A PLANT MIXTURE MODEL AGAINST *PLASMOPARA HALSTEDII* (SUNFLOWER DOWNY MILDEW)

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Abstract: Sunflower downy mildew caused by *Plasmopara halstedii* is one of the most potentially important diseases. So far, a complete, major gene resistance (*Pl*) has been used successfully. But, with the appearance of eight races in France since 2000, research on more durable resistance was undertaken. In this study, we presented new results concerning the evolution of pathogenicity in *P. halstedii* under conditions of re-enforced infection and different *Pl* gene selection pressure. Moreover, we studied the evolution of virulence and aggressiveness of *P. halstedii* under a mixture model of sunflower inbred lines carrying the two types of resistance (qualitative and quantitative). This sunflower model may enhance durable resistance against *P. halstedii*.

Key words: aggressiveness, major gene *Pl*, pathogen evolution, super race, virulence

INTRODUCTION

Plasmopara halstedii (sunflower downy mildew) causes a common disease in many regions where sunflower (*Helianthus annuus* L.) is grown. The pathogen is an obligate parasite. The disease affects young plants when water content of the soil is high and maximum air temperature is between 15 and 18°C. *P. halstedii* propagates asexually by liberation of zoosporangia produced on lower surfaces of sunflower leaves and reproduces sexually by oospores which are found in crop residues.

P. halstedii has 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 (Tourvieille de Labrouhe 1999). Disease resistance in sunflowers to *P. halstedii* can be classed in one of two categories. The first is qualitative resistance which is conferred by the major *Pl* genes and tends to produce a disease-free plant (Radwan *et al*. 2002). The second is quantitative resistance which is controlled by minor genes and tends to impact the rate of disease development (rate reducing) rather than producing a disease-free plant (Vear *et al.* 2007; Tourvieille de Labrouhe *et al.* 2008). Indeed, the use of one type of resistance does not provide a satisfactory durable resistance against *P. halstedii* (Vear *et al.* 2007; Sakr 2008; Tourvieille de Labrouhe *et al.* 2008).

It is more acceptable that selection pressure exerted by qualitative resistance on parasitic populations may lead to the appearance of a new virulence. On the other hand, a wide usage of host plant varieties presenting high levels of quantitative resistance may lead to the increase in aggressiveness in population of a pathogen (Van der Plank 1968; Robinson 1976; Sacristan and Garcia-Arenal 2008; Stukenbrock and McDonald 2008). Indeed, the biological terms used in this study are defined as follows: virulence is defined as specific disease-causing abilities and aggressiveness as non-specific disease-causing abilities. Pathogenicity is used as a general term indicating the ability to cause disease symptoms (Van der Plank 1968). Johnson (1984) defined durable resistance as a resistance that remains effective while being extensively used in agriculture for a long period in an environment conductive to the disease. The mixture is an association of plants which differ in their reaction to a pathogen and reduce disease severity (Mundt 2002). Robinson (1976) considered the competition between super race and simple race as a principal component in the responses of pathogen populations to host mixtures. Super race (showing low aggressiveness) is able to develop on several host genotypes, whereas, simple race (showing high aggressiveness) on few host genotypes.

Actual situation of *P. halstedii* race evolution in French cultivated zones

Race 100 (European race) was present in the sunflower crop since 1965, until 1987, it was the only race identified. But in 1988 and 1989, two new races namely 710 and 703 appeared (Tourvieille de Labrouhe *et al.* 2005; Delmotte *et al.* 2008). Since then, prospecting each year demonstrated the existence in France of 3 races in 1993,

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5 races in 1994, 6 in 2000, 9 in 2002 and 12 in 2004 (Tourvieille de Labrouhe *et al.* 2005; Delmotte *et al.* 2008). The 8 races identified since 2000 (304, 307, 314, 334, 704, 707, 714 and 717) had not been described previously in other countries. This evolution of *P. halstedii* races seems to be linked with a quasi-exclusive use of *Pl6* gene since 1990. This gene was overcome by the parasite races 704 and 714. Moreover, the late use of another gene *Pl5* led to the apparition of new virulence 334 (Tourvieille de Labrouhe *et al.* 2005; Delmotte *et al.* 2008). Consequently, the *Pl* gene life period seems to be very short from the important use of *Pl* gene on a large cultivated zone. Moreover, Gulya (2007) reported 35 races in different parts of the world.

In 1971, Vear and Leclercq suggested cumulating maximum qualitative resistance genes in one genotype in order to avoid a break-down of the resistance by a new race of downy mildew. The French experimentation (Tourvieille de Labrouhe *et al.* 2005) showed that if this strategy did not accompany complementary agronomic (rotation) or genetic measure (quantitative resistance), it would lead to appearance of new virulence in *P. halstedii*. In these conditions, the life period of *Pl* gene was less than 10 years. Indeed, the rapid evolution of virulence observed in this experimentation was attributed to deployment the same genes used in the French sunflower cultivated zones (Tourvieille de Labrouhe *et al.* 2005). However, the same authors showed that whatever the method of management (mixture, alternation or monoculture) of *Pl* genes, their selection pressure led to the appearance of a new virulence.

The present study gives new results concerning the evolution of pathogenicity in *P. halstedii* which is multiplied under several methods of *Pl* genes management (Sakr 2008; Sakr *et al.* 2008b; Sakr 2009b). We propose, based on these results, a sunflower mixture model against *P. halstedii*. It is an association of sunflower inbred lines carrying major genes (Radwan *et al.* 2002; Dussle *et al.* 2004) and quantitative resistance recently identified (Vear *et al.* 2007; Tourvieille de Labrouhe *et al.* 2008). In this model, we imagine the evolution of virulence and aggressiveness of pathogen under conditions of sunflower plants mixture. It may enhance durable resistance against *P. halstedii*. We need such studies because sunflower downy mildew was controlled in France until mid 2000 by using vertical resistance which was led to the appearance of a new virulence in the pathogen (Tourvieille de Labrouhe *et al.* 2005; Delmotte *et al.* 2008).

Sunflower mixture model against *P. halstedii*

The dilution of pathogen inoculum is due to the increase of distance between plants of the same host. This appears to be the most important mechanism which increases durable resistance against pathogens (Mundt 2002).

Theoretically, host-diversity effects in mixtures of cultivars are maximized in the presence of the two following cases together. First, when one host plant expresses differential (qualitative) resistance to pathogen races. Such a genotype acts as a completely resistant host to virulent fraction of the pathogen population (Garrett and Mundt 1999). Second, when another host plant presents various levels of quantitative resistance that reduce disease severity (Garrett *et al.* 2001; Andrivon *et al.* 2003).

In sunflower downy mildew, our experimental model is to make a mixture of plants carrying vertical and horizontal resistances in a given environment, by respecting these two points:

1. The use of sunflower inbred lines carrying resistance genes such as *Pl8* or *Pl*ARg corresponding to virulence genes in *P. halstedii* (Radwan *et al.* 2002; Dussle *et al.* 2004). It prevents the dispersion of pathogen among plants in the field, and in this agro-system, our target is to know whether the conditions of heterogeneity of sunflower lines could be used to increase the level of durable resistance against *P. halstedii*. The sunflower inbred lines carrying effective *Pl* genes would be cultivated alternately with sunflower inbred lines carrying highly and moderately quantitative resistance in the field; whereas the sunflower lines carrying qualitative resistance planted alternately with the sunflower lines carrying quantitative resistance.
2. The use of sunflower inbred lines carrying highly and moderately horizontal resistance (Vear *et al.* 2007; Tourvieille de Labrouhe *et al.* 2008). It decreases quantity of the pathogen.

This strategy supposes that pathogenicity of *P. halstedii* would slowly and difficultly develop on sunflower genotypes carrying qualitative and quantitative resistance. Consequently, it would limit fungal capacity to reproduce and disperse among the plants of a mixture.

Rate of pathogenicity evolution in *P. halstedii*

In the literature, it is widely accepted that stability of a mixture resistance depends on the ability of pathogen populations to evolve their pathogenicity (virulence and aggressiveness) according to Sacristan and Garcia-Arenal; Stukenbrock and McDonald (2008). The virulence is a driving force in host-pathogen co-evolution, since it enables pathogen to overcome qualitative resistance genes *R*. The aggressiveness enables the pathogen to develop within the host plant (Van der Plank 1968; Robinson 1976). *P. halstedii* presents a case with a high level of variability (Albourie *et al.* 1998; Spring and Haas 2002; Gulya 2007; Sakr *et al.* 2007; Delmotte *et al.* 2008; Komjati *et al.* 2008; Sakr 2008; Sakr *et al.* 2008b; Sakr *et al.* 2009a, b) which may help to increase its evolutionary potential.

***P. halstedii* ability to develop more virulent strains**

In *P. halstedii* populations appeared in sunflower parcels (Tourvieille de Labrouhe *et al.* 2005; Sakr 2008, 2009b) showed that the strain (super race) of race 714 was more virulent and less aggressive than the strain (parental race) of race 100 (Table 1). The aggressiveness criteria (Sakr *et al.* 2008; Sakr 2009b; Sakr *et al.* 2009) presented in table 1 were measured on sunflower inbred lines carrying different levels of quantitative resistance (Vear *et al.* 2007; Tourvieille de Labrouhe *et al.* 2008). Short latent period, high sporulation density and, important reduction in the length of hypocotyl represent high aggressiveness (Sakr *et al.* 2008; Sakr 2009b; Sakr *et al.* 2009). Moreover, this strain (super race) was more virulent and did not show any difference of aggressiveness compared to the other strain (parental race) of race 710 (Table 1). Virulence profile of strains (super race, simple race and parental races) was presented in table 2.

Table 1. Responses of *P. halstedii* strains to sunflower inbred lines[v]

P. halstedii strains	Aggressiveness criteria		
	latent period[w] [days]*	sporulation density[x] (zoosporangia per cotyledon)*	hypocotyl length[y] [mm]*
Strain (Parental race) of virulence profile 100	8.51 b	1772.75 10^3 b	28.97 a
Strain (Parental race) of virulence profile 710	9.56 a	764.25 10^3 c	28.92 a
Strain (simple race) of virulence profile 300	8.35 b	1968.5 10^3 a	30.45 a
Strain (Super race) of virulence profile 714	9.82 a	810.00 10^3 c	29.29 a
Populations obtained from parcels (13.51%)[z]	8.48 b	959.50 10^3 a	29.96 b
Populations obtained from parcels (5.88%)[z]	8.77 a	732.00 10^3 b	34.69 a

*values in the column with a letter in common are not significantly different according to Newman-Keuls (p = 0.05)
[v] aggressiveness measured on two sunflower inbred lines FU (highly resistant) and BT (moderately resistant)
[w] latent period: the number of days of incubation period necessary to obtain 80% of sporulating plants
[x] sporulation density: number of zoosporangia produced by a cotyledon
[y] hypocotyl length: corresponds to the distance from the stem base to cotyledon insertion measured after 13 days incubation on diseased plants showing sporulation on a shoot
[z] % of diseased plants in parcels which are taken from *P. halstedii* strains

Table 2. Characterization of virulence of strains (parental races, simple and super race) of *P. halstedii*

Strains	Race	Differential lines								
		D1 Ha-304	D2 Rha-265	D3 Rha-274	D4 PMI3	D5 PM-17	D6 803-1	D7 HAR-4	D8 QHP1	D9 Ha-335
Strain (parent race)	100	S	R	R	R	R	R	R	R	R
Strain (parent race)	710	S	S	S	S	R	R	R	R	R
Strain (simple race)	300	S	S	R	R	R	R	R	R	R
Strain (super race)	714	S	S	S	S	R	R	R	R	S

R – resistant = incompatible interaction; S – susceptible = compatible interaction (Tourvieille de Labrouhe 1999)

Under our sunflower mixture conditions, if the *P. halstedii* super race emerged under the selection pressure of the *Pl* genes, this super race would reproduce on sunflower genotypes carrying different levels of quantitative resistance (high and moderate). These genotypes would not enable *P. halstedii* to have a high level of variability (producing a high quantity of zoosporangia and zoospores). Consequently, the quantity of *P. halstedii* inoculum (oospores) could not be obtained through the period of fungal reproduction. These oospores permit *P. halstedii* to develop during the next generations. Then, the *P. halstedii* super race would disappear because its high level of virulence had not a selective advantage for the multiplication and reproduction according to Robinson's (1976) theory.

Fungus ability to develop more aggressive strains

Sakr *et al.* (2008b) showed in *P. halstedii* populations of virulence profile 710 appeared in sunflower parcels (Tourvieille de Labrouhe *et al.* 2005). Sakr *et al.* (2008b) showed that evolution of aggressiveness in *P. halstedii* populations appears as being linked to the number of diseased plants present in parcels with different strategies of *Pl* gene management (Table 1). The results suggested that the method of *Pl* gene management affects aggressiveness because it determines the number of susceptible plants harbored by the parasite. Moreover, in *P. halstedii* populations it appeared in sunflower parcels (Tourvieille de Labrouhe *et al.* 2005). Sakr (2008a) showed that the strain (simple race) of race 300 was less virulent and more aggressive than the strain (parental race) of race 710 (Table 1).

We will study the evolution of aggressiveness under conditions of our mixture model in the two following cases. First, the evolution of aggressiveness would be limited by the presence of two forms of inbred resistant lines. The mixture model would present weak levels of diseased plants needed by the parasite to develop its aggressiveness. Furthermore, the development of the more aggressive pathotypes in *P. halstedii* might be slowed in the inbred lines showing high and moderate levels of quantitaive resistance. The mechanisms expressed by accumulation of QTL in these sunflower cultivars could explain the low development of parasite (Vear *et al.* 2008; Sakr 2009a). Second, if more *P. halstedii* aggressive isolates (simple race with a low virulence) would emerge under the selection pressure of highly and moderately resistance in sunflower inbred lines, these isolates could not develop on sunflower genotypes carrying effective vertical resistance genes as *Pl8* or *Pl*ARg. Consequently, the quantity of *P. halstedii* simple race inoculum would decrease during the following generations.

Benefits of mixture model against the evolution of pathogenicity in *P. halstedii*

Although *P. halstedii* has an evolutionary capacity to produce new virulence under the selection pressure of *Pl* genes (Sakr 2008; Sakr *et al.* 2008b), it seems that the

ability of pathogen to develop its pathogenicity may be limited by the effects of the presence of the two types of resistances in our mixture model. The sunflower inbred lines carrying effective *Pl* genes could prevent the dispersion of more aggressive pathotypes in *P. halstedii*. And the sunflower inbred lines showing different levels of quantitative resistance could limit the reproduction and dispersion of virulent pathotypes in sunflower downy mildew. Super races present the most difficult hindrance for the cultivation of sunflower on a large surface. Indeed, the agricultural system containing the two types of resistance in a given environment may provide a satisfactory control for the development of parasite. Also, this model may reduce costs of sunflower production by improving the best conditions that limit the reproduction of pathogen.

CONCLUSION

Research on durable resistance to sunflower downy mildew is new enterprise. The best research methods were not yet proven. In our mixture system, the high level of dilution caused by the presence of qualitative resistance genes and different levels of quantitative resistance would assure durable resistance more than one form of resistance. Because the data obtained by Sakr (2008) and Sakr *et al*. (2008b) suggested the use only of major gene resistance, whatever the management system, this may never give satisfactory durable control. It appears very important to include quantitative resistance in integrated control systems. The ability of *P. halstedii* to develop new virulence and aggressiveness under selection pressure renders difficult to predict the evolutionary potential of parasite under traditional agricultural conditions. In these conditions, the influence of environment plays an important role in pathogenicity evolution and the response of host plant to pathogen. Based on the critical analysis in this study, the mixture of different sources of resistance may provide an acceptable level of durable resistance against sunflower downy mildew. Experimental and laboratory tests would be necessary in order to validate our mixture model.

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POLISH SUMMARY

MODEL MIESZANIN ODMIAN UWZGLĘDNIAJĄCY ODPORNOŚĆ NA *PLASMOPARA HALSTEDII* (MĄCZNIAK RZEKOMY SŁONECZNIKA)

Mączniak rzekomy słonecznika wywoływany przez *Plasmopara halstedii* jest jedną z najważniejszych chorób. Dotychczas wykorzystywano, z powodzeniem, całkowitą odporność uwarunkowaną genem głównym (*Pl*). Jednak od roku 2000, gdy we Francji pojawiło się osiem nowych ras, podjęto badania nad poszukiwaniem trwalszej odporności. Przedstawiono nowe wyniki dotyczące ewolucji patogeniczności *P. halstedii*, w warunkach sztucznej infekcji i zróżnicowanej presji infekcyjnej różnych genów *Pl*. Badano również ewolucję wirulencji i agresywności *P. halstedii*, wykorzystując model mieszanin linii wsobnych słonecznika posiadających dwa typy odporności (ilościowy i jakościowy). Model ten może przyczynić się do ulepszenia trwałej odporności słonecznika przeciwko *P. halstedii.*