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Evaluation of parameters accounting for Phomopsis resistance using natural infection and artificial inoculation on recombinant inbred lines from a cross between susceptible and resistant sunflower

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Abstract

HA89, a sunflower line susceptible to Phomopsis, was crossed with a resistant line, LR4-17. Two hundred and forty-one F2/F3 progenies and 232 recombinant inbred (RI) lines were derived from this cross. F2/F3 progenies were tested in semi-natural infections in 1994. F7, F8 and F9 were tested with semi-natural infections in 1997, 1998 and 1999, respectively. F7 RI lines were artificially infected with Phomopsis mycelium on leaves in 1997. Family effects were significant in F2/F3 progenies for attack rates on stems and encircling spots rates on stems after semi-natural infections. Line effects were significant for attack rates on stems and encircling spot rates in F7 and F8. F7 RI lines showed a high heritability for speed of necrosis on leaf blades after artificial infection. Continuous and unimodal distributions were observed for attack rates on stems in all experiments done in semi-natural conditions. In contrast, speed of necrosis on leaf blades showed a bimodal distribution. Significant rank correlations were observed between the speed of necrosis on leaf blades and the attack rates on stems. It is suggested that resistance to Phomopsis is the consequence of several mechanisms, independently inherited. It was concluded that speed of necrosis on leaf blades in artificial infections and attack rates on stems in natural infections should be retained as parameters for quantitative trait locus mapping of Phomopsis resistance.

Abbreviations: FER – final expansion rate of lesions on leaf blades; FFEL – final frequency of encircling stem lesions at maturity; FIF – final infection frequency on stems at maturity; FRES – final ratio of encircling lesion frequency to stem lesion frequency at maturity; IFEL – initial frequency of encircling stem lesions at flowering; IIF – initial infection frequency on stems at flowering; LP – latent period; QTL – quantitative trait locus; RI lines – recombinant inbred lines.

Introduction

Phomopsis stem canker, caused by *Diaporthe helianthi* Munt-Cvet et al. is the most severe fungal disease on sunflower (*Helianthus annuus*) in Europe and it can cause significant damage in cultivation (up to 30% yield loss). In some cases, damage due to lodging is so severe that harvesting is not economical. Natural infections are irregular, but losses justify chemical treatment. However, this is costly and spraying can be difficult due to climatic conditions. Resistance to *D. helianthi* has been found in sunflower germplasm (Skoric, 1985) and in descendants from inter-specific crosses between sunflower and wild *Helianthus* (Griveau et al., 1992; Besnard et al., 1997). Different sources of resistance have been developed with an acceptable level of

resistance. Studies on the mode of inheritance of resistance to *D. helianthi* have revealed continuous variation between fully susceptible and fully resistant, with intermediate levels of resistance in some varieties. Some authors have postulated that the control of resistance to Phomopsis is oligogenic (Vranceanu et al., 1993). In addition, Vear et al. (1997) and Deglene et al. (1999) found a positive correlation between the level of resistance to *D. helianthi* in the parents and that found among the hybrid progeny. Thus, screening the parents should be an effective way of producing hybrids of known resistance.

The inbred line LR4-17 appears to contain most of the resistance factors to *D. helianthi*. In particular, it shows both early and late post-infection resistance (Langar et al., 1997). Moreover, Langar et al. (2000) found two mechanisms of resistance to *D. helianthi* by comparing the rate of lesion expansion on leaves and stems. We therefore developed a population of recombinant inbred (RI) lines issued from a HA89 (susceptible) × LR4-17 (resistant) cross. This population was used to carry out a genetic analysis of factors modifying resistance to *D. helianthi*.

Experiments were carried out to design a reliable routine infection method that would permit the mapping of resistance to *D. helianthi*. Due to the complexity of the trait under study, two types of segregating genetic structure were used for mapping the parameters used to account for the trait: a F2 from a cross between susceptible and resistant lines, and RI lines developed from an equivalent cross. Results confirm that resistance to *D. helianthi* depends on several mechanisms, probably involving several loci, which have to be combined if breeding is to improve resistance levels.

Materials and methods

Sunflower lines

LR4-17 was fixed in the progenies of the hybrid NSH45 (Yugoslavia). The source of resistance is uncertain, but the parental line bringing resistance was derived from a Moroccan population and *Helianthus argophyllus*. This line displays slow necrosis on leaf blades, petioles and stems, whereas the susceptible line, HA89, shows rapid lesion expansion on leaves and stems.

Production of F2 and RI lines

The production of the RI lines and the experimental design are summarised along with the repetitions of the tests for resistance (Figure 1). One plant from the inbred sunflower line HA89, which had been chemically emasculated using gibberellic acid (Piquemal, 1970), was crossed using the pollen from one plant of the line LR4-17. Two F1 plants were obtained and self-pollinated under paper bags. These led to 242 F2 descendants. F3 families were derived from F2 plants by self-pollination. RI lines were developed from the F3 plants (Burr and Burr, 1991). Each generation of RI lines was derived by selfing one plant chosen randomly from each family. To increase the probability of obtaining at least one progeny, 3 plants per family were self-pollinated, but the seeds of only one plant were used to produce the next generation. F3 families and F7, F8 and F9 generations of the RI line were tested in 1994, 1997, 1998 and 1999, respectively, with semi-natural infection by *D. helianthi*. The F7 generation of the RI line was tested in 1997 using artificial inoculation.

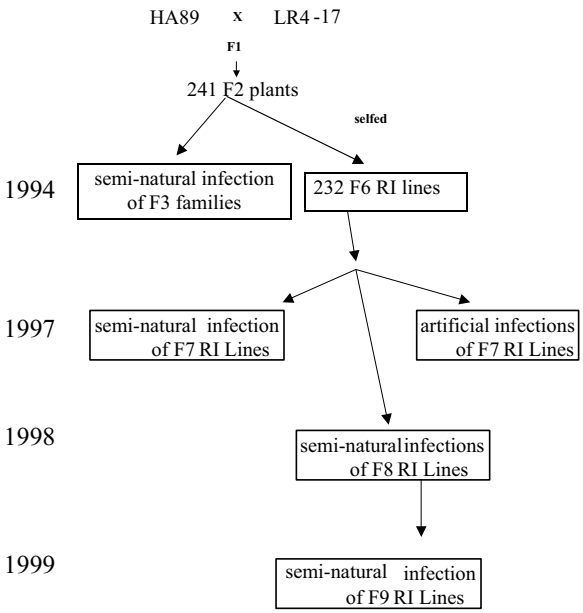

Figure 1. Scheme of generations derived from the cross HA89 × LR4-17.

Pathogenicity tests and measurements

Semi-natural infection

Trials with semi-natural infections were performed at Auzeville, near Toulouse, France. For semi-natural infections of F7 plants, each RI line was represented by two replicate sets of 20 plants. Inoculum was brought with infected pieces of sunflower slems at a density of 2 per plot, at R1 stage (Schneiter and Miller, 1981).

For the F7 and F8 generations, the number of plants attacked by *D. helianthi* on stems, and the number of plants with encircling stem lesions, both at the end of flowering (stage R6) and at physiological maturity and beginning of stage R9 were noted. In the F9 generation of the RI lines, the flowering date, the number of plants attacked and the number of plants with encircling stem lesions at R9 stage were noted. The following observations were made: (1) Initial Infection Frequency (= IIF), that is, percentage of plants at flowering with stem lesions, observed in F3 families, F7 and F8 RI lines; (2) Final Infection Frequency (= FIF), that is, percentage of plants at physiological maturity with stem lesions, noted in F3, F7, F8 and F9; (3) Initial Frequency (percentage) of plants with Encircling stem Lesions at flowering (= IFEL), noted in F3, F7 and F8 and (4) Final Frequency (percentage) of plants at physiological maturity with Encircling Lesions (= FFEL), noted in F3, F7 and F8. At maturity the Final Ratio of Encircling lesion frequency to Stem lesion frequency in F7 (FRES) was calculated.

Artificial inoculation

Leaves were inoculated using mycelium from strain 96001, an aggressive isolate (Viguié et al., 1999) of *D. helianthi*, (Bertrand and Tourvieille, 1987). A 6 mm diameter plug cut from the edge of a 4-day-old Phomopsis culture growing on 1% malt extract + 1.5% agar in a Petri dish was placed on the tip of a leaf and covered with aluminum foil. Inoculations were carried out 75 days after sowing, after all the plants reached or passed the stage R1.

The experiments using artificial inoculation of F7 plants were carried out in a polythene tunnel, in Mauguio (43°34′N, 13°57′E) near Montpellier, France. Each RI line was represented in a plot of three plants. The parental lines, divided into 8 blocks randomly distributed throughout the tunnels, were used as controls. Water was supplied by a sprinkler system regulated to maintain the presence of water drops on the canopy (Tourvieille and Vear, 1986).

The length of lesions on leaf blades, petioles and stems was measured. Symptoms were scored according to the following scale: 0 = no necrosis, 1 = necrosis exceeding the aluminium foil by less than 1 cm, 2 = necrosis exceeding the aluminium foil by more than 1 cm, 3 = necrosis covering a quarter of the vein, 4 = necrosis on half of the vein, 5 = necrosis on three quarters of the vein, 6 = necrosis covering the entire vein, 7 = necrosis on petiole, 8 = necrosis on stem, 9 = lesion encircling the stem and 10 = encircling lesion and broken stem. Observations were made twice a week for 6 weeks from infection until physiological maturity of the flower heads. Those plants whose symptom score did not exceed 2 at the end of the cycle were not included in the analysis because they were assumed to be not infected. For each interval between two measurement dates, the rate of lesion expansion was calculated as being the ratio of the increase in length of the leaf blade lesion over the time interval in days. Thus, the following parameters were taken into account: (1) Latent Period (LP) in days = time separating inoculation from first visible symptoms in F7; (2) Final Expansion Rate (FER) in cm/day = rate of lesion expansion on leaf blades in F7, calculated before lesions appeared on petioles or at maturity.

Statistical analyses

Arcsin $\sqrt{x}$ transformations were computed on the IIF, FIF, IFEL and FFEL. Log x transformation was computed on LP. Before and after transformation, tests (Shapiro and Wilk, 1965) were applied to check whether the parameter data had normal distributions.

Broad sense heritabilities, h^2, were computed using the following model: $P_{ij} = \mu + G_i + \varepsilon_{ij,}$ with P_{ij} the phenotypic value of replicate j of the inbred line i, μ the overall mean of inbred lines, G_i the effect of the line i and ε_{ij} the error term, following the relation $E(\mathrm{CM_g}) = \sigma_e^2 + n\sigma_g^2$, where n is the number of observations per genotype, σ_g^2 is the genetic variance and σ_e^2 the error (Gallais, 1990). Heritability was calculated as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$. Statistical analysis for normality tests, heritabilities and correlations were performed with, respectively, the UNIVARIATE, GLM and CORR procedures of the SAS software, (SAS institute, 1996).

Results

Semi-natural infections

Mean frequencies of infection by *D. helianthi* in the populations varied according the generations, which were tested in different years (Table 1). The strongest attack occurred in the F3 plants, in which 66% of plants had encircling stem lesions at maturity. The weakest attack was observed in the F9 plants, in which only 8.8% of the plants had stem lesions and none had encircling stem lesions (not shown). HA89 was observed as susceptible and LR4-17 as resistant in all experiments

Table 1. Means and heritabilities of symptom criteria measured in F3 families and in RI lines from the cross HA89 × LR4-17

Trait	Unit	Generation	Mean of HA89	Mean of LR4-17	Mean of the population	Heritability
IIF	%	F3	92.6	11.7	53.2	69.4***
		F7	21.0	0.0	9.8	61.7***
		F8	29.4	0.6	6.1	10.5*
IFEL	%	F3	34.0	0.0	3.3	47.7***
		F7	5.0	0.0	1.1	38.7***
		F8	4.6	0.0	0.1	19.8***
FIF	%	F3	100.0	55.4	94.6	74.8 ***
		F7	94.0	0.0	64.0	47.7***
		F8	35.4	4.7	31.7	51.2***
		F9	20.0	0.0	8.8	37.3***
FFEL	%	F3	100.0	31.9	66.0	50.5***
		F7	4.6	1.0	16.4	61.0***
		F8	6.3	0.0	1.5	24.2***
FRES	%	F7	51.8	3.5	28.2	41.2***
LP	days	F7	12.9	16.3	12.5	25.3***
FER	mm/day	F7	7.5	0.8	4.1	82.3***

IIF: initial infection frequency on stems at flowering, IFEL: initial encircling lesion expansion rate on stems at flowering, FIF: final infection frequency on stems at maturity, FFEL: final encircling lesion expansion rate on stems at maturity, FRES: final ratio of encircling lesion frequency to stem lesion frequency at maturity, LP: latent period, FER: final expansion rate of necrosis on leaves.
*, **, ***: family or inbred line effect significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

Table 2. Comparison of infection frequencies by Phomopsis in parent lines and F1 hybrid in 1995

Trait	HA89	HA89 * LR4-17 F1	LR4-17
IIF	29.0 a	9.0 b	1.0 b
IFEL	11.0 a	2.0 b	0.0 b
FIF	56.0 a	11.0 b	2.0 b
FFEL	31.0 a	2.0 b	0.0 b

Details and abbreviations are listed in Table 1. Means followed by the same letter are not significantly different at $p < 0.05$ with a student test.

and for all parameters observed. In 1995, the measurements made on the F1 hybrid showed an intermediate reaction compared with the parents, although the difference was not significant compared to the parent LR4-17 (Table 2).

Family effects were significant ($p < 0.001$) for infection frequencies on stems (IIF and FIF) and IFEL and FFEL encircling lesion expansion rates on stems ($p < 0.001$). Line effects were significant ($p < 0.001$) in F7 plants for infection frequency on stems and encircling lesion expansion rate, both at flowering and at maturity. In F7 plants, the ratio of encircling lesion frequency to stem lesion frequency at maturity FRES, shows significant line effect ($p < 0.001$). In F8 plants, line effects were significant for IIF ($p < 0.001$), FIF and IFEL, but only significant for IFEL at ($p < 0.05$). In F9 plants, only non-encircling lesions on stems were observed at maturity, but line effects were significant for this parameter ($p < 0.001$).

High heritabilities were observed in the F3 (Table 1). They ranged from 47.7% for IFEL to 69.4% for IIF. The highest heritabilities in RI lines were observed in F7. They varied from 38.7% for IFEL to 61.7% for IIF. Low heritabilities were observed in F8 for data collected at flowering, 10.5% for IIF and 19.8% for IFEL, while they were higher for data collected at maturity.

Distributions of infection frequency in the F3 are shown (Figure 2). They differ significantly from normality. No family was more resistant than neither LR4-17 nor more susceptible than HA89. In the F7, some lines reacted as more susceptible than HA89 for encircling lesions expansion rates (IFEL and FFEL), whereas no line was observed as being more resistant than LR4-17.

Artificial inoculations

Line effects were significant for all traits studied in F7 ($p < 0.001$) (Table 1). Heritabilities ranged from

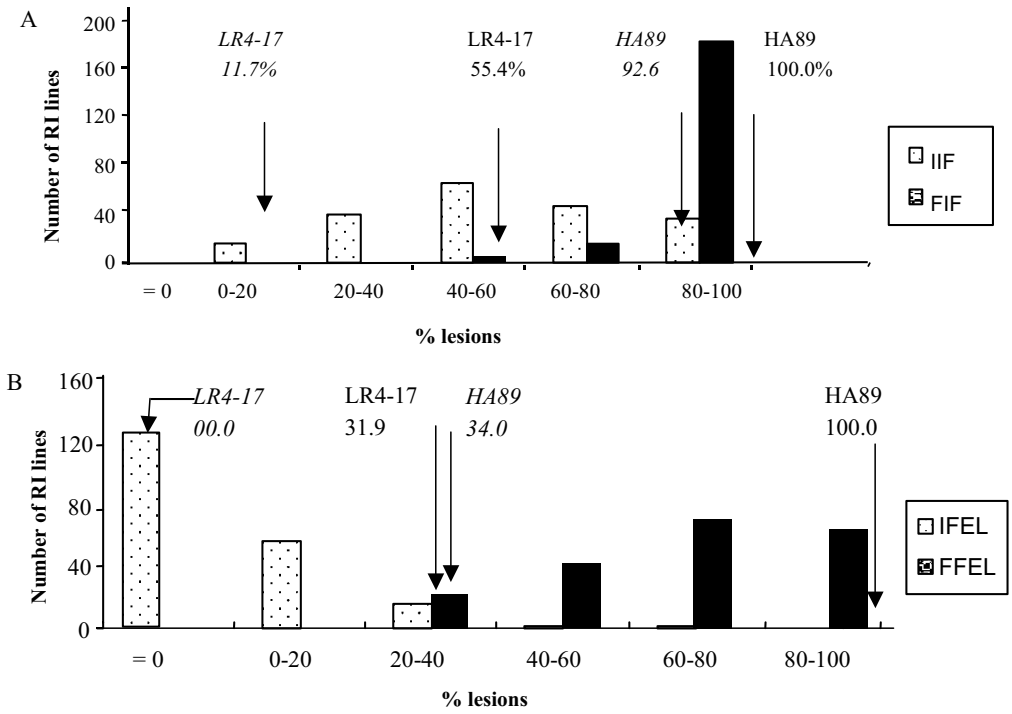

Figure 2. Distribution of % lesion attack by Phomopsis with semi-natural infections in F3 families from the cross HA89 × LR4-17 (A) % lesions (*IIF* and FIF) on stems at flowering and at maturity. Values in italics are controls for IIF. (B) % Encircling lesion (*IFEL* and FFEL) rates on stems at flowering and at maturity. Values in italics are controls for IFEL.

25.3% for the LP to 82.3% for FER. HA89 was susceptible and LR4-17 was resistant in terms of FER as for LP (since the highest scores for LP indicated resistance). Heritability was high for rate of lesion expansion on leaf blades (FER), but much lower for LP and for FER.

No inbred line was found to be more resistant than the resistant parent LR4-17 for any parameter in artificial infections (Figure 4A,B). Some lines appeared to be more susceptible than HA89 regarding and FER, but these differences were not statistically significant. Shapiro and Wilk tests revealed deviations from normal distribution, even after transformation for homoscedasticity. However, log of LP in F7 presented no deviation from normal distribution. Moreover, final and maximum expansion rates showed bimodal distributions in which the highest mode coincided with the value of the susceptible parent HA89 (Figure 4A,B).

Comparison of semi-natural infections and artificial inoculations

Since the data for most of the parameters was not normally distributed, rank correlation was measured between measurements for each experiment with artificial inoculation and semi-natural infection (Table 3). Correlations between the different measures of infection frequencies in semi-natural infections were highly significant. However, they were low between F7, F8 and F9 generations, with a maximum of 0.434 ($p < 0.001$) between FFEL in F7 and FIF in F9, although the maximum correlation in F7 plants were 0.834 ($p < 0.001$) between FIF and FFEL and 0.886 between FRES and FFEL. Correlations between LP and FER after artificial infections were also highly significant. They were negative between LP and rate of lesion expansion, with the highest scores of LP consistently corresponding to resistant lines. The rate of lesion expansion after artificial inoculation, FER, was strongly correlated to disease measurements of semi-natural infections in F7 and F9, as well as the ratio of encircling lesion frequency to stem lesion frequency at maturity. However, correlations between FER and IFEL in F7 were only slightly significant. Significant correlations were observed between LP and infection frequency in semi-natural infections, but not with IFEL in F7.

Table 3. Spearman rank correlations between mean for parameters of RI lines from the cross HA89 × LR4-17 in F7, F8 and F9 generations after semi-natural and artificial infections

Trait	IIF		IFEL		FIF			FFEL		FRES	LP
	F7	F8	F7	F8	F7	F8	F9	F7	F8	F7	F7
IIF											
F7											
F8	0.269***										
IFEL											
F7	0.716***	0.280***									
F8	0.192*	0.400***	0.218**								
FIF											
F7	0.596***	0.347***	0.474***	0.194*							
F8	0.059	0.423***	0.121	0.183**	0.236**						
F9	0.346***	0.264***	0.176***	0.065	0.411***	0.099					
FFEL											
F7	0.719***	0.301***	0.624***	0.216**	0.834***	0.201**	0.434***				
F8	0.078	0.284***	0.150*	0.504***	0.195	0.714***	0.069	0.234*			
FRES											
F7	0.634***	0.265***	0.529***	0.125	0.646***	0.157*	0.423***	0.886***	0.168*		
LP											
F7	−0.159*	−0.076	−0.086	−0.034	−0.212*	0.062	−0.169*	−0.198**	0.050	−0.118	
FER											
F7	0.134	0.157*	0.113	0.096	0.264***	−0.071	0.227**	0.267***	−0.025	0.255***	−0.618***

*, **, ***: Significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. Abbreviations are listed in Table 1.

Discussion

The validity of semi-natural infections for the study of resistance was confirmed. Symptoms shown by the parents HA89 (susceptible) and of LR4-17 (resistant), used as controls, corresponded to their known levels of resistance. This expected behaviour of the controls also validates the significance of effects relating to families or lines. The parameters with the highest agronomic interest for breeders were IFEL and FFEL in F3 and in F7, which had the highest heritabilities. Indeed, Pinochet and Estragnat (1996) observed that the frequency of encircling spots at maturity in semi-natural infections on hybrids with different susceptibility levels was the parameter which best correlated with yield losses. However, the higher infection intensity observed in F3 families compared to RI lines led to heritabilities which were higher in F3 than in F7, F8 and F9, in contrast to that expected. Differences between heritabilities in RI lines tested in different years were probably due to variations in the intensity of infection in semi-natural conditions. Heritability was valuable only when there was a high level of disease.

Artificial inoculation was developed to avoid differences in infection pressure between repeats and the effect of random infections of plants in semi-natural conditions. Indeed, heritabilities computed for parameters with artificial inoculation were actually higher than those for parameters in semi-natural infection conditions. The highest heritability 82.3% for FER with artificial inoculation, and was consistent with this assertion, especially for the rate of necrosis on leaf blades.

The LP showed strong negative correlation to the FER in F7. However, LP represents the addition of two phenomena: (1) penetration of the mycelium into the tissues of the leaf blade and (2) mycelium extension along the main vein. LP was significantly correlated to infection frequencies in semi-natural infections. This suggests that the genetic variation for penetration in leaf blade tissues is a component of the overall variation for *D. helianthi* attack on stems. Artificial infection makes it possible to study the kinetics of symptom evolution. However, as pointed out by Viguié et al. (1999), artificial inoculation cannot reveal the variation between lines regarding resistance to penetration by ascospores. Significant correlations were found between speed of necrosis on leaf blades FER and attack frequencies in semi-natural infection. This suggests that lesion expansion rate on leaf blades was one of the actual components of resistance. However, the weakness of the

correlation suggests that other factors, for example, the ranking for stem symptoms were involved in resistance. Vear et al. (1997) found that measurements taken on artificially inoculated plants were good predictors of behaviour in semi-natural infections for hybrid genotypes of sunflower, but were less reliable for inbred lines.

The ratio of encircling lesion frequency to stem lesion frequency at maturity FRES was calculated to estimate expansion of Phomopsis symptoms on stems. It was highly correlated to FFEL in F7 plants ($r = 0.886$), but FRES was also significantly related to final expansion rates on leaf blades FER ($r = 0.255$). However this ratio could only be calculated for lines presenting symptoms on stems in semi-natural infection that did not concern the most resistant lines.

Continuous and unimodal distribution of attacks on stems (encircling or not, Figures 2 and 3) in semi-natural conditions suggests that numerous factors are involved in the expression of final symptoms. In contrast, the bimodal distribution FER (Figure 4B) in artificial conditions suggests that a major factor segregated in the population. In genetic diversity studies, Viguié et al. (1999) and Langar et al. (2000) studied sampled genotypes that seem to display an accumulation of different factors for resistance. In the present study, no selection pressure was applied to RI lines, enabling us to observe segregation for each factor of resistance. Consequently, recombination between factors controlling resistance on leaves and resistance on stems occurred in RI lines and led to improved material for resistance to *D. helianthi*.

Because it presents a wide range of variability for different mechanisms leading to resistance to Phomopsis, the RI line from the cross HA89 × LR4-17 is a good candidate for detecting quantitative trait loci (QTLs)

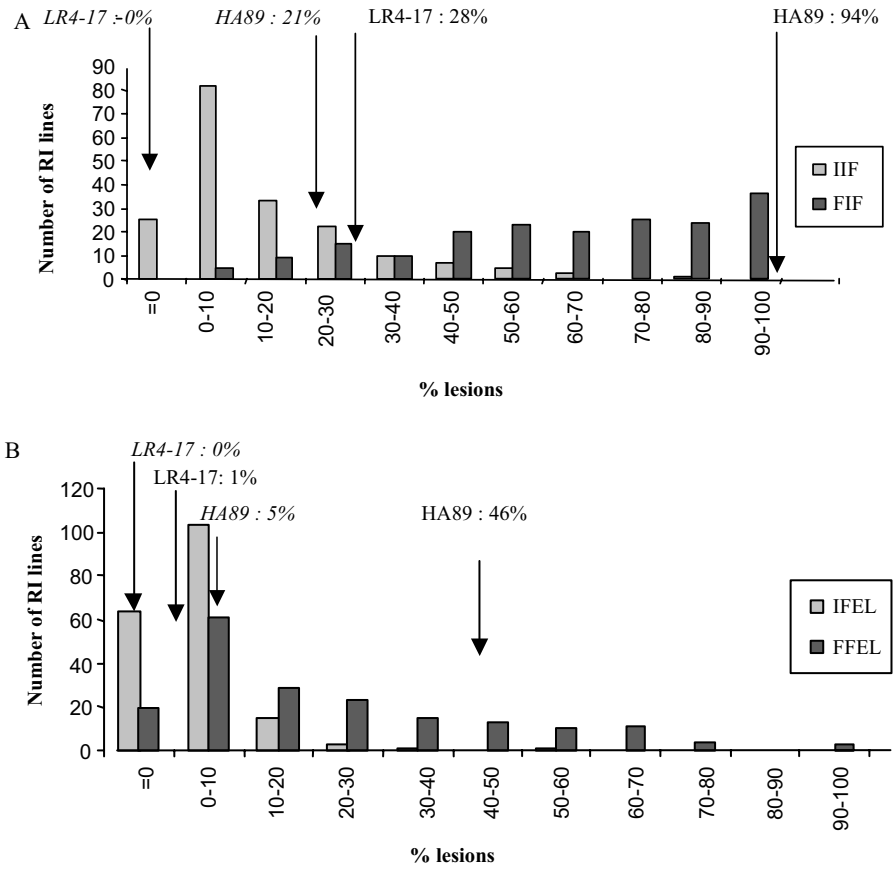

Figure 3. Distributions of % lesion attack by Phomopsis with semi-natural infections in the F7 RI lines. (A) % lesions (IIF and FIF) on stems at flowering and at maturity. Values in italics are controls for IIF. (B) Encircling lesions (IFEL and FFEL) rates on stems at flowering and at maturity. Values in italics are controls for IFEL.

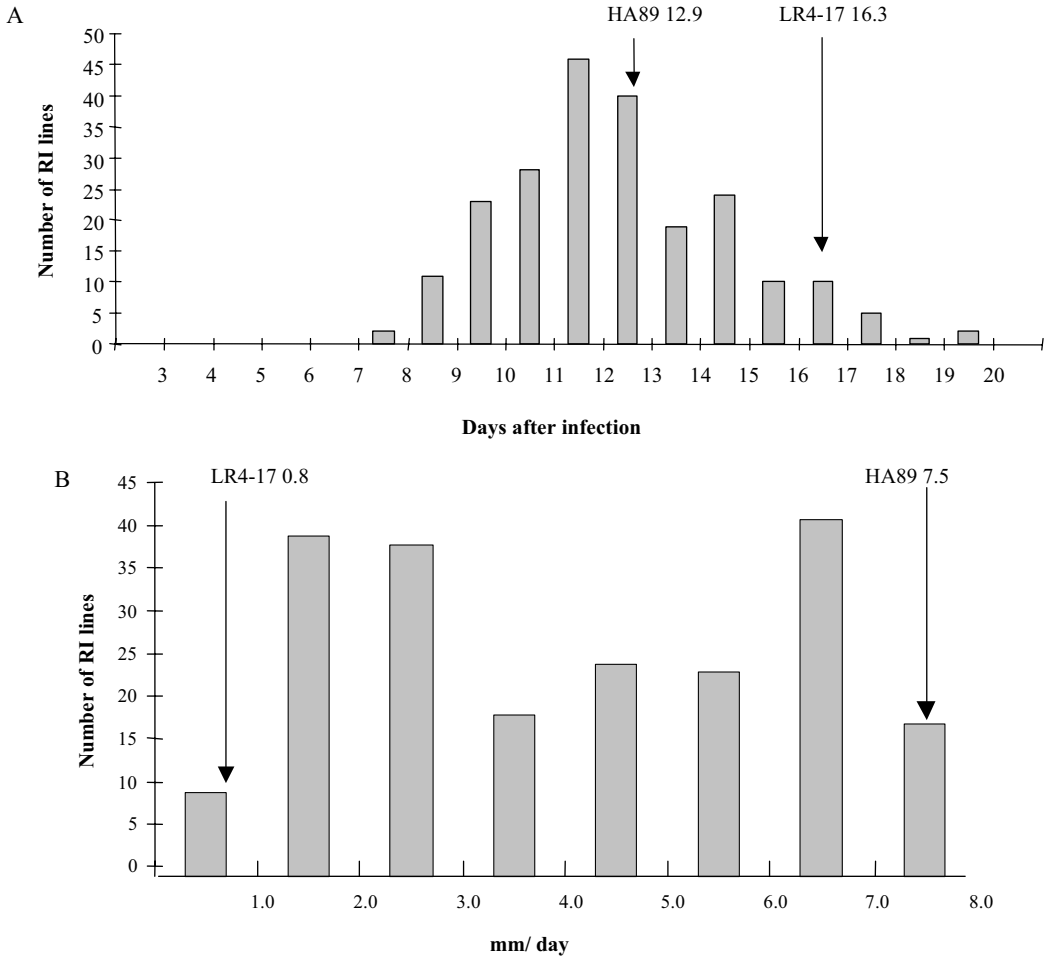

Figure 4. Distributions of traits measured in F7 RI lines with artificial infections. (A) Distribution of the LP. (B) Distribution of the FER of necrosis on leaf blades.

for resistance to Phomopsis. Expansion rate of lesions were highly heritable, but complex to obtain. The segregation of a major factor that would control them has to be confirmed by using genetic markers.

References

Bertrand F and Tourvieille D (1987) Phomopsis tournesol: Tests de sélection. Informations Techniques CETIOM 98: 12–18

Besnard G, Griveau Y, Quillet MC, Serieys H, Lambert P, Varès D and Bervillé A (1997) Specifying of introgressed regions from *H. argophyllus* in cultivated sunflower (*Helianthus annuus*) to mark Phomopsis resistance genes. Theoretical and Applied Genetics 94: 131–138

Burr B and Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends in Genetics 7: 55–60

Degener J, Melchinger EA and Hahn V (1999) Inheritance of resistance to *Phomopsis* in sunflower: Study of leaf and stem resistance after artificial and natural infection, Helia 22: 105–115

Deglène L, Alibert G, Lesigne P, Tourvieille de Labrouhe D and Sarrafi A (1999) Inheritance of resistance to stem canker (*Phomopsis helianthi*) in sunflower. Plant Pathology 48: 559–563

Gallais A (1990) Théorie de la sélection et amélioration des plantes, Masson, Paris, 588 p

Griveau Y, Serieys H and Belhassen E (1992). Resistance evaluation of interspecific and cultivated progenies of sunflower infected by *Diaporthe helianthi*. In: Proceedings of the 13th International Sunflower Conference, Vol II, Pisa, Italy, 7–11 September, (pp 1054–1058)

Langar K, Griveau Y, Vares D and Bervillé A (1997) Evaluation of resistance to Phomopsis on wild species, cultivars and inbreds of sunflower with artificial and seminatural infections. 10th Congress of the Mediterranean

Phytopathological Union, Montpellier, France, 1–5 June (pp 831–837)

Langar K, Griveau Y, Ziercher L, Serieys H and Bervillé A (2000) Comportement de génotypes de tournesol (*Helianthus annuus* L.) vis-à-vis de cinq isolats de Phomopsis (*Diaporthe helianthi* Munt-Cvet. et al.) observé à partir d'infections artificielles sur feuilles, In: Proceedings of the 15th International Sunflower Conference, Vol II, Toulouse, France, (pp K96–101)

Pinochet Xand Estragnat A (1996) Field evaluation of hybrid sensitivity to *Diaporthe helianthi*: relation between symptoms and yield losses. In: Proceedings of the 14th International Sunflower Conference, Beijing, China, Symposium I: Disease tolerance in sunflower, (pp 102–105)

Piquemal G (1970) How to produce hybrid sunflower seeds by inducing male sterility with gibberellic acid. In: Proceedings of the 4th International Sunflower Conference, 23–25 June Memphis, USA, (pp 127–135)

SAS Institute (1996) SAS/STAT language guide for personal computers. Release 6.12.SAS Institute, Cary, NC, USA

Schneiter AA and Miller JF (1981) Description of sunflower growth stages. Crop Science 21: 901–903

Shapiro SS and Wilk MB (1965) An Analysis of Variance Test for Normality (Complete Samples). Biometrika 52: 591–611

Skoric D (1985) Sunflower breeding for resistance to *Diaporthe/Phomopsis helianthi* Munt-Cvet et al. Helia 8: 21–24

Tourvieille D and Vear F (1986) Culture du tournesol sous tunnel en filet avec humectation contrôlée pour l'étude du *Sclerotinia sclerotiorum*. Informations Techniques CETIOM 96: 20–28

Vear F, Garreyn M and Tourvieille D (1997) Inheritance of resistance to Phomopsis (*Diaporthe helianthi*) in sunflower. Plant Breeding, Berlin 116: 277–281

Viguié A, Vear F and Tourvieille de Labrouhe D (1999). Interaction between French isolates of *Phomopsis/Diaporthe helianthi* Munt-Cvet et al. and sunflower (*Helianthus annuus* L.) genotypes. European Journal of Plant Pathology 105: 693–702

Viguié A, Tourvieille de Labrouhe D and Vear F (2000) Inheritance of several sources of resistance to Phomopsis stem canker (*Diaporthe helianthi* Munt-Cvet.) in sunflower (*Helianthus annuus* L.). Euphytica 116: 167–179

Vranceanu AV, Craiciu DS, Soare G, Pacureanu AV, Voinescu G and Sandu I (1993) Sunflower genetic resistance to *Phomopsis helianthi* attack. In: Proceedings of the 13th International Sunflower Conference, Vol II, Pisa, Italy, (pp 1301–1306)