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Interactions between French isolates of *Phomopsis/Diaporthe helianthi* Munt.-Cvet. et al. and sunflower (*Helianthus annuus* L.) genotypes

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Abstract

Three artificial infection tests measuring the rate of mycelial growth of 7 *Phomopsis/Diaporthe helianthi* isolates were used on leaves, stems and capitula of 6 sunflower hybrids. Isolates and hybrids were chosen to cover the range of variability and resistance levels known at the present time. Significant genotype and isolate effects and isolate × genotype interactions were shown in all the tests, with some changes in order of hybrids according to the isolate used for infection. Consequences of interactions in breeding for stable resistance to *P./D. helianthi* are discussed.

Introduction

Phomopsis stem canker, caused by *Phomopsis/ Diaporthe helianthi* (Muntanola-Cvetkovic et al., 1981), has caused yield losses of up to 40% (1 t/ha) in sunflower (*Helianthus annuus* L.) crops in France (Carré, 1993) since its appearance in 1984 (Lamarque and Perny, 1985). However genetic and chemical control methods developed in the last 12 years have been quite efficient in limiting attacks. Sunflower varieties defined as having low susceptibility (peu sensible: PS) or very low susceptibility (très peu sensible: TPS) by CETIOM (1996) and adapted to the most infected regions are grown and may be complemented by chemical control during the periods most favourable for infection (Penaud and Jouffret, 1996; Lespinas, 1998). Genetic studies have shown that sunflower resistance to Phomopsis stem canker is quantitative, mainly under additive control, but probably less polygenic than in the case of sunflower resistance to *Sclerotinia sclerotiorum* (Vranceanu et al., 1983, 1994; Vear et al., 1997). In breeding programmes, the level of resistance of a genotype is determined either in field trials subjected to natural or semi-natural attack (Vear et al., 1997) or by artificial infections with *P./D. helianthi* mycelium (Bertrand and Tourvieille, 1987). For such programmes to give useful results, it is important to know whether any interactions occur between the responses of sunflower genotypes and either climatic conditions, as suggested by Vranceanu et al. (1983), or fungal isolates. A first suggestion that the second type of interaction could occur was made by Benomar (1995), who observed significant interactions in the reactions of 11 sunflower genotypes when treated with culture filtrates from 11 different *P./D. helianthi* isolates. *Phomopsis/Diaporthe helianthi* is a relatively new species, first identified in Yugoslavia in 1980 (Muntanola-Cvetkovic et al., 1981) and it appears to undergo variation and adaptation. Acimovic and Straser (1981) showed differences between Yugoslavian isolates in their morphological development, their biology and their epidemiology. Vukojevic et al. (1996) noted some differences in morphology and *in vitro* rates of mycelial growth between isolates originating from different parts of Europe.

This study was aimed at determining the amount of variation of *P./D. helianthi* in France, and whether interactions occur in the response of cultivated sunflower genotypes to a range of isolates, using three tests measuring the rate of growth of mycelium on leaves, stems and capitula.

Materials and methods

Sunflower genotypes

Initial studies of *P./D. helianthi* growth rates were carried out on a highly susceptible inbred line 2603 (INRA) and on two hybrids with well known resistance levels (CETIOM, 1996): Viki (Maïsadour, susceptible) and Agrisol (Pau Semences, extremely low susceptibility). For studies of possible interactions, 10 F1 hybrids were chosen from those obtained by a factorial cross of 4 female lines and 6 male lines and evaluated in 5 locations in the southwest of France in 1996. The complete results and analyses of the heredity of resistance will be described in another paper. Table 1 gives the origins of the hybrids used for the present study. The choice of these hybrids was based on the results of the 3 most precise trials. Resistance was assessed as the percentage of plants with stem encircling lesions of with stem lesions >5 cm (Montech and Saint-Sauveut 82 respectively) and on capitula (Mondonville 31). These results were the basis of choice of hybrids for this study, in order to cover the widest range of resistance levels while having similar vegetative cycles. Five hybrids were chosen for leaf and petiole infections and 5 different hybrids for capitulum infections. The variety Santiago was included as it is defined as having low susceptibility (CETIOM, 1996), is a control in the official Phomopsis stem canker trials in France, and, in certain years, has appeared to show variations in its reaction.

Phomopsis/Diaporthe helianthi *isolates*

A collection of 98 *P./D. helianthi* isolates was obtained in 1995 and 1996 from sunflower crops in most parts of France. Diseased tissues were surface sterilised with 90% ethanol, rinsed twice with sterile deionized water and a sample placed on 1% malt agar medium with or without 0.1% chloramphenicol. The isolates were maintained at $-80\,^{\circ}$C following the method of B. Grezes-Besset (pers. comm.): the isolates were grown on solid medium with 1% malt extract and 1.5% agar in 9 cm Petri dishes. A cryoprotectant mixture was made up of 15 g l^{-1} aqueous glycerol (RP bidistilled Normapur 99.5% minimum), 20 mM Na_2-MOPS (sodium salt), pH 7, before sterilisation in an autoclave

Table 1. Origin of sunflower hybrids chosen for mycelial tests on leaves, petioles and capitula and behaviour in natural attacks by *Phomopsis/Diaporthe helianthi* in 1996

Hybrids	Origin	% lesions encircling stem (Montech, 82)	% stem lesions >5 cm (Saint-Sauveur, 82)	% symptoms on capitula (Mondonville, 31)	Interaction study[3]
2603 × RHA274	INRA × USDA	51.8 [1]	100.0	4.1	L,P
2603 × LR1	INRA	—[2]	57.9	14.8	L,P
HA74 × 90R18	IFVC × INRA	—	23.4	—	L,P
CMS1.50 × RHA274	IFVC × USDA	8.2	15.8	11.8	L,P
XRQ × PSC8	INRA	7.0	11.9	8.3	L,P
Santiago	Novartis	—	—	—	L,P,C
2603 × 90R18	INRA	43.2	48.0	20.8	C
XRQ × 90R18	INRA	12.1	26.3	18.5	C
XRQ × 83HR4	INRA	8.3	18.2	10.3	C
2603 × 83HR4	INRA	52.7	47.5	6.7	C
CMS1.50 × PSC8	IFVC × INRA	12.0	25.8	0.0	C

[1] Values are the mean of 2 replications of 50 plants.
[2] (—): not tested.
[3] L, P, C: artificial infection tests on leaves, petioles or capitula, respectively.
IFVC = Institute of Food and Vegetable Crops, Novisad, Yugoslavia.
INRA = Institut National de la Recherche Agronomique, France.
USDA = United States Department of Agriculture, USA.

for 20 min at 120 °C. The cultures were covered with 3–5 ml of this mixture and part of the mycelium on their surface was suspended in the cryoprotectant and placed in sterile 2 ml cryotubes. The tubes were placed in a 'Frosty' box one-third filled with 2-propanol. The temperature was reduced by 1 °C min^{-1} to −80 °C. To restart mycelial growth, the contents of each tube were placed on 4 Petri dishes with the usual agar medium, incubated for 7 days at 23 °C and then plated on fresh medium. For the characterisation studies, 21 isolates were chosen, representing the complete range of infected plant parts and geographical zones of the whole collection.

In vitro *characteristics*

Dry weight. A mycelial explant of each isolate was placed in a sterile 250 ml flask containing 80 ml of 0.5% agar – 1% malt medium. This agar concentration is favourable for mycelial growth throughout the medium. The 5 replications for each isolate were incubated for 35 days at 23±1 °C with a photoperiod of 13 h of light per 24 h. Measurement of dry weight followed the method of Mohammed (1987), consisting of melting the agar, then sieving out and drying the mycelium.

Linear growth. The isolates were grown on 1% malt – 1.5% agar medium in 9 cm Petri dishes in the dark at 18 °C, 23 °C and 26 °C, with 13 replications per treatment. The diameters of cultures were measured after 4 days. The difference in growth rate between 26 °C and 18 °C, termed 'cold susceptibility', was calculated as follows:

$$(\text{diameter at } 26\,^{\circ}\text{C} - \text{diameter at } 18\,^{\circ}\text{C})/(\text{diameter at } 26\,^{\circ}\text{C}) \times 100$$

Morphology. After 18 days incubation in the dark at 18 °C, 23 °C and 26 °C, the cultures were either placed in a photoperiod of 13 h of light per 24 h at 23 ± 1 °C or left in the dark at the 3 temperatures. Macroscopic and microscopic observations were made on 3 month-old cultures: the appearance of pycnidia and perithecia, the formation of conidia and ascospores and the macroscopic aspect of the mycelium (diffuseness, pigmentation) were noted.

Mycelial growth on sunflower

Netting cages. The sunflower plants were grown under fine netting cages, as described by Tourvieille et al. (1986) for the study of *S. sclerotiorum.* These cages minimise wind and hail damage and permit dampening of shoots with a fine mist. Small-scale sprinkler irrigation outside the cages was switched on automatically by the drying of a paper inside the cages, and the netting divided the water droplets into a fine mist.

Leaf infections. The leaf infection test developed by Bertrand and Tourvieille (1987) was used. It is based on the measurement of the lesion which develops along the main vein from one mycelial explant placed at the leaf tip and covered with aluminium foil to prevent drying.

Petiole infections. The test measures the passage of *P./D. helianthi* from the leaf to the stem and the rate of extension along the stem (Bertrand and Tourvieille, 1987). Mycelial explants were placed on the transverse sections of petioles cut at 2 cm from the stem and covered with aluminium foil.

Capitulum infections. The test developed by Guillaumin et al. (1974) for studying sunflower resistance to *Botrytis cinerea*, and used also by Vear and Guillaumin (1977) for *S. sclerotiorum*, was applied with slight modifications to *P./D. helianthi*. Capitula at physiological maturity were harvested and placed in a growth chamber at 20 °C, 100% humidity, in the dark. Each capitulum was infected with 3 mycelium explants. The diseased area on the dorsal surface of the capitulum was calculated with a digital planimeter ('Placom' KP-90N).

Experimental plan

Isolate aggressivity. Each of the 21 isolates of *P./D. helianthi* was used to infect 5 plants of Viki and 5 plants of Agrisol sown to form a randomised complete block with 2 cages as replications. The leaf test was used, with 2 leaves infected per plant. Lesion lengths were measured after 10 days. For the petiole test, the 21 isolates were used to infect the inbred line 2603, with one petiole infected per plant and each isolate tested on 2 replications of 5 plants grown in the field. Measurements of lesions on the stem were made 15 days later.

Sunflower × P./D. helianthi *interactions.* The 6 sunflower genotypes described in Table 1 were infected with each of the 7 *P./D. helianthi* isolates chosen from

the preliminary studies of the 21 to represent the greatest diversity available in both *in vitro* characteristics and aggressivity on sunflower. The plants were grown under netting cages to give a randomised block design with each of the 3 cages forming a replication. The leaf and petiole tests were made on 3 leaves or 2 petioles per plant and 3 plants for each isolate. For the capitulum test, 5 capitula of each sunflower genotype were infected with each of the 7 isolates. Lesions were measured 15 days after infection on leaves, 18 days after infection on petioles and 6 days after infection on capitula. The percentage of successful infections was calculated for each test and only the measurable lesions were used in the calculation of mean lengths or areas per plant or per treatment. Normality of distributions were tested and data transformed when necessary: arcsine square root was applied for percentage successful infections.

Statgraphics Plus, version 7.1 was used for statistical analyses. Factorial analyses of variance were used to determine main and interaction effects. Correlations between test results for each *P./D. helianthi* isolate and the observations of semi-natural attack presented in Table 1 (one location for leaf/stem infections and one location for capitulum infections) were calculated in order to obtain some information about relations between the origin of an isolate and its aggressivity on a sunflower plant part.

Results

In vitro *characteristics of* P./D. helianthi *isolates*

The linear growth and dry weight measurements for the 21 isolates (Table 2) showed that there were considerable variations. For example, at 23 °C, minimum and maximum colony diameters after 4 days growth were 29.8 ± 1.2 and 66.9 ± 1.1 mm. For dry weights, the extremes were 145.8 ± 7.7 and 216.6 ± 7.7 mg after 35 days. There was a highly significant effect of temperature on colony diameter: after 4 days growth the mean diameter (for all isolates) was 25.2 ± 0.2 mm at 18 °C, 43.2 ± 0.2 mm at 23 °C and 54.0 ± 0.2 mm at 26 °C (detailed data not shown). There was a significant interaction between isolate and temperature ($F = 5.02$), but this interaction effect was 25 times smaller than the isolate main effect and 613 times smaller than the temperature main effect. Thus, isolates do not react in exactly the same manner to temperature differences. The isolates obtained in the more northern regions of France were generally less sensitive to lower

Table 2. Analysis of 21 *Phomopsis/Diaporthe helianthi* isolates; *in vitro* characteristics and aggressivity on sunflower. Details of the 7 isolates used for the interaction study

Isolates	Origin characteristics		Linear growth days (mm)	Characteristics *in vitro* (malt extract 1%, 23 °C)		Susceptibility to low temperatures	Stem 2603 15 days	Aggressivity (lesions in mm)	
	Origin (France)	Infected plant part		Dry weight 35 days (mg)	Pseudothecia appearance			Leaf Viki 10 days	Leaf Agrisol 10 days
Mean[1]			43.2	191.8	14/21	54.6	98.7	23.9	14.0
Min.			29.8	145.8		24.5	52.0	2.5	2.0
Max.			66.9	216.6		67.6	126.0	45.5	31.0
F isolates[2]			50.20**	5.86**		13.45**	5.02**	6.92**	
LSD (5%)			3.2	21.7		7.8	22.4	11.1	
cv (%)			9.6	9.0		18.5	25.7	78.9	
95031	South	Leaf	41.4	184.4	Present	61.9	85.5	36.5	9.5
95049	North	Petiole	29.8	216.6	Present	53.1	75.0	2.5	2.0
95057	South	Capitulum	40.1	195.2	Present	53.7	104.0	29.5	14.0
95066	South	Stem	41.4	199.4	Present	56.2	114.5	16.0	10.5
95082	North	Capitulum	33.6	195.2	Present	58.5	67.0	10.5	7.0
95100	Centre	Stem	41.3	197.8	Absent	50.9	125.5	39.5	25.5
96001	North	Seed	66.9	214.4	Present	24.5	104.0	45.5	26.0

[1]Means were calculated on 21 isolates.
[2]**: $P < 0.01$.

Table 3. Susceptibility to low temperatures and differentiation of pseudothecia within 21 *Phomopsis/Diaporthe helianthi* isolates according to their geographic origin

	South[1]	Centre	North
Cold susceptibility (%):	56.9 ± 2.8	51.6 ± 2.8	51.6 ± 2.8
Pseudothecia appearance:			
$-18\,^{\circ}$C	5/12[2]	2/4	5/5
$-23\,^{\circ}$C	5/12	2/4	4/5
$-26\,^{\circ}$C	1/12	2/4	3/5

[1] 'South': latitude $<45\,^{\circ}$; 'North': $>47\,^{\circ}$.
[2] Appearance of pseudothecia in light at $23\,^{\circ}$C after a period in darkness at $18\,^{\circ}$C, $23\,^{\circ}$C or $26\,^{\circ}$C: ratios are number of isolates producing pseudothecia (in less than 3 months) as a fraction of total number of isolates sampled in the same region.

temperatures (Table 3). There were variations between isolates for all the morphological characters studied. For isolates whose mycelium grew within the culture medium, there was generally no colouration, whereas aerial mycelium was either white or pigmented with brown shades. These characters also varied for single isolates grown on 1% malt medium according to temperature and lighting.

Observations of the fructifications produced by the different isolates with and without lighting and at the 3 temperatures, indicated that light was favourable for differentiation of pycnidia, pseudothecia and ascospore production. None of the isolates observed formed pseudothecia in the dark at $26\,^{\circ}$C. The cultures incubated at $18\,^{\circ}$C before a period in the light at $23\,^{\circ}$C produced pycnidia and pseudothecia more rapidly than those maintained at $23\,^{\circ}$C or $26\,^{\circ}$C. In contrast, the production of conidia was influenced neither by light nor by temperature, and they appeared on almost all cultures. There was some variation between isolates: those from most northern areas produced pseudothecia under the widest range of temperatures. Of the 21 isolates studied, 14 produced pseudothecia in at least one of the experimental conditions in less than 3 months. All isolates produced pycnidia in at least one culture condition.

Isolate aggressivity on sunflower

The infection tests on leaves and petioles showed that there were highly significant differences in isolate aggressivities ($F = 6.92$, Table 2). The mean lesion length after the leaf test on the variety Viki varied from 2.5 to 45.5 mm and from 2.0 to 31.0 mm on Agrisol, with a significant difference between the two varieties, but no significant isolate $\times$ variety interaction ($F = 1.50$). The petiole test gave stem lesions on 2603 varying from 52.0 to 126.0 mm. There was no correlation between growth rates *in vitro* and those on sunflower leaf and stem ($r < 0.37$, $n = 21$ ns).

Sunflower genotype $\times$ P./D. helianthi *interactions*

The behaviour of the 7 isolates chosen for this study according to the first results are given in Table 2. Isolates 96001 and 95100 were the most aggressive on leaves (on both Viki and Agrisol), isolates 95100 and 95066 on stems, whereas isolates 95049 and 95082 were the least aggressive on both plant parts. Of those chosen, only isolate 95100 did not produce pseudothecia in the experimental conditions used.

Leaf test. The mean percentage of successful infections on leaves was 54.1% (Table 4), with significant differences between hybrids (means ranging from 32% to 68%) and between isolates (means ranging from 26% to 87%). Replication and interaction effects were not significant. Lesion lengths on leaves are presented in Table 5. There were significant differences between genotypes and between isolates, and the isolate $\times$ genotype interaction was significant. (a) The hybrid HA74 $\times$ 90R18 had the smallest lesions with all 7 isolates; (b) The least aggressive isolates 95031, 95049 and 95057 did not distinguish the sunflower genotypes; (c) The differences in order of the hybrids were significant only for the two susceptible genotypes 2603 $\times$ RHA274 and 2603 $\times$ LR1. In this case, lesion sizes were not significantly different with isolate 95100, but when 96001 was used lesion sizes were significantly smaller for 2603 $\times$ LR1 than for 2603 $\times$ RHA274. The order of the resistant genotypes Santiago and XRQ $\times$ PSC8 differed, but not significantly, with isolates 95100 and 96001. For both percentage infection and lesion length, isolate 95066 showed the closest correlation with percentage of plants with stem lesions >5 cm observed in semi-natural attack at the trial of Saint-Sauveur: $r = 0.80$ ns and $r = 0.98^{**}$ respectively.

Petiole test. The mean rate of successful infection was 91.7%, with no significant differences between treatments (data not shown). Lesion lengths on the stem 18 days after infection are presented in Table 6. For this character, there were significant genotype, isolate and

Table 4. Percentage infections on leaves for sunflower hybrids and *Phomopsis/Diaporthe helianthi* isolates (means of 3 replications)

Hybrids\Isolates	95100	96001	95066	95057	95082	95049	95031	Mean
2603 × RHA274	92.6	100.0	78.0	61.3	49.6	44.3	35.3	65.9
2603 × LR1	86.3	65.6	57.6	51.6	36.0	37.0	11.0	49.3
CMS1.50 × RHA274	93.6	85.3	59.3	27.6	49.3	18.0	22.3	50.8
XRQ × PSC8	88.0	77.6	51.6	83.3	71.3	55.6	48.0	67.9
Santiago	89.0	91.0	61.6	49.6	51.0	42.3	28.6	59.0
HA74 × 90R18	70.3	54.6	40.6	7.3	25.6	11.3	13.0	31.8
Mean	86.6	79.0	58.1	46.8	47.1	34.8	26.4	54.1
r[1]	0.30 ns	0.51 ns	0.80 ns	0.24 ns	−0.16 ns	0.30 ns	−0.01 ns	0.32 ns

Analysis of variance

F hybrids = 8.64**	LSD (5%) treatment (hybrid/isolate) = 32.3
F isolates = 20.44**	LSD (5%) hybrids = 12.2
F interaction = 0.91 ns	LSD (5%) isolates = 13.2

c.v. = 36.6%.

[1] r = correlation with the trial under natural infection at Saint-Sauveur; **: $P < 0.01$; ns: not significant.

Table 5. Lesion lengths on leaves (mm) observed 15 days after infection of leaves for sunflower hybrids and *Phomopsis/Diaporthe helianthi* isolates (means of 9 replications)

Hybrids\Isolates	95100	96001	95066	95057	95082	95049	95031	Mean
2603 × RHA274	68.1	73.8	45.2	32.5	34.5	32.5	26.3	44.7
2603 × LR1	80.0	56.4	38.2	30.3	30.5	31.6	24.5	41.6
CMS1.50 × RHA274	55.2	56.1	28.5	25.6	27.1	26.6	23.3	34.6
XRQ × PSC8	42.8	46.6	27.3	30.3	29.5	28.7	26.2	33.1
Santiago	47.6	41.5	31.8	30.1	30.1	27.5	27.3	33.7
HA74 × 90R18	42.0	30.1	27.2	23.2	25.6	22.0	24.9	27.9
Mean	56.0	50.8	33.0	28.7	29.6	28.1	25.4	35.9
r[1]	0.72 ns	0.75 ns	0.98**	0.66 ns	0.86*	0.70 ns	0.40 ns	0.86*

Analysis of variance

F hybrids = 8.40**	LSD (5%) treatment = 12.7
F isolates = 38.11**	LSD (5%) hybrids = 5.0
F interaction = 2.11**	LSD (5%) isolates = 5.4

c.v. = 35.5%.

[1] r = correlation with the trial under natural infection at Saint-Sauveur; **: $P < 0.01$; *: $P < 0.05$; ns: not significant.

replication effects and a significant isolate × genotype interaction. It may be noted that the hybrids Santiago and CMS1.50 × RHA274 showed smaller stem lesions than HA74 × 90R18 which had the smallest leaf lesions. As for the leaf infections, the less aggressive isolates did not give different lesion sizes on the different hybrids. Hybrids 2603 × LR1 and 2603 × RHA274 were not significantly differentiated with the aggressive isolate 96001, whereas the second was significantly less susceptible with isolate 95100. Hybrids 2603 × LR1 and CMS1.50 × RHA274, one of the most resistant, did not develop different lesion sizes with isolate 95082, whereas all other isolates differentiated between them. For the 5 hybrids that had been observed in semi-natural attack, the results of this test with isolate 96001 were significantly correlated with semi-natural attack ($r = 0.83$*), and isolates 95066 and 95049 gave similar results ($r = 0.79$ ns and $r = 0.77$ ns, respectively).

Capitulum test. The mean successful infection rate was 43.4%. There were significant differences between hybrids (means ranging from 32% to 55%) and between

Table 6. Lesion lengths on stems (mm), observed 18 days after infection of petioles, for sunflower hybrids and *Phomopsis/Diaporthe helianthi* isolates (means of 9 replications)

Hybrids\Isolates	95100	96001	95066	95057	95082	95049	95031	Mean
2603 × LR1	152.2	149.4	151.9	131.6	99.4	133.8	78.4	128.1
2603 × RHA274	123.6	138.6	137.2	145.2	123.6	116.6	86.7	124.5
CMS1.50 × RHA274	100.0	101.9	102.2	90.5	108.0	79.1	51.6	90.5
XRQ × PSC8	142.7	127.7	132.2	128.3	93.0	110.2	80.0	116.3
Santiago	97.5	101.3	97.2	78.0	80.5	86.6	66.6	86.8
HA74 × 90R18	120.2	116.6	121.9	104.5	123.0	110.0	74.4	110.1
Mean	122.7	122.6	123.7	113.0	104.6	106.1	73.0	109.4
r[1]	0.58 ns	0.83*	0.79 ns	0.57 ns	−0.03 ns	0.77 ns	0.43 ns	0.65 ns

Analysis of variance

F hybrids = 39.55**	LSD (5%) treatment = 20.4
F isolates = 35.03**	LSD (5%) hybrids = 7.7
F interaction = 2.37**	LSD (5%) isolates = 8.3

c.v. = 19.8%.

[1] r = correlation with the trial under natural infection at Saint-Sauveur; **: $P < 0.01$; *: $P < 0.05$; ns: not significant.

Table 7. Percentage infections on capitula for sunflower hybrids and *Phomopsis/Diaporthe helianthi* isolates (means of 2 replications)

Hybrids\Isolates	95100	96001	95066	95057	95082	95049	95031	Mean
CMS1.50 × PSC8	54.1	88.9	75.0	28.5	0.0	16.6	0.0	36.3
2603 × 83HR4	86.1	94.4	75.0	87.5	19.4	5.5	20.8	55.5
2603 × 90R18	75.0	86.1	100.0	37.5	15.5	0.0	11.1	46.5
XRQ × 83HR4	83.3	100.0	66.7	42.2	0.0	0.0	11.1	43.3
Santiago	70.7	91.6	62.5	0.0	0.0	0.0	0.0	32.1
XRQ × 90R18	70.8	94.4	58.3	58.3	22.2	4.1	19.4	46.8
Mean	73.3	92.6	72.9	42.3	8.0	4.4	10.4	43.4
r[1]	0.33 ns	−0.12 ns	0.23 ns	0.02 ns	0.60 ns	−0.78 ns	0.49 ns	0.33 ns

Analysis of variance

F hybrids = 4.01**	LSD (5%) treatment = 32.6
F isolates = 33.69**	LSD (5%) hybrids = 12.3
F interaction = 1.19 ns	LSD (5%) isolates = 13.3

c.v. = 37.5%.

[1] r = correlation with the trial under natural infection at Mondonville; **: $P < 0.01$; ns: not significant.

isolates (means ranging from 4% to 93%) and no isolate × genotype interaction (Table 7). The mean capitulum lesion areas per treatment are given in Table 8. Genotype and isolate effects were highly significant, as was the interaction isolate × genotype. The same variation in aggressivity as for the leaf and petiole test was observed and generally the order of isolates was similar to that for the two other tests. The hybrid CMS1.50 × PSC8 showed a specific interaction with isolate 96001, which caused extremely large lesions on this hybrid whereas with the other isolates it showed an intermediate level of resistance. Correlations with the observation of natural attack of capitula at Mondonville were not significant, but it may be noted that the most similar result was obtained with isolate 95082 for both level of infection and lesion size ($r = 0.60$ ns and $r = 0.48$ ns, respectively).

Discussion

The *in vitro* studies showed that French isolates of *P./D. helianthi* varied both in their growth rates and morphology and in their aggressivity on sunflower leaves and stems. The present results of *in vitro* variability are in agreement with the variability of

Table 8. Lesion areas on capitula (mm^2), observed 6 days after infection of the dorsal surface of capitula for sunflower hybrids and *Phomopsis/Diaporthe helianthi* isolates (means of 5 replications)

Hybrids\Isolates	95100	96001	95066	95057	95082	95049	95031	Mean
CMS1.50 × PSC8	479.3	1490.5	88.3	386.3	102.4	157.3	0.0	382.2
2603 × 83HR4	684.9	363.9	430.5	549.5	48.6	5.3	40.1	303.3
2603 × 90R18	294.8	326.0	429.4	258.0	269.2	0.0	15.0	225.0
XRQ × 83HR4	444.0	210.8	179.6	20.7	9.1	0.0	137.6	142.0
Santiago	60.7	130.1	175.8	30.3	0.0	2.6	1.9	56.0
XRQ × 90R18	260.6	159.1	204.3	34.1	65.8	0.0	87.4	112.9
Mean	370.7	446.7	251.3	213.2	82.1	20.7	42.2	203.8
r[1]	−0.73 ns	−0.75 ns	0.46 ns	−0.53 ns	0.48 ns	−0.75 ns	0.23 ns	−0.75 ns

Analysis of variance

F hybrids = 4.52**	LSD (5%) treatment = 409.4
F isolates = 7.18**	LSD (5%) hybrids = 154.7
F interaction = 2.01**	LSD (5%) isolates = 167.1

c.v. = 158.7%.

[1] r = correlation with the trial under natural infection at Mondonville; **: $P < 0.01$; ns: not significant.

P./D. helianthi reported by Vukojevic et al. (1996). Growth rates on agar were not correlated with lesion sizes on sunflower, indicating that *in vitro* studies cannot replace those *in vivo*. This phenotypic variability has been confirmed by molecular studies on the genotypes of the same collection of isolates (V. Says-Lesage, pers. comm.).

The isolates and the sunflower genotypes studied were chosen to represent the range of pathogen and sunflower found or likely to be used in France. The tests covered the different plant parts which may be infected. The mycelium tests on leaves and petioles have already been proposed for use in resistance breeding programmes (Tourvieille et al., 1988; Vear et al., 1997), in particular, the leaf test which this study confirms as showing the best correlation with natural attack. The two tests measure different resistance factors, and it may be that resistance factors which control the passage of *P./D. helianthi* from the petiole to the stem exist less frequently in the sunflower genotypes studied than those controlling fungal progression in the leaves. This is probably the first report of use of the capitulum test with *P./D. helianthi*. Compared with *S. sclerotiorum*, mycelial growth is slower; 6 days are necessary for the development of lesions the size of those *S. sclerotiorum* shows after 3 days. This test appears complementary to the other two, indicating reaction to capitulum attack, which has been reported in France since 1993 (Quenin et al., 1993). However, it will be necessary to confirm results and it appears very important to choose the right isolate. Results with isolate 96001 showed a negative relationship compared with the observations of natural attack ($r = -0.75$) whereas isolate 95082 gave results which agreed with the relative behaviours of the sunflower genotypes observed in the field. Thus isolate origin is not the best criterion to choose the right isolate.

The restriction in the value of these tests is that inoculum is always mycelium; the conclusions on isolate effects and isolate × genotype interactions could be quite different if ascospores were used as inoculum, as in natural attacks and as proposed by Bertrand and Tourvieille (1987). Although correlated significantly with natural attack, mycelium tests cannot measure all possible resistance processes. A comparison may be made with *S. sclerotiorum*: the test using ascospores of this pathogen gives results closer to those observed with natural capitulum attack than the mycelium test, but both show significant correlations with field results (Vear and Tourvieille, 1988). However, at present, *P./D. helianthi* ascospores are difficult to obtain with certainty at a given date and in large quantities. Moreover, such studies of isolate × genotype interactions with ascospore suspensions as inoculum would require larger experimental plans than for mycelial tests where aerial spread is not a problem.

For the present mycelium tests, using a single isolate, the results indicate that choice of the right isolate (or small number of isolates), representative of the known pathogenic variability (Tourvieille and Guiard, 1992), is essential for resistance tests to be efficient. In tests using ascospore suspensions as inoculum, the

appropriate choice of a representative series of isolates would also simulate as nearly as possible natural infections. The *P./D. helianthi* isolate must be sufficiently aggressive to permit ranking of genotypes according to their resistance level. In this study, isolate 95066 appears to represent natural attacks on leaves and stems most closely and isolate 95082 those on capitula, although these isolates were not the most aggressive on average. It may be noted that they were obtained from stems and capitula respectively. However, these specificities need confirming. The presence of an interaction also appears to depend on the type of observation made. On both leaves and capitula, isolates have the same order of aggressivity measured by percentage infection (mycelial penetration) and by lesion size (mycelial extension), but only the latter showed significant interactions. Further, the isolates did not show the same order on leaves, stems and capitula. On capitula, it may be noted that isolate 95082 penetrated sunflower with some difficulty, as for isolates 95031 and 95049, but then extended much more rapidly than the latter two.

The real importance of interactions remains to be confirmed. The trials with the 21 isolates showed no interaction in the rather extreme varieties Viki and Agrisol. In the study of 6 sunflower genotypes and 7 isolates, only one case of complete opposition (capitula of CMS1.50 × PSC8 infected with isolate 96001 compared with the other 6 isolates) was observed. The other changes of order were only between similar hybrids, either rather susceptible or quite resistant. The isolate × genotype interaction was on average 7.5 times smaller than the genotype effect and 12 times less than that for isolates. Thus the question is raised of whether breeding programmes with tests using only one isolate will permit selection of genotypes with usable resistance. Similar interactions have already been reported for various crop diseases. For resistance to *Fusarium* head blight in wheat, Atanassov et al. (1994) explained part of the isolate × genotype interaction by the considerable variability of the pathogen, with an isolate effect much greater than that of any of the other effects (observations were partly of mycotoxin production). In contrast, Sah and Fehrmann (1992) demonstrated an interaction between 8 wheat varieties and 20 monoascospore or monoconidial isolates of *Pyrenophora tritici-repentis* in spite of the weak variability in aggressivity of the isolates from very different geographical origins. In the case of poplar rust, Hamelin et al. (1992) reported different behaviours of host genotypes according to the origin of isolates, and linked this adaptation of the parasite to its reproductive system, which may be sexual or asexual according to the environment, thus modifying its genotypic diversity.

All reports of isolate x genotype interactions draw the same conclusion concerning inoculum to be used when breeding for durable resistance. In studies of strawberry resistance to *Colletotrichum acutatum*, Denoyes-Rothan and Guérin (1996) observed fluctuations in disease response and warned breeders of the necessity of using strains representative of the population to screen for stable resistant cultivars. Baergen et al. (1993), working on *Verticillium dahliae* on tomato, suggested that several isolates should be used to improve resistance to race 2 of the pathogen. Reid et al. (1993) reported a significant isolate × genotype interaction in two years of trials with 3 isolates of *Fusarium graminearum* and a wide range of maize inbred lines and hybrids. Although this interaction did not change the ranking of genotypes, the authors suggested using either one aggressive isolate (to determine the complete range of resistance and susceptibility) or a mixture of isolates (to have results closer to natural attacks) to obtain a valid evaluation of resistance.

In breeding for stable resistance, it is necessary to take into consideration host interactions with both pathogen isolates and the environment over several years. Miedaner (1997) in studies on *F. graminearum* on wheat and rice, for which there was no isolate × genotype interaction, nevertheless proposed the use of a mixture of isolates with different levels of aggressivity to cover possible genotype × environment effects thus making artificial infections more comparable to natural attack. Cardwell and Wehrly (1997) suggested that, to breed for resistance to pathogens that show natural variability, it is necessary: (a) to choose host genotypes with different origins and reactions, but not showing interactions with the environment; (b) to make trials over several years in different locations with these 'differential genotypes', so that variety × location interactions observed would indicate different pathogen populations. Such locations should then be used in programmes breeding for stable resistance.

At present isolate × genotype interactions for *P./D. helianthi* on sunflower do not appear very important, but they do exist. In addition some isolates are not sufficiently aggressive to differentiate between sunflower genotypes. Thus it appears important to take into consideration both aggressivity and diversity of isolates used for testing Phomopsis stem canker resistance in

sunflower and then to check the behaviour of the genotypes selected on the basis of artificial infections, under conditions of natural field attack.

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