Can management of *Pl* genes influence aggressiveness in *Plasmopara halstedii* (sunflower downy mildew)?

Nachaat Sakr[1], Jeanne Tourvieille[2], Pascal Walser[1], Félicity Vear[1], Mireille Ducher[2], Denis Tourvieille de Labrouhe[1]

[1]INRA-UBP, UMR 1095, 234 Avenue du Brézet, 63100 Clermont-Ferrand, France, E-mail: tourvie@clermont.inra.fr

[2]INRA-UBP, UMR 1095, 24 Avenue des Landais, 63177 Clermont-Ferrand, France

ABSTRACT

Evolution of aggressiveness in populations of race 710 of *Plasmopara halstedii* was measured under different strategies of *Pl* gene management: mixture, alternation and monoculture of major resistance genes in comparison with a population under no selection pressure. Two sunflower lines showing different levels of non-race-specific resistance were used to measure four aggressiveness criteria: length of latent period, sporulation density, percentage infection, and hypocotyl length. The sunflower inbred line BT, rather susceptible in the field, presented a higher percentage infection, a higher sporulation density, a lower latent period and less reduced hypocotyl length than inbred line FU, which has greater resistance in the field. Differences were observed between *P. halstedii* populations under different strategies of *Pl* gene management. Strains multiplied under varietal mixtures gave the greatest sporulation densities and shortest hypocotyl lengths, those multiplied under alternation gave a reduced latent period and shorter hypocotyl lengths compared with those not influenced by selection pressure. There were no significant differences between populations multiplied under monoculture of resistance genes and those under no selection pressure. These differences appear to be linked to the number of diseased plants present. The results suggested that the method of *Pl* gene management affects aggressiveness because it determines the number of susceptible plants harbouring the parasite. Applications of these strategies of *Pl* gene management are discussed.

Key words: alternation – mixture– monoculture – pathogenicity – *Pl* gene

RESUME

L'évolution de l'agressivité des populations du profil 710 de *Plasmopara halstedii* a été mesurée sous différentes stratégies de gestion de gènes *Pl*: l'alternance, l'assemblage et la monoculture de source de la résistance en comparaison avec une population n'ayant subit aucune pression. Deux génotypes du tournesol présentant des niveaux différents de résistance non-race spécifique ont été utilisés pour mesurer quatre facteurs de l'agressivité: le taux de réussite de l'infection, la durée de latence, la densité de sporulation et la longueur de l'hypocotyle. Les souches récoltées sous les systèmes de l'alternance et de l'assemblage présentent des durées de latence les plus courtes significativement sur le génotype résistant et des longueurs de l'hypocotyle les moins grandes sur le génotype le plus sensible par rapport aux souches multipliées sous la monoculture de source résistant. De plus, les souches récoltées sous le système de l'alternance présentent des densités de sporulation plus élevées sur les deux génotypes. Cette évolution semble directement liée à la présence de nombreuses plantes malades dans ces dispositifs. Nos résultats suggèrent l'existence d'un impact du mode de gestion des gènes *Pl* sur l'évolution de l'agressivité. Seules les stratégies qui maintiennent des effectifs de la population parasitaire assez élevés permettent une évolution de l'agressivité de *P.halstedii*. Les résultats sont discutés aux regards à la mise en œuvre de ces méthodes de gestion.

Mots-clés: alternance – assemblage – monoculture – pathogenicité – *Pl* gene

INTRODUCTION

Selective effects on pathogenicity due to host resistance are an important aspect of plant-pathogen interactions, which can be divided into two parts: virulence (specific disease-causing abilities) and aggressiveness (non-specific disease-causing abilities) according to Van der Plank (1968). There have been many reports concerning increase of virulence in relation to host resistance in pathogens of economically important crops (McDonald and Linde, 2002). Similarly, Gandon and Michalakis (2000) predicted that increased levels of quantitative host resistance may select for increased aggressiveness of parasites, leading to increased crop losses. Cowger and Mundt (2002) showed that wheat cultivars with good partial resistance selected more aggressive isolates of *Mycospharella graminicola*. However, this is not always true, Sullivan et al. (2005) reported that tobacco cultivars with high levels of quantitative resistance did not select for more aggressive isolates of *Phytophthora parasitica* var. *nicotiana*e. Also, Flier et al. (2007) showed that, following large-scale introduction of more resistant potato varieties in organic production systems in Europe, there was no shift towards increased levels of aggressiveness of *Phytophthora infestans* populations.

Plasmopara halstedii (downy mildew) is a pathogen specific to sunflower, present in most areas of the world where this crop is grown. It shows physiological races (pathotypes) capable of infecting a variable range of sunflower genotypes. The nomenclature of these races is based on the reaction of a series of differential lines (Gulya et al., 1998). Race specific resistance is controlled by major genes, denoted *Pl*. Tourvieille de Labrouhe et al. (2005) showed that whatever the method of management (mixture, alternation, monoculture) of *Pl* genes, their selection pressure led to appearance of new virulences.

This paper reports studies of levels of aggressiveness in 3 populations of *P. halstedii,* race 710, obtained under different strategies of *Pl* gene management: mixture, alternance and monoculture, in comparison with a population obtained in the absence of any effective *Pl* gene.

MATERIALS AND METHODS

Sunflower genotypes

Four quasi-isogenic hybrids were used, obtained from crosses of 2 forms each of two inbred lines:

- L1a: carrying resistance gene *Pl2,* resistant to race 100 and susceptible to race 710,
- L1b: carrying resistance genes *Pl2* and *Pl8,* resistant to races100 and 710,
- L2a: carrying no known resistance gene,
- L2b: carrying resistance gene *Pl6,* resistant to races 100 and 710.

The four hybrids were produced as follows: H1= L1a x L2a, H2= L1a x L2b, H3= L1b x L2a and H4= L1b x L2b.

P. halstedii strains present in the soil were trapped with a sunflower hybrid (Airelle), carrying no downy mildew resistant gene. To characterise aggressiveness of *P. halstedii* strains, two inbred lines not carrying any *Pl* gene and known to have different levels of non race specific resistance (Vear et al., 2007) were studied: FU and BT.

Experimental protocol

The protocol was developed by Tourvieille de Labrouhe et al. (2005) to determine durability of resistance. Four plots constituted by netting cages were maintained with climate conditions favourable for expression of disease. Plot P1 was planted in four consecutive years with H1 (no effective resistance against race 710). Plot P2 was planted all years with an equal mixture of the four hybrids. Plot P3 was planted in first year with H1, then successively with H2, H3 and H4. Plot P4 was planted with H2, resistant to race 710, in all 4 years.

P. halstedii *strains*

After 4 years, *P.halstedii* strains were collected from soil according to the method described by Tourvieille de Labrouhe et al. (2008) and their virulence profile characterised by the method of Gulya et al. (1998). For plots 1, 2 and 3, four strains were analysed and for P4, 3 strains.

Measurements of aggressiveness

The protocols developed by Sakr et al. (2008) were used, determining:

- Length of period between infection and sporulation on 80% of infected plants = latent period,
- Maximal sporulation density on cotyledons obtained 12 and 13 days after infection = sporulation density,

- Percentage infection = % infection,
- Hypocotyl length 13 days after infection, calculated by a percentage of the hypocotyl length of healthy plants = hypocotyl length.

All tests were carried out in growth chambers respecting European regulations (No 2003/DRAF/70).

Statistical Analyses

All statistical analyses of the phenotypic data were performed using StatBox 6.7® (GimmerSoft) software. To compare strains and genotypes, there were 2 replications for sporulation density and 3 replications for percentage infection, latent period and hypocotyl length. To compare each characteristic in the different plots, the means of each strain were used as replications in one-way analyses of variance (ANOVA). The Newman-Keuls test was used to compare the means at P=0.05

RESULTS

Changes in percentage attack in the 4 plots

Data are presented in Table 1.

Table 1. Changes in downy mildew attack in 4 plots observed over 5 years.

Plots	**2001**[1]	**2002** (*)	**2003** (*)	**2004** (*)	**2005**
P1					
% diseased plants	71.5	37.4	75.4	60.3	
Number of diseased plants	203	125	215	194	
% of race 710	100	100	100	100	84.0
P2					
% diseased plants	13.9	6.5	9.9	15.1	
Number of diseased plants	43	19	33	51	
% of race 710	100	100	81.0	91.3	48.9
P3					
% diseased plants	75.2	1.1	1.5	1.1	
Number of diseased plants	236	4	5	11	
% of race 710	100	100	100	9.1	12.5
P4					
% diseased plants	2.7	1.1	4.9	14.8	
Number of diseased plants	10	4	16	52	
% of race 710	100	100	16.7	30.0	34.5

[1]Tourvieille de Labrouhe et al. 2005

Table 1 shows that total numbers of diseased plants differed between plots (from 737 for plot P1 to 82 for plot P4). There was a continued reduction in percentage of *P.halstedii* samples of race 710 especially in the absence of susceptible sunflower genotypes in plots P3 and P4. Nevertheless this race was present in soil samples taken in 2005 from all plots.

Comparison of aggressiveness of 15 strains of race 710 on inbred lines FU and BT

The two sunflower lines gave a significantly different response (Table 2).

Table 2. Anova on aggressive criteria of 15 strains of *P. halstedii* measured on two sunflower lines.

% infection	Line effect			Strain effect			Interaction		
	BT	FU	Significant	Mini	Maxi	Significant	Min.	Max.	Significant
	100%	99.3%	P<0.001	98.6%	100%	NS	97.2%	100%	NS
Sporulation density (zoosporangia per cotyledon)	963 10^5	788 10^5	P<0.001	677 10^5	1264 10^5	P<0.001	562 10^5	1343 10^5	NS
Latent period (days)	8.1 d.	9.0 d.	P<0.001	8.3 d.	8.9 d.	NS	7.8 d.	9.7 d.	NS
Hypocotyl length (% of length of healthy plants)	33.0%	40.1%	P<0.001	31.1%	40.3%	NS	26.7%	43.7%	NS

The inbred line BT showed a higher percentage infection, a higher sporulation density, a shorter latent period and less reduced hypocotyl length than FU. The 15 strains appeared as being homogeneous for all criteria analysed except spore density. There was no interaction between parasite strains and host genotypes.

Comparison of strain aggressiveness in each plot

Plot P4 was not distinct from P1 whereas P2 presented greater mean sporulation density and reduction in hypocotyl length, and P3 showed a shorter latent period and greater reduction in hypocotyl length (Table 3).

Table 3. Comparison of means observed for isolates from each plot compared with P1 (no effective *Pl* gene).

	% infection		Latent period (days)		Sporulation density (zoosporangia per cotyledon)		Hypocotyl length (% of length of healthy plants)	
	mean	reference[1]	mean	/reference[1]	mean	/reference[1]	mean	/reference[1]
P1 (reference)	99.65		8.83		8.15		40.00	
P2	99.84	NS	8.55	NS	10.89	S	35.11	S
P3	99.86	NS	8.41	S	8.30	NS	35.10	S
P4	99.09	NS	8.71	NS	7.32	NS	38.03	NS

[1]Test of Newman Keuls, P=0.05

DISCUSSION

The presence of strains of race 710 in plots not grown with a susceptible genotype for 3 (P3) or 4 years (P4) trapped by a susceptible genotype in soil tests may be explained by the maintenance of the inoculum in the soil and/or hybrid seed impurities susceptible to isolates sampled in 2005. With the first hypothesis, the evolution of parasitic populations may depend on characters linked to fitness but independent of aggressiveness, such as their capacity to survive for a long time as oospores. With the second hypothesis, the level of susceptible seed impurities would be the important factor which intervenes in the evolution of parasitic populations.

Study of the reaction of two inbred lines to 15 strains underlined their differences in behaviour. The very good resistance of inbred line FU observed in the field was confirmed by the measurements of aggressiveness criteria described by Sakr et al. (2008). These methods can be used to characterise non-race-specific partial resistance since there were no interactions between genotypes and strains. For the 15 strains analysed, only sporulation density varied (from 1 to 2), overall, the *P. halstedii* strains appeared to be quite homogeneous.

Comparison of parasite populations isolated from the 4 plots showed that strains of race 710 from plot P4 (monoculture of *Pl*6) were not different from the population isolated from P1, with no efficient *Pl* gene. This could be explained on one hand by selection of strains which survive in the soil, independently from the factors of aggressiveness measured, or, on the other hand, by a weak level of parasitic multiplication linked to a small number of plants susceptible to race 710, thus giving incomplete expression of parasitic diversity. This second hypothesis appears most likely because the number of plants infected with race 710 was always very low in plot P4. Plot P2 was grown with a mixture of different hybrid forms, giving 25% of plants susceptible to race 710, one third of which contributed to parasitic multiplication (Table 1). Compared with plot P1, and with few infected plants, it is reasonable to suggest that isolates with a high sporulation capacity could have been favoured and may have caused the secondary infections shown by 20% of infested plants in this plot between 2001 and 2004 (Tourvieille de Labrouhe et al., 2005). These secondary infections contributed to the stock of inoculum which may explain why strains isolated from this plot showed a significantly higher sporulation density. In plot P3 (alternation), the abundant downy mildew population created in the first year, from more than 230 diseased plants, was confronted with new resistance genes every year but race 710 remained in 2005, although at a lower level than in the other 3 plots. This population evolved towards increased aggressiveness as measured by latent period. Compared with plot P4, it had a wider genetic base. Differences in aggressiveness, as compared with plot P1, were weak but significant for latent period, suggesting that, from a similar number of diseased plants, different aggressiveness factors could be

selected if the number of diseased plants is small. The 2 plots that significantly differed for either latent period or sporulation density (i.e., P3 and P2) also differed for hypocotyl length.

It is commonly admitted that non-race specific partial resistance applies selection pressure on parasitic populations, which may lead to more aggressive strains. An example was maize resistance against *Cochliobolus heterostrophus* (Kolmer and Leonard, 1986). In contrast, many authors report that use of race specific resistance does not lead to modifications in aggressiveness. Sullivan et al., (2005) showed that race specific resistance in tobacco did not exert a selective effect on aggressiveness of *Phytophthora parasitica* var. *nicotiana*e and in the pathosystem *Venturia inaequalis* / apple, Parisi et al. (2004) found that virulent strains taken from cultivars carrying vertical resistance genes were highly aggressive. Since the four sunflower hybrids in the present study were isogenic except for their *Pl* genes, it appears reasonable to consider that selection pressure was mainly applied on criteria linked to virulence (Tourvieille de Labrouhe et al., 2005). The results obtained showed positive effects of certain modes of *Pl* gene management on aggressiveness factors. This effect no doubt depends more on the number of susceptible plants than on direct selection pressure of monogenic resistances. It could be suggested that management of *Pl* genes which reduce the number of susceptible plants, limits selection pressure for more aggressive strains, but increases the risk of appearance of new virulence. In contrast, management modes which lead to a non negligible number of diseased plants (mixtures and alternation), may slow down the appearance of new virulence (Tourvieille de Labrouhe et al., 2005), but could favour more aggressive strains. This conclusion must be taken into account in the choice of methods to obtain durable control of sunflower downy mildew with both race-specific and non-race-specific resistance.

ACKNOWLEDGEMENTS

We would like to thank the Syrian Commission of Energy Atomic for a Doctoral Scholarship favouring the first author. This work was carried out with the financial support of the ANR- Agence Nationale de la Recherche (The French National Research Agency) under the Programme Agriculture et Développement Durable, project "ANR-05-PADD-0705, Cedre", and the French Centre Technique Interprofessionnel des Oléagineux Métropolitains (CETIOM).

REFERENCES

Cowger, C., and C.C. Mundt. 2002. Aggressiveness of *Mycosphaerella graminicola* Isolates from Suseptible and Partially Resistant Wheat Cultivars. Phytopathology 92:624-630.

Flier, W.G., L.P.N.M. Kroon, A. Hermansen, H.M.G.van. Raaij, B. Speiser, L. Tamm, J.G. Fuchs, J. Lambion, J. Razzaghian, D. Andrivon, S. Wilcockson, and C. Leifert. 2007. Genetic structure and pathogenicity of populations of *Phytophthora infestans* from organic potato crops in France, Norway, Switzerland and the United Kingdome. Plant Pathol. 56:562-572.

Gandon, S., and Y. Michalakis. 2000. Evolution of parasite virulence against qualitative or quantitative host resistance. Proc. Royal Soc. London, 267:985-990.

Gulya, T.J., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K. Rashid, and F. Viranyi. 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii* (sunflower downy mildew). p. 130-136. In: Proc. of the ISA Symposium. III: Sunflower Downy Mildew. Fargo, ND, USA.

McDonald, B.A., and C. Linde. 2002. Pathogen population genetics and the durability of disease resistance. Euphytica 124:163-180.

Kolmer, J.A., and K.J. Leonard. 1986. Genetic selection and adaptation of *Cochliobolus heterostrphus* to corn hosts with partial resistance. Phytopathology 76:774-777.

Parisi, L., V. Fouillet, H.J. Schouten, R. Groenwoil, F. Laurens, F. Didelot, K. Evans, C. Fischer, F. Gennari, H. Kemp, M. Lateur, A. Patocchi, J. Thissen, and C. Tsipouridis. 2004. Variability of the pathogenicity of *Venturia inaequalis* in Europe. Acta Horticulturae 663:107-113.

Sakr, N., M. Ducher, J. Tourvieille, P. Walser, and D. Tourvieille de Labrouhe. 2007. A new method to obtain monozoosporangial isolates of sunflower downy mildew (*Plasmopara halstedii*). Cryptogamie, Mycologie 28:123-131.

Sakr, N., X. Giresse, P. Walser, S. Richard-Cervera, J. Tourvieille, F. Vear, F. Delmotte, M. Ducher, and D. Tourvieille de Labrouhe. 2008. Variation of aggressiveness of different pathotypes of *Plasmopara halstedii* (agent of Sunflower Downy Mildew). Ms submitted.

Sullivan, M.J., T.A. Melton, and H.D. Shew. 2005. Managing the race structure of *Phytophthora parasitica* var. *nicotiana*e with cultivar rotation. Plant Disease 89:1285-1294.

Tourvieille de Labrouhe, D., E. Mestries, and P. Walser. 2005. Quelles perspectives pour la lutte génétique vis-à-vis du mildiou du tournesol? Oleagineux, Corps Gras, Lipides 12:85-93.

Tourvieille de Labrouhe, D., A. Penaud, P. Walser, E. Mestries, J. Moinard, S. Frederic, Thiery, C.B. Garric, and X. Pinochet. 2008. Determine the «sunflower downy mildew» risk by soil analysis. In: Proc. 17th Int. Sunflower Conf., Cordoba, Spain.

Vanderplank, J.E. 1968. Disease resistance in plants. Academic Press, New York, USA.

Vear, F., F. Serre, S. Roche, P. Walser, and D. Tourvieille de Labrouhe. 2007. Recent research on downy mildew resistance useful for breeding industrial-use sunflower. Helia 30:45-54.