of this technique it should be possible to obtain a better understanding of disease resistance. The next stages of work include studies of : 1. the best conditions and reproductibility of results, 2. the amplitude of variation among a wide range of sunflower genotypes and their correlation with field observations of *Sclerotinia* reaction, 3. the possibility of distinguishing, among all the peaks of a profile, a few representative markers and 4. the development of a model applicable in breeding for resistance to other diseases of sunflowers, such as *Botrytis*, *Phomopsis* and *Macrophomina*.

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