USE OF TWO MYCELIUM TESTS IN BREEDING SUNFLOWER RESISTANT TO PHOMOPSIS

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SUMMARY

Breeders use observations of natural attack or ascospore tests to select Phomopsis resistant sunflowers, but the methods are limited in their use. Two mycelium tests on leaves and petioles were studied to determine individual plant response and the existence of different types of resistance. In each case, lengths of recroses were measured after 20 and 10 days respectively. The leaf test gave the same genotypic classification as natural attack. The petiole test was less well correlated but demonstrates a different mode of resistance. The percentages of sucessful inoculation (leaf 72 p. cent, petiole 95 p. cent mean that with a maximum of 2 inoculations a plant can be judged. The tests were used on F2 progenies from crosses and F3 progenies from selfing resistant hybrids. The results showed that resistance may be recessive or depend on interactions between genes and that the tests to be employed depend on the genetic origin of resistance. Selection of Phomopsis resistant genotypes using mycelium tests appears possible and can take into account the polygenic nature of resistance.

INTRODUCTION

Diaporthe helianthi, with the condial form Phomopsis helianthi has caused economic damage on sunflowers since 1985 (Regnault, 1985). In the absence of any very efficient chemical treatment, use at least partially resistant hybrids will limit losses and diminish the risk that Phomopsis will spread throughout the sunflower crop in France. At present breeders use observations of natural attack or artificial infections with ascospores to select interesting genotypes (Mihaljcevic et al., 1982 ; Peres and Regnault, 1986 ; Tourvieille and Pelletier, 1988). These techniques have their limits : natural attack can only be observd in naturally contaminated areas and neither technique permits efficient selection of individual plants.

A method based on two mycelium tests has been developed which makes is possible to judge individual plants for two resistance criteria. This method has the advantage that it can be used each year in uncontaminated areas without causing risks of epidemic. This article describes the results for hybrids with good levels of resistance and for segregating F2 and F3 genotypes which form the basis of a breeding programme for Phomopsis resistance.

MATERIAL AND METHODS

1 - Sunflower genotypes

The hybrids studied represented a wide range of susceptibility to Phomopsis : the French variety Elia, the Yugoslave varieties NSH43 (Condor), NSH44 (Helios), NSH45 (Flower), and the Rumanian variety Sélect. The segregating progenies were (a) F2 plants obtained from crosses between French inbred lines and cultivars with good resistance (Select, NSH15, NSH44). (b) F3 progenies obtained from selfing Select, NSH15 and NSH44. The F2 generation was selected for oil content and downy mildew resistance only.

2 - Pathogen

The isolate of D. helianthi (PHf) was isolated on sunflowers in July 1985 in the

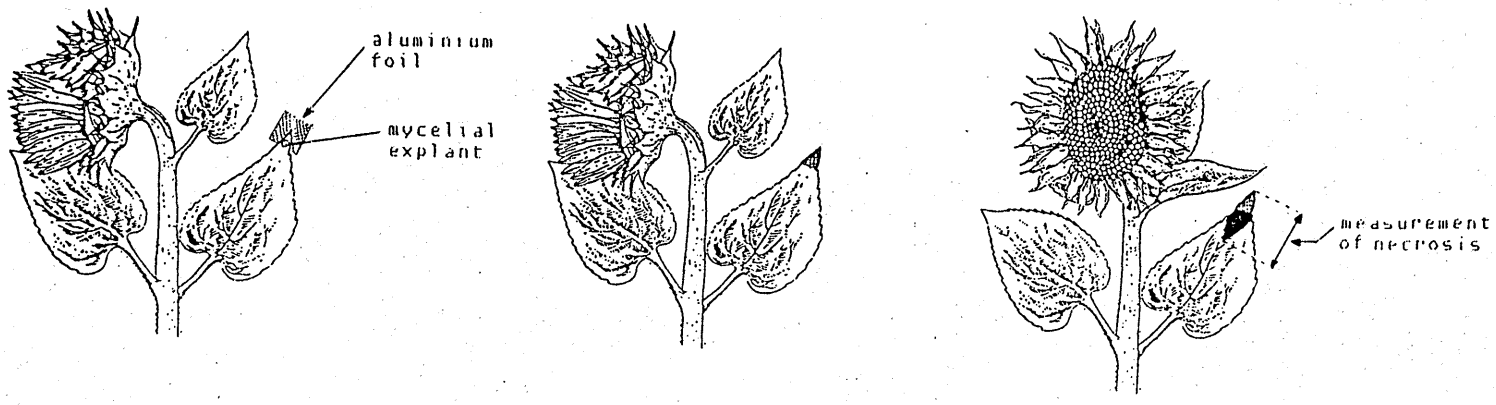

FIGURE I : Inoculation with mycelial explant placed on the extremity of the main vein.

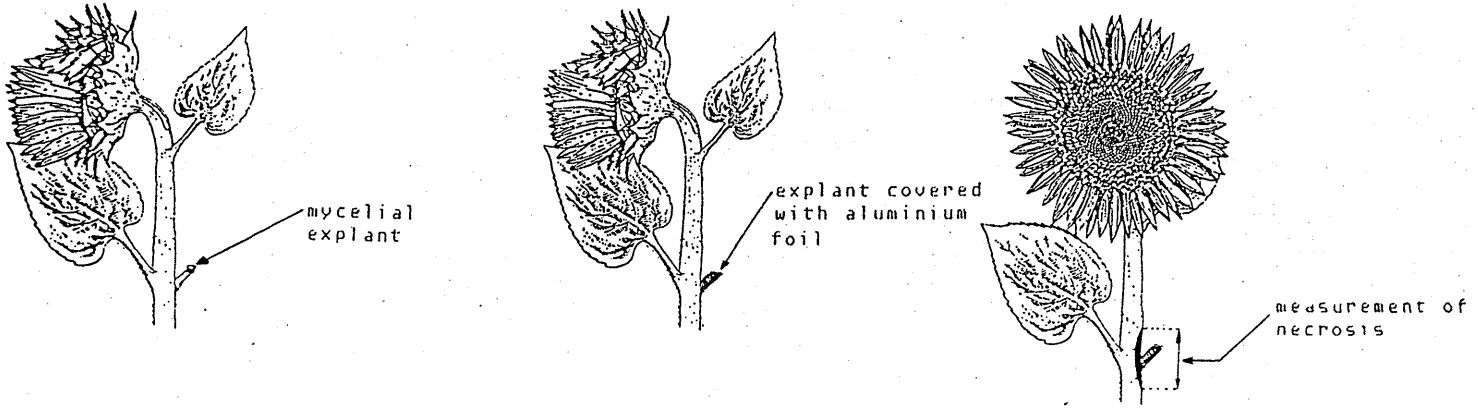

FIGURE II : Inoculation by mycelial explant placed on the cut petiole 2 cm from the stem.

Lauragais, France. Taxonomic determination was carried out by Acimovic. The inoculum was cultured on 1 p. cent malt medium with 1,5 p. cent agar at 23° C ± 1° in darkness.

3 - Contamination methods (Bertrand and Tourvieille, 1987)

Plant development - The sunflowers were grown in the field. Their normal requirements were filled until the beginning of flowering when the tests were carried out. Then they were irrigated each day (5 mm). Controls were inoculated at each date in order to permit comparison of genotypes with different earliness.

Leaf test (figure 1) - A mycelial explant of 6 mm diameter cut from the edge of a 4 day-old Diaporthe culture is placed on the extremity of the main vein of a leaf. The surface of the explant carrying the mycelium must be in contact with the upper surface of the lamina. Aluminium foil protects the contamination site from drying. Measurements are made of the length of necroses visible on leaves 20 days after contamination.

Petiole test (figure 2) - The petiole is cut across 2 cm from the stem. A mycelial explant obtained in the same conditions as for the leaf test, is placed in contact with the cut surface. The petiole and inoculum are protected from drying by aluminium foil. The length of necroses visible on the stem, 10 days after infection, are measured.

RESULTS

1 - Success rate of inoculations.

Table 1 carries the rates of sucessful inoculations on leaves and petioles.

Table 1 - Success rate of Phomopsis inoculations in Leaf (L) and Petiole (P) tests

Tests	Leaf level	Successful/Total Infections	%	means
L	lower	357/423	84.4	71.9
	middle	306/423	72.3	
	upper	249/423	58.9	
P	lower	385/412	93.4	94.9
	middle	388/412	94.2	
	upper	400/412	97.1	

The inoculation on petioles almost always results in successful infection. The 25 p. cent of the leaf tests which were unsuccessful mayy be explained by :
- poor contact between the leaf and inoculum
- premature drying of the leaf
- leaf drop due to breakage of the petiole.

2 - Reaction of different genotypes

The responses of 6 hybrids to the tests are compared in Table 2 with observations of natural attack. The classifications obtained with the two tests are not the same, indicating that several resistance mechanisms are involved in the different phases of the infection process. The leaf test is the better correlated with natural infection and explains the good behaviour of NSH43. However alone,

it does not explain the extremely good behaviour in the field of NSH45. The petiole test (length of necrosis in the field) explains the second part of the resistance of NSH45 and also the resistance of NSH15, whose susceptibility to the leaf test does not explain its level of resistance in the field.

Table 2 - Reaction of 6 hybrids to 2 Phomopsis test (lengths of necrosis in cm) and natural attack.

Hybrids	Tests*		Natural ** infection (p. cent)
	L	P	
NSH45	4.45	1.89	14.0
NSH43	4.14	4.73	27.5
NSH44	4.66	3.66	28.3
SELECT	5.71	4.64	30.2
NSH15	6.80	3.86	79.7

* means of 30 infections ** means of 4 trials.

3 - Behaviour of progenies obtained from susceptible inbred x resistant hybrid crosses.

The results obtained with the two tests are given in Table 3. With the leaf test the progenies show an increase in variation coefficient (from 1.08 to 1.60) compared with the F1 hybrids. A few plants show the same level of resistance as the resistant parents (hybrids) but the mean level of resistance is low with the mean necrosis length varying from 1.87 to 3.17 times those on the parental hybrids.

Table 3 - Behaviour of 4 F2 progenies (inbred x hybrid) to the two Phomopsis mycelial tests (necrosis in cm).

Genotypes	Test F *	Test P *
PR56 x NSH15	9.85	6.24
PRS7 x SELECT	15.21	5.10
PK1 x NSH45	6.40	5.96
84HR2 x NSH43	7.67	5.63

* means of 30 inoculations.

4 - Response of F3 progenies obtained from selfing the resistant hybrids

The behaviour of the F3 families in response to the test are grouped in Table 4.

Table 4 - Response to 2 mycelium tests of F3 progenies from selfing of hybrids with good resistance to Phomopsis.

Parental hybrids	Tests	Necroses (in cm) min	max	mean*	sig. dif. between F3
SELECT	F	5.49	12.73	7.76	+
	P	3.37	5.46	4.47	+
NSH44	F	3.53	5.84	4.36	-
	P	1.23	2.75	2.19	-
NSH15	F	6.80	11.03	9.37	-
	P	3.66	6.29	4.77	+

* means of 30 infections (10 plants per progeny).

There are significant differences in the reactions to the tests between the progenies from Select (0.96 to 2.23 times the necrosis on the F1 for the leaf test) and from NSH15 (1.27 to 2.19 times the necrosis on the F1 for the petiole test). In contrast, the F3 progenies from NSH44 do not differ significantly for either of the tests, they all show a good level of resistance : Leaf test 0.75 to 1.25 times the necrosis on F1 ; Petiole test 0.33 to 0.75 times the necrosis on F1.

DISCUSSION

The proportion of successful inoculations, 72 p. cent for the leaf test and 95 p. cent for the petiole test, indicate that it is possible to judge, with certitude, individual plants with a maximum of 2 infections per plant. The leaf infections should be applied on "adult" leaves for which the success rate attained was 84 p. cent whereas for growing leaves it was only 59 p. cent.

The different behaviours of the hybrid varieties to the two tests demonstrates the partial and polygenic nature of the resistance of sunflowers to Phomopsis. They justify the utilisation in breeding of two tests which measure different resistance factors. Thus NSH43 is characterised by a high level of leaf resistance but is very susceptible if infection reaches the stem.

The behaviour of the F2 progenies obtained from crosses of Select, NSH43 and NSH45 with susceptible inbreds, with an increase in the variation coefficient and the mean level of susceptibility, suggests that the resistance is partly under control of recessive genes and that it depends also on interactions between a number of genes which may be separated by cross-pollination.

The significant differences between F3 progenies from Select and NSH15 indicating segregation for resistance, suggests that only one of the parents of these hybrids had useful levels of resistance. On the contrary the satisfactory behaviour of all the progenies from NSH44 and the absence of significant differences between them suggests that these genotypes are homozygous for the more important resistance genes.

In conclusion, test using artificial infections with mycelial explants can be used in breeding for resistance to Phomopsis. They permit definition of different types of resistance and they indicate that breeding programmes taking account the polygenic nature of resistance are possible.

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