Research on a growth chamber test to measure quantitative resistance to sunflower downy mildew

Frédéric Serre, Pascal Walser, Sylvie Roche, Félicity Vear, Denis Tourvieille de Labrouhe
INRA, UMR 1095, 234 avenue du Brézet, 63100 Clermont-Ferrand,
France, tourvie@clermont.inra.fr

ABSTRACT

A test on sunflower seedlings in a growth chamber would facilitate measurement of factors determining quantitative resistance to downy mildew compared with the complex field trials necessary at present. Tests based on the usual method to determine major gene resistance were made on genotypes with no efficient major gene. Observations were made of the percentage of plants showing either damping off or sporulation on cotyledons and true leaves, with the aim of representing the percentage of systemically diseased plants observed in field trials. It was found that radicle length needs to be between 3 and 10mm at infection to obtain the closest correlation between field and growth chamber results for inbred lines. For hybrids, there was no significant correlation with field results and hybrids made between one tester line and varied inbreds showed reduced variability compared with per se values. Use of this test to understand quantitative resistance and to improve durability of resistance is discussed.

Key words: germination – *Helianthus annuus* – *Plasmopara halstedii* – quantitative resistance – sporulation

RESUME

Afin de phénotyper des génotypes de tournesol pour leur caractère de résistance partielle non-race spécifique, nous avons recherché un test en laboratoire. L'intérêt du protocole de notation a été validé par comparaison avec le comportement des génotypes observé en infections naturelles en absence de toutes méthodes de lutte culturale ou chimique. Les tests sont réalisés en chambre de culture dans les conditions identiques à celles qui sont utilisées pour la caractérisation des résistances monogéniques dominantes**.** Nous utilisons un pathotype virulent vis-à-vis des génotypes en évaluation. Les observations portent sur l'importance des symptômes: la fonte de semis et l'étendue des sporulations sur les organes aériens. Le taux de plantules présentant des symptômes forts et caractéristiques de mildiou lors du test en laboratoire est bien corrélé avec le taux de plantes montrant une infection primaire (infection tellurique) lors des observations réalisées en plein champ. L'intérêt de ce test en laboratoire pour sélectionner des variétés de tournesol présentant un bon niveau de résistance non race spécifique au mildiou est discuté.

INTRODUCTION

Downy mildew resistance in sunflowers has mainly been based on use of major resistance genes, denoted *Pl*. However, selection pressure on the parasite, *Plasmopara halstedii* has led to the appearance of new races (Tourvieille de Labrouhe et al., 2005) which could cause reduced crop yields in areas where weather conditions at sowing are favourable to the disease (Délos et al., 2000). To obtain more durable resistance, research was made for quantitative resistance and field trials showed high levels of partial resistance in cultivated sunflower which would be useful in breeding (Tourvieille de Labrouhe et al., 2008). However, field trials with downy mildew are complex and limited to the naturally occurring race. To make possible studies of reaction to different races, or large scale early breeding tests, a laboratory test on sunflower seedlings, measuring frequency or extent of downy mildew symptoms, is required. This paper reports experiments measuring the frequency of sporulation on the first true leaves, and the effects of radicle length when infected, for both inbred lines and hybrids. The results were compared with those obtained in field trials.

MATERIALS AND METHODS

Plasmopara halstedii race. Race 710 was shown to be naturally present in the field at Clermont-Ferrand, by observation of differential lines (Gulya et al., 1998). In the growth chamber, the same race was used.

Sunflower genotypes. Two series of genotypes were used (Table 1):

- 44 inbred lines and 45 hybrids obtained from crosses between these lines, considered as representing the variability present in modern cultivated sunflower.
- 40 recombinant inbred lines (RIL) chosen, among a population obtained from a cross between 2 INRA lines, XRQ and PSC8, for their diversity of reaction in downy mildew field trials. They carried either no *Pl* gene or *Pl*2, which is not effective against race 710. Hybrids between these lines and a very susceptible line GB (Vear et al., 2006) were also studied.

Table 1. Numbers of inbred lines and hybrids used in each experiment

Germination stage	Radicle length	Inbred - hybrid comparison	Heredity
44 inbreds	40 RIL	40 RIL	11 inbreds
45 hybrids	40 GB x RIL	40 GBxRIL	28 hybrids

Field trials. Methodology used was that of Tourvieille de Labrouhe et al. (2008). The level of attack of each genotype was defined according to the percentage showing damping off, yellowed leaves or dwarfing, characteristic of primary downy mildew attacks (Tourvieille de Labrouhe et al., 2000). Three to 4 weeks after sowing (cotyledon stage), the number of plants emerged in each plot was counted (including those showing symptoms of damping off). Two to 3 weeks later (2-3 pairs of leaves), the number of healthy plants per plot were counted (rather than the number of diseased plants since some of these had already withered). Percentage infection was then calculated (from 100-% healthy plants).

Growth chamber experiments. Growth chambers, in accordance with quarantine regulations, had 16h light (12000 lux) with a temperature of 18±1°C and 65-90% RH. Methodology was based on that of Roche et al. (2005) for testing major gene resistance. Sunflower seeds with radicle lengths between 1 and 30mm were soaked for 3h in fresh zoosporangia suspensions (100,000/ml), obtained from infected seedlings covered with polythene bags for 48h. For the germination stage trial, germinating seeds were divided into 2 groups: those with radicles of <5mm and those with radicles of >5mm. For the radicle length trial: germinated seeds were photographed before infection to permit measurement of radicle lengths. Seedlings were then pricked out in trays with Klasmann Seedlingsubstrat NF U 44-551 compost.

After 12 days, seedlings were maintained at 100%RH for 48h. Since all genotypes were susceptible to race 710, they all showed some sporulation on the shoot. However, symptom intensity varied and plants were placed in 1 of 3 classes: 1= Damped off (rotting before or after emergence); 2= Sporulation on cotyledons and at least 1 true leaf; 3= More or less sporulation on cotyledons but no sporulation on true leaves. From these observations, the percentage of "completely susceptible" (%CS) was calculated from the sum of classes 1 and 2 compared with the total.

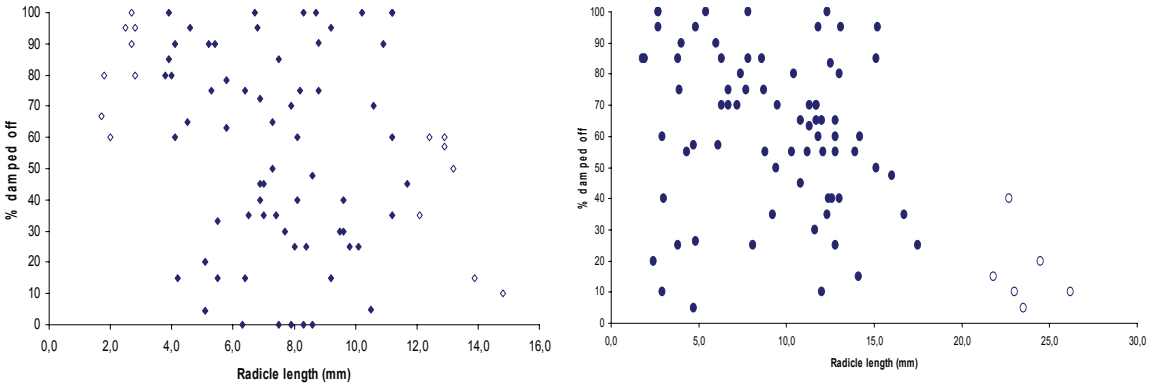
RESULTS

Effect of germination stage. The %CS was significantly higher for seedlings with radicles <5mm (Table 2) but the results of the 2 series were significantly correlated (Pearson correlation): inbred lines r= 0.774** , hybrids: r= 0.572**. However, 7 inbred lines and 15 hybrids showed more symptoms with radicles >5mm.

Table 2. Percent of seedlings showing complete susceptibility (%CS) according to germination stage when infected

		Radicle length	
		< 5 mm	≥ 5 mm
44 inbred lines			
	Mean	73.1%	58.2%
Extremes			
	inbred F340	100.0	35.7
	inbred MO502	44.0	65.0
45 hybrids			
	Mean	65.2%	52.8%
Extremes			
	hybrid CD x 90R18	100.0	0.0
	hybrid SL x PAZ2	47.6	75.0

Effect of radicle length on proportion of damped off seedlings. Fig. 1a and 1b present the relations between radicle length measured from photographs before infection and proportion of damped off seedlings of RIL and their hybrids, respectively. For the RIL, the 8 genotypes showing the shortest radicles (<3mm) were all damped off at >60% whereas the 8 with radicles of >12mm always showed less than 60% damping off.



a.) b.)

Fig. 1. Proportion of damping off according to radicle length: a.) 40 RIL; b.) 40 hybrids (2 replications)

The hybrids showed a more rapid germination than the inbred lines and there was no clear effect of short radicles, but the hybrids with long radicles (>16mm) again had a low level of damping off (<50%).
Comparison RIL - hybrids: Since germination rate appeared to be important in measurement of quantitative resistance, the vigour provided by hybrid seed could help to provide uniformity between genotypes. The reactions of 40RIL were thus compared with those of their hybrids with GB (Fig. 2). The hybrids showed a greater proportion of CS (68.2%) than the RIL (56.2%), probably related to the high susceptibility of GB. As could be expected from crosses with a single tester line, there was less variation among the hybrids than among RIL, but the results were significantly correlated (r= 0.392*).

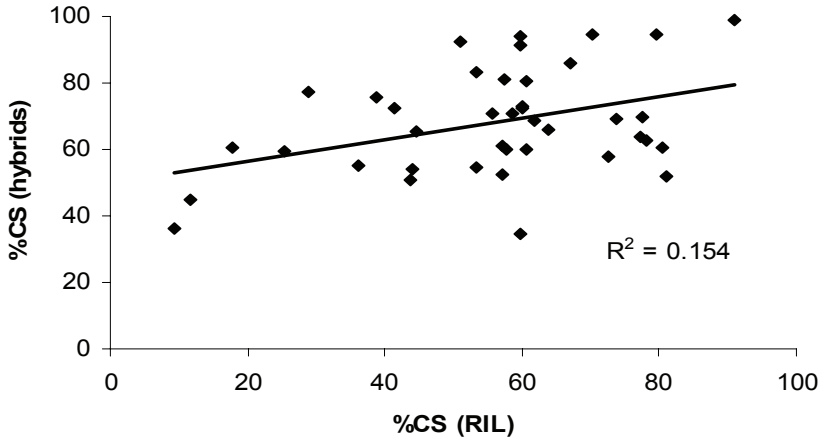


Fig. 2. Relations between 40 RIL and 40 (GB x RIL) hybrids: mean % completely susceptible in 4 tests.

Choice of test conditions according to relations between growth chamber tests and field trials. Fig. 3a and 3b show that when the germinated seed was divided into 2 groups, radicles <5mm or >5mm, the closest correlation between %CS in growth chamber and % infection in the field was obtained with radicles <5mm. For the RIL and their hybrids, where radicle length was measured at infection and long radicles showed little damping off whereas short radicles of inbred lines showed a high level of damping

off, correlations were made with field attack, including and excluding the genotypes concerned. Exclusion of seedlings which had probably not been infected correctly did not exclude extreme reactions and improved the correlation for inbred lines but made no difference for hybrids (Table 3). In addition, when reactions of inbred lines in the field were compared with those of their hybrids under test, there were no significant correlations. It may thus be concluded that to judge inbred lines it is better to test the lines than hybrids made with a single tester genotype.

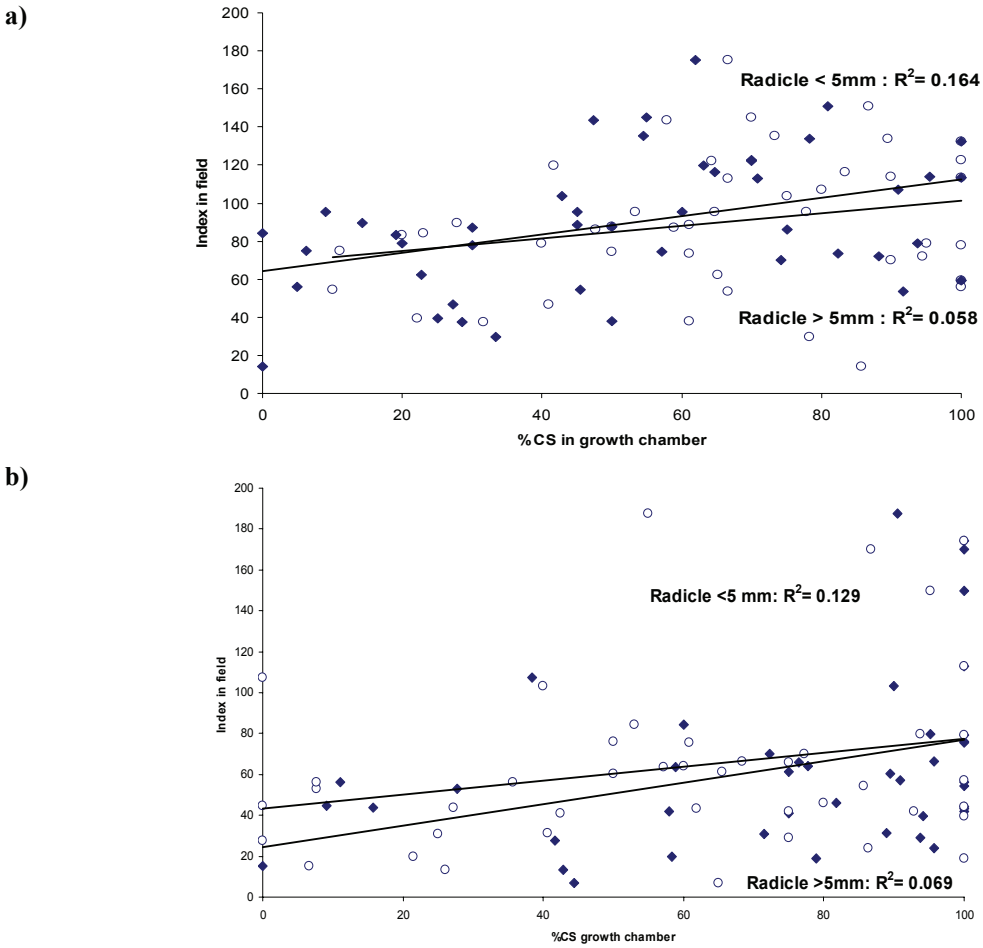


Fig. 3. Comparison of downy mildew attack on 45 hybrids (a) and 44 inbred lines (b) in the field and in the growth chamber test according to radicle length in the test.

Table 3. Correlations between percent downy mildew attack in field trials and in growth chamber tests, including and excluding genotypes with very short or very long radicles when infected in the growth chamber

	Complete series		Excluding genotypes with very short or very long radicles.	
	Nb genotypes	Pearson correlation	Nb genotypes	Pearson correlation
RIL in growth Chamber[(2)] / RIL in field trials[(1)]	40	r= **0.484****	35	r= **0.548****
Hybrids in growth Chamber[(2)] / Hybrids in field trials[(1)]	40	r= 0.188 ns	34	r= 0.274 ns
Hybrids in growth Chamber[(2)] / RIL in field trials[(1)]	40	r= 0.214 ns	35	r= 0.248 ns

[(1)] % attack compared with mean of 4 check lines (4 replications of 30 plants)
[(2)] % completely susceptible plants (%CS) (2 replications of at least 10 plants)

Inheritance of percent completely susceptible plants in growth chamber tests. The 28 hybrids from a factorial cross of 7 female lines and 4 restorers, and the parental lines were tested in the growth chamber. Results are presented in Table 4.

Table 4. Percent completely susceptible plants in growth chamber tests on hybrids of a factorial cross and their parental lines.

Males / Females	83HR4	PR56	PAZ2	90R18	Mean hybrid value	Inbred line
FU	61.1	4.2	11.1	22.2	24.7	15.8
FRIGA	80.0	42.9	10.0	100.0	58.2	38.5
IR	66.7	78.3	40.9	85.7	67.9	100.0
GX	58.8	31.6	90.0	65.2	61.4	89.5
SL72	100.0	83.3	47.6	100.0	82.7	100.0
GU	86.7	75.0	89.5	95.0	86.5	90.5
HA89	40.0	23.1	20.0	100.0	45.8	100.0
Mean hybrid value	70.5	48.3	44.2	81.2		
Inbred line	100.0	57.9	11.1	95.7		

The female line FU appears best with hybrids showing low percent of completely susceptible plants, and this is also true for the restorers PR56 and PAZ2. The mean parent - hybrid correlation was highly significant (r= 0.617**) (Fig. 4).

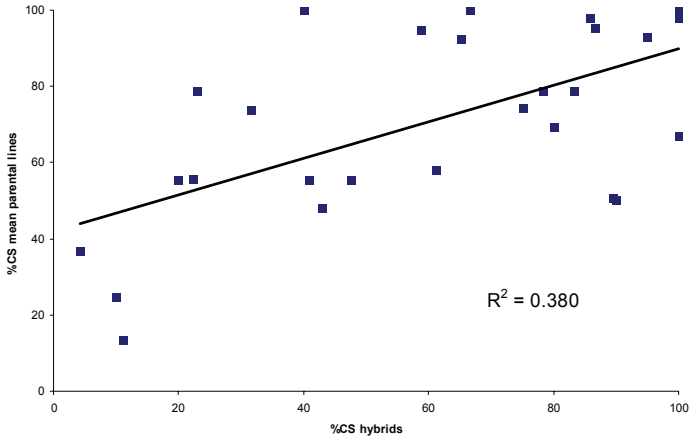


Fig. 4. Relations between percent completely susceptible plants in growth chamber test of hybrids compared with parental inbred lines

DISCUSSION

Horizontal reactions (according to Robinson, 1973) of sunflower genotypes to parasites depend on many environmental factors. It is often difficult to design a laboratory test which gives a good indication of the mean response from multi-location trials over several years in the field (Ladsous et al., 1991; Viguié et al., 2000; Eva, 2002; Serre et al., 2004). However, in controlled conditions, it may be possible to obtain more detailed knowledge of the processes involved in resistance even if the results of a single test do not represent the overall field reaction. The results reported here concern the probability that a systemic downy mildew attack will develop after infection of sunflower seedlings at their most susceptible stage under environmental conditions favourable for the disease.

The percentage of completely susceptible plants for each genotype depends clearly on radicle length at infection. This is probably because the pathogen infects plant tissues through the extremities of root hairs (Allard, 1978) and to provoke symptoms characteristic of systemic infection, it must reach the apical meristem very quickly. The test described here measures this possibility and the results show that for the germination stage to be the most uniform possible, infection should be made of seedlings with radicles

measuring between 3 and 10mm. This measurement of the proportion of completely susceptible seedlings in the growth chamber is indicative of reaction in field trials for many inbred lines, but it does not represent all possible resistance factors. Genotypes such as "IR" and "90R18", which have high levels of resistance in the field, appear very susceptible in the growth chamber. Factors other than the germination rate are certainly involved; some examples could be resistance to infection or tissue receptivity (Mazeyrat et al., 1999). These cannot be studied in the field and will require additional growth chamber or laboratory tests.

In 2000, changes in downy mildew races in France suggested that sole use of monogenic resistances was a strategic error (Tourvieille de Labrouhe, 2000) and more recently it has been confirmed that major gene resistance alone does not provide durable control of downy mildew (Tourvieille de Labrouhe et al., 2005). Quantitative resistance is most often non-race specific and the levels observed in cultivated sunflowers suggest that this type of resistance should be used in breeding, at least to complement major genes. The test proposed here is a first step in understanding this resistance and making routine breeding programmes for this character possible.

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