Emergence of New Virulence in *Plasmopara halstedii* (Sunflower Downy Mildew)

N. SAKR*

Department of Agriculture, Syrian Atomic Energy Commission, P.O. Box 6091, Damascus, Syria
Previous address: INRA-UBP, UMR 1095, 234 Avenue du Brézet, 63100 Clermont-Ferrand, France

The fast evolution of *Plasmopara halstedii* (downy mildew) remains a major risk for sunflower crop, as new races of the pathogen are bypassing the resistance of sunflower hybrids. In order to understand the processes which led a new virulence to appear in a local *P. halstedii* population, the genetic relationships were studied using 12 EST (Expressed Sequence Tag)-derived markers between five progeny isolates of races 300, 304, 314, 704 and 714 and two parental ones of races 100 and 710. All genetic analyses were carried out using five single zoosporangium isolates per *P. halstedii* isolate. There was no *intra-isolate* genetic variation among the seven pathogen isolates and five multilocus genotypes (MLG) were identified among the 35 *P. halstedii* single zoosporangium isolates. The single zoosporangium isolates of races 314, 704 and 714 had an intermediary genetic position between the single zoosporangium isolates of two parental isolates. The single zoosporangium isolates of three isolates of races 100, 300 and 304 were localized in the same genetic clade. Two genetic mechanisms could explain the emergence of new virulence in *P. halstedii* as a recombination between races and mutations in a clonal lineage.

Keywords: *Avr* gene, EST-derived markers, *Helianthus annuus*, *Pl* gene, race.

Costs of virulence adaptation measured by the appearance of new races play a major role in host-pathogen co-evolution and thus take a central place in the evolutionary theory (Brown and Tellier, 2011). Many plant parasites interact with their hosts according to the widely accepted gene-for-gene model, which predicts that successful disease resistance is triggered only if a resistance *R* gene product in the host plant recognizes, directly or indirectly, a specific avirulence *Avr* gene product from the pathogen (Flor, 1971). Both the resistance and virulence are inherited as single genes (Pedley and Martin, 2003). There are many recorded examples of directional selection leading to invasion of pathogen populations by virulent isolates, and hence resistance breakdown (Jacobs and Parlevliet, 1993; Bayles et al., 2000). However, there are some cases demonstrating that single *R* gene can conifer highly durable resistance (Kolmer, 1993; Parisi et al., 1993). For the genetic bases of new virulence emergence, Van der Hoorn et al. (2002) predicted that direct recognition of avirulence *Avr* gene in pathogen by resistance *R* gene in plants may lead to a relatively rapid evolution of new virulence phenotypes by the alteration of *Avr* structure. On the other hand, the recombination following crosses between different races caused the appearance of a new virulence in pathogen isolates as observed for *Phytophthora infestans* (Brasier et al., 1999). However, in the case of *Plasmopara halstedii*, characterized by a

* E-mails: snachaat@hotmail.com; snachaat@gmail.com

high level of evolutionary potential and the apparition of many races (Sakr, 2011a, 2011b, 2012, 2014a, 2014b, 2014c, 2015), no studies are extant on the genetic background for avirulence *Avr* genes and the mechanisms explaining the emergence of new virulence. Virulence is a quantitative character to describe degrees of pathogenicity (according to the Terminology Sub-Committee of the Federation of British Plant Pathologist definition).

Sunflower downy mildew is caused by *P. halstedii* [(Farl.) Berl. et Toni], an invasive species which grows where the sunflower (*Helianthus annuus* L.) is planted. *P. halstedii* is a homothallic endoparasite to Oomycetes and its cycle is made up of a single sexual generation permitting overwintering and one or perhaps two asexual generations which occur during the growing season. Qualitative resistance in sunflower is conferred by the major *Pl* genes and tends to produce a disease-free plant (Tourvieille de Labrouhe et al., 2000).

P. halstedii displays a gene-for-gene interaction with its host plant and 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 et al., 2000). The interaction of *Avr* avirulence genes of *P. halstedii* and sunflower genotypes carrying *Pl* effective genes caused the appearance of a new virulence in field pathogen isolates. Indeed, it has been possible to identify up to 35 races, with different virulence patterns (As-Sadi et al., 2011; Sakr, 2014a).

Concerning genetic characteristics in *P. halstedii*, Spring et al. (2006) differentiated between two *P. halstedii* populations. Moreover, Delmotte et al. (2008) identified three genetically different groups of isolates organized around the first three races described in France. Indeed, the interest of Internal Transcribed Spacer (ITS) (Spring et al., 2006) and Expressed Sequence Tag (EST) (Delmotte et al., 2008; Sakr, 2011a, 2014b, 2014c, 2015) sequences to characterize *P. halstedii* isolates have been shown even though races are still not defined with certainty. However, studies have only started recently on effectors of pathogenicity (As-Sadi et al., 2011). Recently, Ahmed et al. (2012) and Sakr (2014a) suggested that multiple introductions have aided the establishment of *P. halstedii* in France, and noted that recombination facilitated by these introductions is driving the emergence of new and endemic races in response to host resistance. So that the emergence of new virulence could be understood, genetic analyses were carried out in a local *P. halstedii* population obtained under *Pl* genes selection pressure for five consecutive years (Tourvieille de Labrouhe et al., 2010; Sakr, 2011b, 2014a). Our study dealt with single zoosporangium isolates to avoid the problem incurred by the presence of more than one virulent pathotype in a complex virulent profile (Sakr et al., 2007). In current paper, our target was (i) to study the genetic origin of five progeny *P. halstedii* isolates of five races arising from two parental ones derived from races 100 and 710 and (ii) to underline the genetic mechanisms explaining the emergence of new virulence in *P. halstedii*.

Materials and Methods

Oomycete isolates and race identification

P. halstedii isolates used in this study were collected in France and maintained at INRA, Clermont-Ferrand. Manipulation of this quarantine pathogen followed European

regulations (no. 2003/DRAF/70). Isolate MIL 001 (race 100) was sampled in 1966 and isolate MIL 002 (race 710) in 1988. The progeny isolates originated from an initial mixture of pathotypes 100 and 710 and were multiplied in sunflower cages isolated from the exterior environment (Sakr, 2011b; Tourvieille de Labrouhe et al., 2010) and their races (Table 1) were determined by method reported by Tourvieille de Labrouhe et al. (2000): isolate DU 1842 (race 300); isolate DU 1767 (race 304); isolate 1943 (race 314); isolate 1734 (race 704) and isolate 1915 (race 714). Our study dealt with single zoosporangium isolates to avoid the problem incurred by the presence of more than one virulent pathotype in a complex virulent profile (Sakr et al., 2007). For each *P. halstedii* isolate, five single zoosporangium isolates were obtained according to method described by Sakr et al. (2007). The characterization of the race for 35 single zoosporangium isolates was carried out with the same method adapted in this study. There were three replications for each differential line (10 plants in each replication) and the entire experiment was repeated twice for seven *P. halstedii* isolates and 35 *P. halstedii* single zoosporangium isolates.

DNA extraction and molecular typing

For 35 single zoosporangium isolates tested, DNA was isolated from infected plant tissue as previously described for *Plasmopara viticola* by Delmotte et al. (2008). The 12 polymorphic EST (Expressed Sequence Tag)-derived markers (Delmotte et al., 2008) were then used to genotype *P. halstedii* isolates. The polygenetic relations between the 35 single zoosporangium isolates were obtained by building a Neighbour-joining (NJ) tree (Jin and Chakraborty, 1993) using DnaSP v5 Software (Librado and Rozas, 2009). A Bootstrap analysis was performed on 10,000 replicates.

Table 1

Virulence of seven *Plasmopara halstedii* parental isolates on nine sunflower differential lines

Parental isolates	Race	Year isolated	Differential lines								
			D1 Ha-304 without *Pl* gene	D2 Rha-265 *Pl1*	D3 Rha-274 *Pl2*	D4 PMI3 *Pl(?)*	D5 PM-17 *Pl(?)*	D6 803-1 *Pl(?)*	D7 HAR-4 *Pl(?)*	D8 QHP1 *Pl(?)*	D9 Ha-335 *Pl6*
MIL001	100	1960	S	R	R	R	R	R	R	R	R
DU1842	300	2005	S	S	R	R	R	R	R	R	R
DU1943	314	2005	S	S	R	S	R	R	R	R	S
DU1767	304	2005	S	S	R	R	R	R	R	R	S
MIL002	710	1988	S	S	S	S	R	R	R	R	R
DU1915	714	2005	S	S	S	S	R	R	R	R	S
DU1734	704	2005	S	S	S	R	R	R	R	R	S

S: susceptible = sporulation on cotyledons; R: resistant = no sporulation; Pl (?) = Pl gene not identified, data from Tourvieille de Labrouhe et al. (2000)

Results and Discussion

Our understanding of the recurrent breakdown of sunflower major resistance genes may be improved by new findings concerning the key processes governing the evolution of *Plasmopara halstedii* populations. Recent evidence for race emergence in *P. halstedii* was demonstrated by infecting an experimental plot with races 100 and 710. After 5 years of selective pressure exerted by sunflower *Pl* resistance genes, six other races (300, 304, 314, 700, 704, 714) that had not been present at the start of the study, were reported (Tourvieille de Labrouhe et al., 2010; Sakr, 2011b, 2014a). Furthermore, in the absence of selection pressure, Sakr et al. (2011) and Sakr (2014a) stated that *P. halstedii* did not evolve its virulence. This rapid evolution of virulence in *P. halstedii* was revealed in France, exerted by intensive *Pl* genes resistance selection pressure. Fourteen different races of this pathogen have now been characterized, nine of which emerged in the last ten years (Delmotte et al., 2008; Sakr, 2014a).

Table 2 demonstrates that single zoosporangium isolates were of the same race as the parental isolates previously identified in Table 1. Indeed, *P. halstedii* isolates of seven races used were uncloned. Consequently, they represented a mixture of organisms with different virulence profiles which may show the same race of the phenotype. Analyses were subsequently performed with single zoosporangium isolates to diminish this problem (Sakr et al., 2007). Furthermore, we confirmed that single zoosporangium isolates were of the same race as the pathogen isolates (Tables 1 and 2). Our data were not revealed within the same pathogenic system in which Molinero-Ruiz et al. (2002) found that some single zoosporangium *P. halstedii* isolates, obtained from one parental isolate, showed differences in the virulence profile when compared with the parental one. However, there is a possibility of genetic inhomogenity of single zoosporangium isolates (in contrast to single zoospore isolates). In fact, indications now exist (Spring and Zipper, 2006) that two or even more nuclei immigrate into a single zoosporangium and these nuclei may mitotically divide there. This may cause that, in a heterokaryotic mycelium, genetically inhomogeneous zoospores may develop into a single zoosporangium.

The combination of 12 EST (Expressed Sequence Tag)-derived markers revealed five multilocus genotypes (MLG) among 35 *P. halstedii* single zoosporangium isolates (Table 3). There was no *intra-genetic* variation for all pathogen isolates tested. With the exception of Pha54, the single zoosporangium isolates of MIL 001 and MIL 002 were different for all other genomic markers. The single zoosporangium isolates MIL 001, DU 1842 and DU 1767 had the same genetic background. The Neighbour-joining tree showed that single zoosporangium isolates of DU 1915, DU 1734 and DU 1943 had an intermediary genetic position between single zoosporangium isolates of MIL 001 and MIL 002 (Fig. 1). Our results observed genetic distances between two races 100 and 710 corroborating conclusions by Delmotte et al. (2008), As-Sadi et al. (2011) and Sakr (2011a, 2014b, 2015). By using the same EST-derived markers, Delmotte et al. (2008) and Sakr (2011a, 2014b) found that races 100, 300 and 304 had the same genetic clade as observed in our study (Fig. 1). However, As-Sadi et al. (2011) reported that certain single nucleotide polymorphisms (SNPs) might allow for clear differentiation between races 304 and 100, which has not been detected in current research (Fig. 1) and in previous studies

Table 2

Virulence of 35 single zoosporangium *Plasmopara halstedii* isolates on nine sunflower differential lines

Single zoo-sporangium isolates	Race	Year isolated	Differential lines								
			D1 Ha-304 without *Pl* gene	D2 Rha-265 *Pl1*	D3 Rha-274 *Pl2*	D4 PMI3 *Pl(?)*	D5 PM-17 *Pl(?)*	D6 803-1 *Pl(?)*	D7 HAR-4 *Pl(?)*	D8 QHP1 *Pl(?)*	D9 Ha-335 *Pl6*
MIL001 M2	100	2006	S	R	R	R	R	R	R	R	R
MIL001 M3	100	2006	S	R	R	R	R	R	R	R	R
MIL001 M4	100	2006	S	R	R	R	R	R	R	R	R
MIL001 M5	100	2006	S	R	R	R	R	R	R	R	R
MIL001 M6	100	2006	S	R	R	R	R	R	R	R	R
DU1842 M1	300	2006	S	S	R	R	R	R	R	R	R
DU1842 M2	300	2005	S	S	R	R	R	R	R	R	R
DU1842 M3	300	2006	S	S	R	R	R	R	R	R	R
DU1842 M4	300	2006	S	S	R	R	R	R	R	R	R
DU1842 M5	300	2006	S	S	R	R	R	R	R	R	R
DU1943 M1	314	2006	S	S	R	S	R	R	R	R	S
DU1943 M2	314	2006	S	S	R	S	R	R	R	R	S
DU1943 M3	314	2005	S	S	R	S	R	R	R	R	S
DU1943 M4	314	2006	S	S	R	S	R	R	R	R	S
DU1943 M5	314	2006	S	S	R	S	R	R	R	R	S
DU1767 M1	304	2006	S	S	R	R	R	R	R	R	S
DU1767 M2	304	2006	S	S	R	R	R	R	R	R	S
DU1767 M3	304	2006	S	S	R	R	R	R	R	R	S
DU1767 M4	304	2005	S	S	R	R	R	R	R	R	S
DU1767 M5	304	2006	S	S	R	R	R	R	R	R	S
MIL002 M1	710	2006	S	S	S	S	R	R	R	R	R
MIL002 M2	710	2006	S	S	S	S	R	R	R	R	R
MIL002 M3	710	2006	S	S	S	S	R	R	R	R	R
MIL002 M4	710	2006	S	S	S	S	R	R	R	R	R
MIL002 M5	710	2006	S	S	S	S	R	R	R	R	R
DU1915 M1	714	2005	S	S	S	S	R	R	R	R	S
DU1915 M2	714	2005	S	S	S	S	R	R	R	R	S
DU1915 M3	714	2005	S	S	S	S	R	R	R	R	S
DU1915 M5	714	2005	S	S	S	S	R	R	R	R	S
DU1915 M6	714	2005	S	S	S	S	R	R	R	R	S
DU1734 M1	704	2005	S	S	S	R	R	R	R	R	S
DU1734 M2	704	2005	S	S	S	R	R	R	R	R	S
DU1734 M3	704	2005	S	S	S	R	R	R	R	R	S
DU1734 M7	704	2005	S	S	S	R	R	R	R	R	S
DU1734 M8	704	2005	S	S	S	R	R	R	R	R	S

S: susceptible = sporulation on cotyledons; R: resistant = no sporulation; *Pl (?)* = *Pl* gene not identified, data from Tourvieille de Labrouhe et al. (2000)

(Delmotte et al., 2008; Sakr, 2011a, 2014b). Although Delmotte et al. (2008) grouped races 704, 710 and 714 together within the same genetic clade, this association was not identified in current work and in data by Sakr (2011a, 2014b, 2014c). Absence of genetic variation among single zoosporangium isolates of the same race (Table 3) for the seven pathotypes (Tables 1 and 2) may be due to the fact that molecular markers used in current study were non-specific and insufficiently polymorphic within *P. halstedii* to detect differences among pathogen isolates of the same race, this observation was detected in the same pathosystem (Sakr, 2014c, 2015). However, based on ITS (Internal Transcribed Spacer) sequence data, the distinctiveness of the 7xx races compared to those races of 100 or 3xx has recently been shown (Spring et al., 2006).

Oomycetes have already been reported to display a large range of processes generating phenotypic variability, including interspecific hybridization (Brasier et al., 1999), recombination resulting from sexual reproduction, and genome instability caused by mitotic recombination, gene conversion, transposable elements or dispensable chromosomes (Kamoun, 2003). Progress in genetic studies on *Phytophthora* is very fast but very little is known about the population genetics of downy mildews which are obligate parasite. Our study provides several important insights into the genetic basis of phenotypic evolution in a local *P. halstedii* population obtained under qualitative resistance selection pressure. Based on the genetic results for parental and progeny 35 single zoosporangium isolates (Table 3 and Fig. 1), two mechanisms could explain the emergence of new virulence in *P. halstedii*.

First, our data indicated that recombination events between genetically differentiated parental isolates of races 100 and 710 may have led to the emergence of new races: races 314, 704 and 714 are intermediate between races 100 and 710 (Fig. 1). The above indicated that it may have resulted from crosses between two parental isolates. These examples clearly illustrated the contribution of outcrossing events to the emergence of a new race in the local population of *P. halstedii*. In this case, the recombination of genetically differentiated races generated new phenotypic profiles, characterized by the ability of races 314, 704 and 714 to overcome the resistance gene of the differential host D9 (Table 1).

Second, our results provided evidence to suggest that a clonal lineage (one multilocus genotype) may include several races (Table 3). This fact suggests that mutations in a clonal lineage may lead to the emergence of new races in the same genetic background: this is the cases for races 100, 300 and 304 (Fig. 1). Two non-exclusive mechanisms may be responsible for this phenotypic variability in avirulence determinants. The first of these mechanisms is recombination within the lineage via the homothallic fusion of gametangia. This hypothesis is certainly the most likely, given the high selfing rate of sunflower downy mildew inferred from our analysis. Alternatively, genetic recombination via parasexual events may have generated this variability as has been shown recently that mitotic recombination between different isolates of *P. halstedii* may be involved in the generation of new phenotypes within a population. In this case, mitotic recombination within a genetic lineage would be required. However, no such phenomenon has ever been described.

The importance of mutation events in race evolution has already been highlighted in several Oomycetes plant pathogens such as *Plasmopara viticola* (Delmotte et al., 2011),
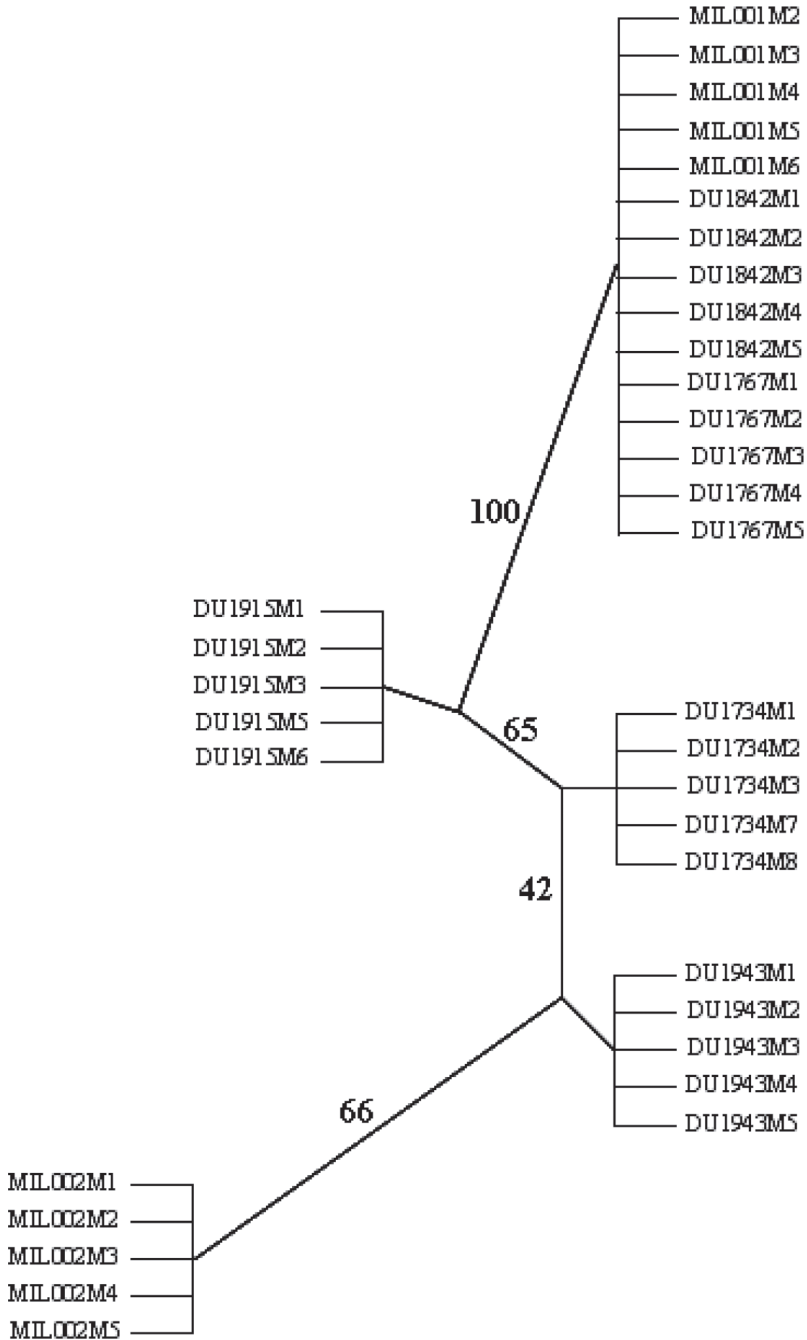
Fig. 1. Phylogenetic tree according to Neighbour-joining analysis of 12 EST (Expressed Sequence Tag)-derived markers. Figures on branches indicate bootstrap values (10,000 replicates)

Table 3

Genotypes multilocus (GML) characterized using 12 EST (Expressed Sequence Tag)-derived genomic markers on 35 single zoosporangium isolates of *Plasmopara halstedii*

Isolate	Race	EST-derived markers											
		Pha6	Pha39	Pha42	Pha43	Pha54	Pha56	Pha74	Pha79	Pha82	Pha99	Pha106	Pha120
MIL001 M2	100	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001 M3	100	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001 M4	100	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001 M5	100	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001 M6	100	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1842 M1	300	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1842 M2	300	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1842 M3	300	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1842 M4	300	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1842 M5	300	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1943 M1	314	1/1	2/2	1/1	2/2	1/1	2/2	2/2	3/3	2/2	1/1	2/2	1/1
DU1943 M2	314	1/1	2/2	1/1	2/2	1/1	2/2	2/2	3/3	2/2	1/1	2/2	1/1
DU1943 M3	314	1/1	2/2	1/1	2/2	1/1	2/2	2/2	3/3	2/2	1/1	2/2	1/1
DU1943 M4	314	1/1	2/2	1/1	2/2	1/1	2/2	2/2	3/3	2/2	1/1	2/2	1/1
DU1943 M5	314	1/1	2/2	1/1	2/2	1/1	2/2	2/2	3/3	2/2	1/1	2/2	1/1
DU1767 M1	304	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1767 M2	304	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1767 M3	304	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1767 M4	304	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
DU1767 M5	304	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2

Table 3 continued

Isolate	Race	EST-derived markers											
		Pha6	Pha39	Pha42	Pha43	Pha54	Pha56	Pha74	Pha79	Pha82	Pha99	Pha106	Pha120
MIL002 M1	710	1/1	1/1	2/2	2/2	1/1	2/2	2/2	1/1	1/1	1/1	2/2	1/1
MIL002 M2	710	1/1	1/1	2/2	2/2	1/1	2/2	2/2	1/1	1/1	1/1	2/2	1/1
MIL002 M3	710	1/1	1/1	2/2	2/2	1/1	2/2	2/2	1/1	1/1	1/1	2/2	1/1
MIL002 M4	710	1/1	1/1	2/2	2/2	1/1	2/2	2/2	1/1	1/1	1/1	2/2	1/1
MIL002 M5	710	1/1	1/1	2/2	2/2	1/1	2/2	2/2	1/1	1/1	1/1	2/2	1/1
DU1915 M1	714	1/1	2/2	1/2	1/1	1/1	1/1	1/1	3/3	2/2	1/1	2/2	1/1
DU1915 M2	714	1/1	2/2	1/2	1/1	1/1	1/1	1/1	3/3	2/2	1/1	2/2	1/1
DU1915 M3	714	1/1	2/2	1/2	1/1	1/1	1/1	1/1	3/3	2/2	1/1	2/2	1/1
DU1915 M5	714	1/1	2/2	1/2	1/1	1/1	1/1	1/1	3/3	2/2	1/1	2/2	1/1
DU1915 M6	714	1/1	2/2	1/2	1/1	1/1	1/1	1/1	3/3	2/2	1/1	2/2	1/1
DU1734 M1	704	2/2	2/2	2/2	1/1	1/1	1/1	2/2	3/3	1/1	1/1	2/2	1/1
DU1734 M2	704	2/2	2/2	2/2	1/1	1/1	1/1	2/2	3/3	1/1	1/1	2/2	1/1
DU1734 M3	704	2/2	2/2	2/2	1/1	1/1	1/1	2/2	3/3	1/1	1/1	2/2	1/1
DU1734 M7	704	2/2	2/2	2/2	1/1	1/1	1/1	2/2	3/3	1/1	1/1	2/2	1/1
DU1734 M8	704	2/2	2/2	2/2	1/1	1/1	1/1	2/2	3/3	1/1	1/1	2/2	1/1

The values of two figures indicate to the alleles of 35 *Plasmopara halstedii* single zoosporangium isolates for 12 EST-derived genomic markers. For each isolate, the race and the two alleles at each derived genomic marker were indicated

Bremia lactucae (Lebeda and Petrzelova, 2004), and *P. infestans* (Andrivon, 1994). Although surprising at first sight, the importance of mutation greater than that of recombination in populations able to outcross may be related to the large number of clonal cycles of fungal multiplication during epidemics. The combination of large populations, due to asexual reproduction of the pathogen, with strong selective pressures induced by resistance genes in the plant is likely to favor the emergence of new virulent races.

Conclusion

Our results evidence that the recombination events and mutation were the main factors to explain the emergence of new virulence in a local pathogen population. We consider that pathosystem of *P. halstedii* and *H. annuus* will continue to evolve new virulence in pathogen unless it was reengineered to make it less conductive to pathogen evolution (Sakr, 2014a) in accordance with Brown and Tellier's (2011) hypothesis. Sakr (2014b) found a trade-off between virulence and aggressiveness which probably has considerable consequences for *P. halstedii* evolution, because races that accumulate a large number of virulence genes might never be the most aggressive on sunflower genotypes. Moreover, the negative relationship between the two components of pathogenicity (Sakr 2014a, 2014b) may play an important role in generating local adaptation in the pathosystem of *P. halstedii* and sunflower by impeding the emergence and evolution of races that are both highly aggressive and capable of multiplying on all sunflower genotypes. However, Ahmed et al. (2012) and Sakr (2011b, 2014a) suggested another mechanism: multiple introductions of several pathogen races to underline the establishment of *P. halstedii* in France. The experimental conditions in sunflower cages isolated from exterior environment (Sakr, 2011b; Tourvieille de Labrouhe et al., 2010) excluded the introduction of new races. The potential of *P. halstedii* to evolve new races has also been enhanced by the introduction of several genetically differentiated genotypes: repeated introductions of *P. halstedii* isolates, combined with the selective pressure exerted by host resistance genes, may have greatly accelerated the evolution of new races (Novak, 2007; Sakr, 2014a, 2014b). To improve our knowledgee on the emergence of new virulence of sunflower downy mildew populations, it is necessary to obtain genetic structure data for all the recorded races and to include different geographical isolates for each race.

Acknowledgements

Literature

Ahmed, S., Delmotte, F. and Tourvieille de Labrouhe, D. (2012): Emerging virulence arising from hybridisation facilitated by multiple introductions of the sunflower downy mildew pathogen *Plasmopara halstedii*. Fungal Genet. Biol. 49, 847–855.

Andrivon, D. (1994): Race structure and dynamics in populations of *Phytophthora infestans*. Can. J. Botany 72, 1681–1687.

As-Sadi, F., Carrere, S., Gascuel, Q., Hourlier, T., Rengel, D., Le Paslier, M. C., Bordat, A., Boniface, M. C., Brunel, D., Gouzy, J., Godiard, L. and Vincourt, P. (2011): Transcriptomic analysis of the interaction between *Helianthus annuus* and its obligate parasite *Plasmopara halstedii* shows single nucleotide polymorphisms in CRN sequences. BMC Genomics 12, 498.

Bayles, R. A., Flath, K., Hovmoller, M. S. and de Vallavieille-Pope, C. (2000): Breakdown of the *Yr17* resistance to yellow rust of wheat in northern Europe. Agronomie 20, 805–811.

Brasier, C. M., Cooke, D. E. L. and Duncan, J. M. (1999): Origin of a new *Phytophthora* pathogen through interspecific hybridization. P. Nat. Acad. Sci. USA 96, 5878–5883.

Brown, J. K. M. and Tellier, A. (2011): Plant-parasite coevolution: Bridging the gap between genetics and ecology. Annu. Rev. Phytopathol. 49, 345–367.

Delmotte, F., Giresse, X., Richard-Cervera, S., M'baya, J., Vear, F., Tourvieille, J., Walser, P. and Tourvieille de Labrouhe, D. (2008): Single nucleotide polymorphisms reveal multiple introductions into France of *Plasmopara halstedii*, the plant pathogen causing sunflower downy mildew. Infect. Genet. Evol. 8, 534–540.

Delmotte, F., Machefer, V., Giresse, X., Richard-Cervera, S., Latorse, M. P. and Beffa, R. (2011): Characterization of single-nucleotide-polymorphism markers for *Plasmopara viticola*, the causal agent of grapevine downy mildew. Appl. Environ. Microbiol. 77, 7861–7863.

Flor, H. H. (1971): Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9, 275–296.

Jacobs, T. H. and Parlevliet, J. E. (1993): Durability of disease resistance. Springer-Verlag Publications, pp. 1–375.

Jin, L. and Chakraborty, R. (1993): Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting data. Mol. Biol. Evol. 11, 120–127.

Kamoun, S. (2003): Molecular genetics of pathogenic oomycetes. Eukaryot. Cell 2, 191–199.

Kolmer, J. A. (1993): Selection in a heterogeneous populations of *Puccinia recondita* f.sp. *tritici*. Phytopathology 83, 909–914.

Lebeda, A. and Petrzelova, I. (2004): Variation and distribution of virulence phenotypes of *Bremia lactucae* in natural populations of *Lactuca serriola*. Plant Pathol. 53, 316–324.

Librado, P. and Rozas, J. (2009): DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Molinero-Ruiz, M. L., Dominguez, J. and Melero-Vars, J. M. (2002): Races of isolates of *Plasmopara halstedii* from Spain and studies on their virulence. Plant Dis. 86, 736–740.

Novak, S. J. (2007): The role of evolution in the invasion process. P. Nat. Acad. Sci. USA 104, 3671–3672.

Parisi, L., Lespinasse, Y., Guillaumes, J. and Kruger, J. (1993): A new race of *Venturia inaequalis* virulent to apples with resistance due to the *Vf* gene. Phytopathology 839, 533–537.

Pedley, K. F. and Martin, G. B. (2003): Molecular basis of Pto-mediated resistance to bacterial speck disease in tomato. Annu. Rev. Phytopathol. 14, 215–243.

Sakr, N. (2011a): Aggressiveness in *Plasmopara halstedii* (sunflower downy mildew). Plant Pathol. J. 27, 110–115.

Sakr, N. (2011b): Evolution of pathogenicity in *Plasmopara halstedii* (sunflower downy mildew). Arch. Phytopathol. Plant Protect. 44, 1432–1436.

Sakr, N. (2012): Virulence cost in *Plasmopara halstedii* (sunflower downy mildew). Arch. Phytopathol. Plant Protect. 45, 646–651.

Sakr, N. (2014a): Evolution of new *Plasmopara halstedii* races under the selection pressure with resistant sunflower plants: A review. Hellenic Plant Protect. J. l7, 1–13.

Sakr, N. (2014b): Determining the fitness cost associated with virulence in *Plasmopara halstedii* (sunflower downy mildew). Acta Phytopathol. et Entomol. Hung. 49, 201–210.

Sakr, N. (2014c): Pathogenicity in *Plasmopara halstedii* (sunflower downy mildew) races 710, 714 and 704. Acta Phytopathol. et Entomol. Hung. 49, 11–24.

Sakr, N. (2015): *Intra-* and *inter-isolate* variation in two predominant *Plasmopara halstedii* (sunflower downy mildew) races in Europe. Botanica Serbica 39, 1–8.

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

Sakr, N., Tourvieille de Labrouhe, D., Walser, P., Ducher, M., Delmotte, F., Tourvieille, J. and Vear, F. (2011): Analyzing the influence of qualitative resistance selection pressure on variation of aggressiveness in *Plasmopara halstedii*. J. Plant Protect. Res. 51, 140–144.

Spring, O. and Zipper, R. (2006): Evidence for asexual genetic recombination in sunflower downy mildew, *Plasmopara halstedii*. Mycol. Res. 110, 657–663.

Spring, O., Bachofer, M., Thines, M., Riethmuller, A., Goker, M. and Oberwinkler, F. (2006): Intraspecific relationship of *Plasmopara halstedii* isolates differing in pathogenicity and geographic origin based on ITS sequence data. Eur. J. Plant Pathol. 114, 309–315.

Tourvieille de Labrouhe, D., Pilorge, E., Nicolas, P. and Vear, F. (2000): Le mildiou du tournesol. CETIOM-INRA Publications, pp. 1–176.

Tourvieille de Labrouhe, D., Bordat, A., Tourvieille, J., Mestries, E., Walser, P., Sakr, N., Ducher, M., Delmotte, F. and Vear, F. (2010): Impact of major gene resistance management for sunflower on fitness of *Plasmopara halstedii* (downy mildew) populations. Oleagineux, Corps Gras, Lipides 17, 56–64.

Van der Hoorn, R. A. L., De Wit, P. J. G. M. and Joosten, M. H. A. (2002): Balancing selection favours guarding resistance proteins. Trends Plant Sci. 7, 67–71.