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THEORETICAL BASES ASSUMING DURABLE RESISTANCE TO *PLASMOPARA HALSTEDII* (SUNFLOWER DOWNY MILDEW)

Nachaat Sakr

INRA-UBP, UMR 1095, 234 Avenue du Brézet, 63100 Clermont-Ferrand, France Present address *Department of Agriculture, Syrian Atomic Energy Commission, Damascus, P.O. Box 6091, Syria,* Author for correspondence
snachaat@hotmail.com; snachaat@gmail.com

ABSTRACT

Sunflower downy mildew is considered as one of the most serious diseases. Therefore, vertical resistance has been used intensively, but with the appearance of many races since 2000, research on more durable resistance has been undertaken. In this review, we present new results concerning the evolution of pathogenicity under artificial conditions in order to underline a mixture model assuming durable resistance against *Plasmopara halstedii*. Examples of host-parasite interactions including the influence of plant mixture against pathogens and durable resistance are presented to integrate in our couple *P. halstedii* / *Helianthus annuus*.

Key Words: Aggressiveness, horizontal resistance, *Pl* gene, super race, vertical resistance, virulence

1. INTRODUCTION

In order to maintain the survival of plants, they were forced to ensure a constant surveillance against pathogens. The plants and their pathogens co-excited in a relentlessly battle for better adaptation in a new environment. Within in vegetal species, some genotypes are susceptible, the pathogen invades the

plant and relationship is compatible. However, the others are resistant, the plant resist and relationship is incompatible (Parlevliet 2002). The association of different resistance sources within a same plant permit to enhance his durability against pathogens (Mundt 2002). The pathogens classed in two groups (Parlevliet 2002): 1- pathogens that attack a limited number of hosts such as *Plasmopara halstedii* and *Puccinia hordei*, pathogenic on sunflower and barley respectively, 2- pathogens characterised with a high number of hosts as *Sclerotinia sclerotiorum*, that attack more than 60 families. We use terms according to the definitions of Van der Plank (1968). Aggressiveness is used to indicate the quantitative component of pathogenicity that is expressed horizontally irrespective of plant cultivars or species, while virulence is used to indicate the qualitative component of pathogenicity that is expressed vertically.

This review presents theoretical bases and several studies for host-parasite interactions which affect durable resistance. From these examples, we propose a mixture model against *Plasmopara halstedii* (sunflower downy mildew), by using an association of sunflower inbred lines carrying major genes (Radwan et al. 2002; Dussle et al. 2004) and quantitative resistance newly discovered (Tourvieille de Labrouhe et al. 2008). New results concerning the evolution of pathogenicity in *P. halstedii* which multiplied under several methods of *Pl* genes management are presented equally (Sakr 2011a,b; Sakr et al. 2011). With this in mind, we underline hypotheses explicating durable resistance in consideration that sunflower downy mildew has been controlled in France until middle 90 years by using a complete, major gene resistance (*Pl*).

2. RESISTANCE DURABILITY

The importance of resistance for controlling disease has frequently been demonstrated in agricultural systems (Wolfe 1985; McDonald and Linde 2002; Mundt 2002; Burdon et al. 2006). Van der Plank (1968) postulated that disease

resistance in plants can be classed in one of two categories 1- vertical resistance (race-specific, qualitative resistance) which is conferred by major specific genes and tends to produce a disease-free plant. Vertical resistance is generally unstable, because resistance genes are quickly overcome by compatible races in the pathogen population, and too short-lived to be considered durable and 2-horizontal resistance (partial, field, non-race-specific, or quantitative resistance) which is controlled by minor genes and tends to impact the rate of disease development (rate reducing) rather than produce a disease-free plant. Horizontal resistance generally is more stable and equally active against many races of pathogen. However, the resistance conferred by many sources is not durable as a result of rapid changes in the pathogen (Leach et al. 2001). Durable resistance is a long – sought goal of crop production and plant breeding programmes. Johnson (1984) defined it as a resistant that remains effective while being extensively used in agriculture for a long period in an environment conductive to the disease. Major gene resistance durability is affected by the evolutionary potential of pathogen populations. Several factors intervening pathogen evolution: the mutation, the genetic drift, gene flow, reproduction system and the selection (McDonald and Linde 2002). Selection by hosts probably plays the most prominent role among evolutionary forces shaping pathogen populations structures (McDonald and Linde 2002; Montarry et al. 2006), and it is also the force that can be best manipulated by man, through plant breeding and cultivar deployment (Palumbi 2001). Limited durability of the resistance, however, remains a major problem in the deployment of resistant cultivars (Van den Bosch and Gilligan 2003).

There are some cases demonstrating that single *R* gene can conifer highly durable resistance. Resistance to wheat leaf rust, caused by *Puccinia triticina* that is conditioned by the single adult plant resistance gene *Lr* 34 has provided highly effective control for more than 30 years in North America (Kolmer 1993). More than, the gene *Vf* from *Malus floribunda* 821 for resistance to scab

(*Venturia inaequalis*) has been used successfully for 50 years in apple breeding programmes (Parisi et al. 1993). Other are remarkably ephemeral, for example, *Yr17* for the control of yellow rust on wheat was rapidly overcome in two to three seasons by virulent isolates of *Puccinia striiformis* f. sp. *tritici* in the UK followed by Denmark, France and Germany (Bayles et al. 2000). There is evidence that resistance durability can be enhanced by using of mixture of plants (Mundt 2002).

3. HOST -PLANT MIXTURE AGAINST PATHOGENS

A negative relationship was demonstrated between levels of foliar fungal and species richness according to Knops et al. (1999). However, the use of mixtures in agricultural situation is that mimic natural systems where disease is often less apparent (Thrall and Burdon 2000). Mundt (2002) defined the mixture as association of plants which differ in their reaction to a pathogen and that reduce disease severity. Although individual plants might be susceptible to a component of the pathogen population, the host mixture as a whole develops a significant resistance level resulting mainly from host diversification (Wolfe 1985; Lannou et al. 2005). Host diversification is to use different resistance levels of plants against a given pathogen (Finckh and Wolfe 1998; Mundt 2002). Several studies showed a positive correlation between host-plant diversity and resistance to pathogen (Wolfe 1985; Thrall et Burdon 2000; Zhu et al. 2000). Duffy and Sivars-Becker (2007) found that increased diversity of the host population increases the speed at which the population evolves resistance, decreases the length of a disease and decreases its effect on host population. Higher diversity resulted in a greater probability that individual genotypes of either host or pathogen would be lost, and also resulted in greater (and more unpredictable) fluctuations in pathogen abundance (Nowak and May 1994). Agricultural systems involving the use of varietal mixtures have repeatedly demonstrated the linkage between host and pathogen genetic structure and the capacity of a diversified host population, albeit limited and artificial, to reduce

disease severity and consequent pathogen-induced selection (Wolfe 1985; Zhu et al. 2000; Burdon et al. 2006). Diseased levels in mixtures are typically 10-70% of incidence levels seen in pure lines of their components (Burdon 1987). More than, Smithson and Lenne (1996) noted disease reductions relative to pure stands ranging from 4% to 89%. However, not all mixtures lead to disease reduction (Groenewegen and Zadoks 1979).

In a mixture of cultivars with different resistant genes, pathogens are restricted to susceptible hosts and spore losses on resistant plants results in considerable reduction in disease severity (Lannou 2001). Molecular analyses of pathogens populations (Zeigler et al. 1995) and individual avirulence genes (Cruz et al. 2000; Leach et al. 2001) may provide further insights into the most appropriate resistance genes to deploy in host mixtures to maximize durability. A resistance gene deployed in a mixture will have less exposure to the pathogen population than if the same gene were deployed in monoculture of the same total crop area (de Vallavieille-Pope 2004). This would be expected to reduce selection pressure and increase durability of that gene; limited field observations seem to support this view (Mundt 2002).

Van der Plank (1968) predicted that a mutation to virulence is associated with an increase in fitness if the *R* gene is present. However, if the mutation in the avirulence gene also carries a fitness penalty, the pathogen will suffer from a reduction in fitness on the host without the *R* gene; this is a concept of virulence cost. All the response of pathogen populations to host mixtures based on this hypothesis (Lannou 2001; Thrall and Burdon 2002; Lannou et al. 2005). They described the competition between simple pathotypes, which have greater specificity on one of the host genotypes but are unable to develop on several other hosts, and complex race (super race according to Robinson 1976) which are able to develop on several host genotypes but have a fitness disadvantage related to the number of virulences they carry. More than, A pathogen cost of virulence parameter is often a component in models of factors affecting

durability of host resistance (Cruz et al. 2000 ; Leach et al. 2001 ; Huang et al. 2006).

The majority of studies have been interested to measure the effect of plant mixture in rusts and powdery mildew pathosystems (Mundt 2002). However, a little observation carries on the downy mildews and particularly in the case of sunflower downy mildew according to Tourvieille de Labrouhe et al. (2010).

4. *PLASMOPARA HALSTEDII* IS A MODEL FOR PHYSIOLOGICAL RACE DEVELOPMENT

Sunflower downy mildew is a common disease in many regions where sunflowers (*Helianthus annuus* L.) are grown. The pathogen, *Plasmopara halstedii* (Farlow) Berles & de Toni, is an obligate parasite. The disease affects young plants when the water content of the soil is high and the maximum temperature is between 15 and 18°C. *P. halstedii* is an Oomycete with asexual multiplication by liberation of zoosporangia produced on the under surfaces of sunflower leaves and sexual reproduction giving oospores which are found in crop residues.

P. halstedii 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 (Tourvieille de Labrouhe 1999). Race specific resistance against is controlled by major genes, denoted *Pl*. Pathogen – specific recognition occurs following a gene-for-gene model (Radwan et al. 2002), which predicted that the outcome of the interaction is disease resistance when corresponding *R* and *Avr* genes are present in both host plant and pathogen. In all other cases, plants do not sense pathogens and disease results (Flor 1971).

In France, race 100 (European race) has been present in the sunflower crop since 1965. Until 1987, it was the only race identified but in 1988 and 1989, two

new races, 710 and 703 appeared (Tourvieille de Labrouhe et al. 2010). Since then, prospecting each year have demonstrated the existence in France of 3 races in 1993, 5 races in 1994, 6 in 2000, 9 in 2002 and 12 in 2004 (Tourvieille de Labrouhe et al. 2010). The 8 races identified since 2000 (304, 307, 314, 334, 704, 707, 714 and 717) and purified on specific host differentials had not been described previously in other countries. They appear to result from evolution of the parasite in France. This evolution seems linked with a quasi exclusive use of *Pl6* gene from 1990 which have been overcame by the parasite races 704 and 714. The late use of another gene *Pl5* was lead to an apparition of new virulence 334 (Tourvieille de Labrouhe et al. 2010). This apparition was lead to intense use of cluster *Pl5*/*Pl8* in sunflower zones cultivated. The life period of *Pl* gene seems very short from its important use on a large soil. To date, there are at least 35 races in different parts of the world (Gulya 2007).

In 1971, Vear and Leclercq suggested to cumulate maximum of resistance genes in one genotype in order to avoid the break-down of the resistance by a new race of downy mildew. The France experimentation (Tourvieille de Labrouhe et al. 2010) show that if this strategy does not accompany by complementary agronomic measure (rotation) or genetic measure (quantitative resistance); it will lead to appear new virulence. In these conditions, the life period of *Pl* gene is inferior to 10 years. The quick evolution of virulence observed in France attributes to deploy the same genes used in this experimentation (Tourvieille de Labrouhe et al. 2010). However, the same authors showed that whatever the method of management (mixture, alternation, and monoculture) of *Pl* genes, their selection pressure led to appearance of new virulence.

5. MIXTURE MODEL

The dilution of pathogen inoculum that occurs due to increased distance between plants of the same genotype appears the most important mechanism to

increase durable resistance in genotype mixture against pathogens (Wolfe 1985; Mundt 2002). We can provoke this dilution by decreasing the proportions of auto-infection (the dispersion of pathogen within host plant) and the proportion of allo-infection (the dispersion of pathogen from plant to another).

Theoretically, host-diversity effects in mixtures of cultivars are maximized both when one host genotype expresses differential (vertical) resistance to pathogen races, because such a genotype acts as a completely resistant host to avirulent fraction of the pathogen population (Garrett and Mundt 1999) and with various levels of horizontal resistance shown the potential to 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 the following points:

1. Plant density must to be sufficiently high which presents first mechanism for decreasing auto-infection by indirect intervening in reduction of nutritive elements necessary to fungus development in plant tissues (Mundt 2002).

2. Nitrogen fertilization must to be sufficiently low because there is a negative correlation between nitrogen leaves content and symptom severity caused by rusts and mildews pathogens on cereals (Danial and Parlevliet 1995; Olesen et al. 2003).

3. Use of sunflower inbred lines carrying resistance genes correspond to avirulence genes in *P. halstedii* such as *Pl8* or *Pl*ARg (Radwan et al. 2002; Dussle et al. 2004) which presents the first mechanism for decreasing allo-infection.

4. Use of sunflower inbred lines carrying highly and moderately horizontal resistant (Tourvieille de Labrouhe et al. 2008) which presents the second mechanism for decreasing auto-infection.

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

6. RATE OF FUNGAL EVOLUTION

The stability of the mixture's resistance depends on the ability of the pathogen populations to evolve their pathogenicity including virulence and aggressiveness (Lannou and Mundt 1996; Lannou 2001; Lannou et al. 2005; Sacristan and Garcia-Arenal 2008). Lenski and May (1994) showed that virulence is an unavoidable consequence of parasite reproduction within the infected host, because it does not represent any clear advantage for parasites which depend on their hosts for survival (Leach et al. 2001; McDonald and Linde 2002; Mundt 2002; Sacristan and Garcia-Arenal 2008). In the other hand, aggressiveness presents an advantage because this capacity enables the pathogen to develop within the host plant (Van der Plank 1968; Robinson 1976).

1- Fungus ability to develop super race that alter their *Avr* factors to avoid *R*-dependant recognition and this mechanism determine *Avr* evolution (Sacristan and Garcia-Arenal 2008). However, direct recognition of *Avr* by *R* can lead to relatively rapid evolution of new virulence phenotypes by alteration of the *Avr* structure (Van der Hoorn et al. 2002). For a given pathogen, the breakdown of *R* gene resistance can be obtained by a mutation of avirulence gene. This can vary from a mutation at nucleotide-level (Joosten et al. 1994) to a complete deletion of the avirulence gene (Joosten and de Wit 1999). Gout et al. (2007) showed that gain of virulence was linked to a 260 kb deletion of a chromosomal segment spanning *AvrLm1* in *Leptoshaeria maculans*. The selection of super race

depends on the environment, host genotypes presented in the mixture, fungal capacity to develop new mechanisms for spore dispersion and stimulation of auto-infection and finally the genetic background of super race (Mundt 2002; Lannou et al. 2005). However, it is more difficult to determine the selective influence of mixtures for clonal pathogens because virulence genes will not be randomly associated with other genes that influence pathogen fitness (Leach et al. 2001). In a very carefully designed greenhouse experiment with populations of *Puccinia graminis* on three different wheat mixtures over 12 generations, Kolmer (1995) found strong selection for pathogen genotypes with virulence corresponding to three of the five resistance genes present in the mixture populations, but genotypes with four to five corresponding virulence represented a small minority and occurred at frequencies lower than predicted by mathematical model.

The aggressiveness of super race is weaker than a simple race which overcomes a limited number of vertical resistance genes (Robinson 1976; Lannou and Mundt 1996; Lannou 2001). Our results show that super race of virulence profile 714 in *P. halstedii* appeared in sunflower parcels (Tourvieille de Labrouhe et al. 2010) was more virulent and less aggressive than parent race of profile virulence 100 (Table 1). In similarly conditions, Murakami et al. (2007) found the same case in the pathosystem *Magnaporthe oryzae* / the wheat and the millet. The aggressiveness criteria presented in Table 1 (Sakr 2011a, Sakr et al. 2011) were measured on sunflower inbred lines carrying different levels of horizontal resistance (Tourvieille de Labrouhe et al. 2008). On the other hand, this super race was more virulent (Table 2) and did not show any difference of aggressiveness than the other parent of virulence profile 710 (Sakr 2011). Damgaard et al. (1999) suggested in a theoretical model the absence of difference of aggressiveness between virulent strain and avirulent.

Table 1: Responses of Plasmopara halstedii strains on sunflower inbred lines (data from Sakr 2011a, Sakr et al. 2011)

Plasmopara 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 (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 *Plasmopara halstedii* strains.

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.

Table 2. Characterization of the virulence of strains (parental races and super race) of Plasmopara halstedii

Strains	Race	Differential lines								
		D1	D2	D3	D4	D5	D6	D7	D8	D9
		Ha-304	Rha-265	Rha-274	PMI3	PM-17	803-1	HAR-4	QHP1	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 (Super race)	714	S	S	S	S	R	R	R	R	S

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

2- Fungus ability to develop more aggressive strains. Aggressiveness is linked to the transmission of parasite, it is defined by Robinson (1976) as the capacity of pathogens to develop an epidemic in function to their host plants. The increasing of transmission is balanced by the disappearance of the host. Aggressiveness should not increase without limits and it should stabilise to an intermediate value. Heterogeneous conditions of host plants must lead to decrees pathogen aggressiveness; these are considered as vareital mixture of efficacy control (Villareal and Lannou 2000; de Vallavieille-Pope 2004). Wolfe and Barrett (1980) showed that reduction of symptom severity reaches 50% in the couple *Blumeria graminis* / barley in mixture conditions of plants carrying vertical genes resistance. However, several studies found that mixture of genotypes carrying different levels of quantitative resistance can equally

decreases symptom severity caused by pathohens on wheat *Pseudcercsporella herportrichoides* (Mundt et al. 1995), *Mycosphaerella graminicola* (Cowger and Mundt 2002) and *Phytophthora infestans* on potato (Andrivon et al. 2003). More than, Finckh and Mundt (1992) showed that mixture of plants carrying qualitative and quantitative resistance reduces 50% of symptom severity in *Puccinia striiformis* on the wheat.

Sakr et al. (2011) showed in *P. halstedii* populations 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, Sakr (2011a) showed in *P. halstedii* populations 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 horizontal resistance. The mechanisms expressed by accumulation of QTL in these sunflower cultivars could explain the low development of parasite (Vear et al. 2008). 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.

7. 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 2011a,b; Sakr et al. 2011), 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 recently studied. 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 (2011a,b) and Sakr et al. (2011) 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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