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Intra- and *inter-isolate* variation in two predominant *Plasmopara halstedii* (sunflower downy mildew) races in Europe

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ABSTRACT: Morphological, pathogenic and genetic variation was studied in two *Plasmopara halstedii* (sunflower downy mildew) races 100 and 710 using five single zoosporangium isolates per pathogen isolate. Aggressiveness criteria were analysed in one sunflower inbred line showing a high level of quantitative resistance. Morphological analyses were carried out on zoosporangia and sporangiophores for single zoosporangium isolates. Genetic relationships were detected among the single zoosporangium isolates using 12 EST (Expressed Sequence Tag)-derived markers. Index of aggressiveness was calculated for each isolate and revealed the presence of significant differences in and between single zoosporangium isolates of two races 100 and 710. There were significant morphological differences for pathogen single zoosporangium isolates. There was no relation between zoosporangia and sporangiophore morphology and race virulence profiles or aggressiveness criteria. There was no *intra-isolate* genetic variation for the two pathogen races, but major genetic variation was observed between single zoosporangium isolates of the two races 100 and 710. A correlation was detected between pathogenicity traits and EST genotypes.

Key words: *Plasmopara halstedii*, sunflower downy mildew, EST-derived markers, morphology of zoosporangia and sporangiophores, aggressiveness, virulence, *Helianthus annuus*

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INTRODUCTION

Downy mildew is caused by the obligate Oomycete parasite *Plasmopara halstedii*, an invasive species where sunflower (*Helianthus annuus L.*) is grown. Its life cycle is made up of a single sexual generation permitting overwintering and one or perhaps two asexual generations which occur during the growing season (SPRING & ZIPPER 2006). Disease resistance in sunflower to *P. halstedii* can be classified into 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 (Tourvieille de Labrouhe *et al.* 2000). The second is quantitative resistance which is controlled by minor genes and influences the rate of disease development (rate reducing) rather than producing a disease-free plant (Tourvieille de Labrouhe *et al.* 2008).

P. halstedii shows physiological races (pathotypes) capable of infecting a variable range of sunflower genotypes. This species is widespread in all sunflower-growing countries with the exception of Australia. *P. halstedii* has been known since before 1950, both in North America and Russia, and since 1966 in France (Tourvieille de Labrouhe *et al.*, 2000; DELMOTTE *et al.* 2008; JOCIC *et al.* 2012), corresponding to development of the sunflower crop. Major gene resistance played a very important role in the development of sunflower acreages from 1974, particularly in Europe and USA and all modern varieties carry some major resistance genes. This resistance allowed the demonstration of physiological specialization

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of the pathogen, into two races, race 100 in Europe, and race 300 in North America. Race 710 was introduced into European sunflower cultivated zones from the USA during the 1980s (Tourvieille de Labrouhe *et al.* 2000; DELMOTTE *et al.* 2008; JOCIC *et al.* 2012). In Europe, races 100 and 710 could be considered as the dominant pathotypes (Gulya 2007; JOCIC *et al.* 2012).

Variability of virulence in *P. halstedii* has been the subject of several studies (Tourvieille de Labrouhe *et al.* 2000; Gulya 2007; SAKR 2011a, 2012) because major monogenic resistances have been overcome rapidly by the parasite. Downy mildew races have been observed by SAKR (2009, 2011a,b,c, 2012) to show considerable differences in aggressiveness and this affects host response, the most aggressive races often producing symptoms which cannot be compared with those produced by those that are less aggressive. Virulence has been defined as specific disease-causing abilities and aggressiveness as non-specific disease-causing abilities (Van der Plank 1968). For morphology in *P. halstedii*, SPRING & THINES (2004) found phenotypic-limited tools for analyzing *P. halstedii*. Concerning genotypic diversity in *P. halstedii*, Internal Transcribed Spacer (ITS) (SPRING *et al.* 2006) and Expressed Sequence Tag (EST) (DELMOTTE *et al.* 2008) sequences have been used to characterize *P. halstedii* isolates, but races can still not be defined with certainty. However, AS-SADI *et al.* (2011) reported that genetic distance between four *P. halstedii* races could be detected using Single Nucleotide Polymorphisms (SNPs) discovered in CRN (a cell-death-inducing protein that causes crinkling and necrosis phenotypes in the leaves of infected plants) effector sequences. To generate information about the *intra-* and *inter-isolate* variation in *P. halstedii*, the aim of this study was to analyze morphological, pathogenic and genetic variation in two *P. halstedii* predominant pathotypes 100 and 710.

MATERIALS and METHODS

Fungal isolates. The two *P. halstedii* isolates used in this study were collected in France and maintained at INRA, Clermont–Ferrand. Manipulation of this quarantine parasite respected European regulations (No 2003/DRAF/70). Isolate MIL 001 (race 100) was isolated in 1966 and isolate MIL 002 (710) in 1988. Their virulence characteristics (Table 1) were defined by Tourvieille de Labrouhe *et al.* (2000) which are based on the reaction of a series of differential lines. For each isolate, five single zoosporangium isolates were obtained according to the method described by SAKR *et al.* (2007). This study dealt with five single zoosporangium isolates per pathogen isolate, giving a total of 10 single zoosporangium isolates. The characterization of the race for 10 single zoosporangium isolates (Table 2) was determined using the same method adapted in the study by Tourvieille de Labrouhe *et al.* (2000). For race identification, there were three replications for each sunflower differential line (10 plants in each replication) and the entire experiment was repeated twice for *P. halstedii* isolates.

Measurement of aggressiveness in *P. halstedii* single zoosporangium isolates. To characterize aggressiveness criteria: percentage infection, latent period, sporulation density and reduction of hypocotyl length (SAKR 2009, 2011a,b,c, 2012) for 10 *P. halstedii* single zoosporangium isolates, one INRA inbred line FU was used. It carried no *Pl* gene, but is known to a have a high level of quantitative resistance (Tourvieille de Labrouhe *et al.* 2008). The index of aggressiveness of *P. halstedii* single zoosporangium isolates was calculated as the ratio of (percentage infection × sporulation density) / (latent period × dwarfing) (SAKR 2011a,c, 2012). All the pathogenic tests were carried out in growth chambers regulated at 18 h of light, 18±1 °C and RH of 65 - 90%. All statistical analysis of aggressiveness data were performed using Stat Box 6.7® (GimmerSoft) software. The values obtained were submitted to one-way analysis of variance (ANOVA). The Newman-Keuls test (SNEDECOR & COCHRAN 1986) was used to compare the means at $p > 0.05$. Sample correlation coefficients (Pearson *r*) were calculated at $p > 0.05$ and $p > 0.01$.

Morphological observations. After 13 d of infection of sunflower inbred line `FU`, the zoosporangia and sporangiophores suspensions were obtained by grouping all sporulated cotyledons for each single zoosporangium isolate in a small container and adding 1 ml of physiological water for each cotyledon (9g NaCl + 1L sterilized water). This slowed zoosporangia maturation to facilitate observations before liberation of zoospores (SAKR *et al.* 2007). Identification of form and measurement of size was carried out on 50 zoosporangia per treatment under a light microscope (magnification X400) with 2 replications. Zoosporangia size was calculated from an oval $\pi \times a \times b$, a = ½ length, b = ½ width. Furthermore, sporangiophore dimensions were observed by measuring 50 fresh sporangiophores in physiological water under a light microscope (magnification X400) with 2 replications.

DNA extraction and molecular typing. For 10 single zoosporangium isolates tested, DNA was isolated from infected plant tissue and 12 polymorphic EST-derived markers were used to genotype the *P. halstedii* isolates (DELMOTTE *et al.* 2008).

RESULTS

Analysis of aggressiveness criteria. Table 2 presents aggressiveness criteria of ten *P. halstedii* single zoosporangium isolates. All tested isolates showed high percentage infections (Table 2). MIL 001 showed the greatest variability with the widest range: from 92 to 100%. MIL 002 showed no significant variability: from 90

Table 1. Virulence of two *Plasmopara halstedii* isolates of races 100 and 710 on nine sunflower differential lines

Isolates	Race	Year isolated	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
MIL001	100	1966	S	R	R	R	R	R	R	R	R
MIL002	710	1988	S	S	S	S	R	R	R	R	R

S: susceptible, sporulation on cotyledons. R: resistant, no sporulation data from TOURVIEILLE DE LABROUHE *et al.* (2000), identification of virulence profile for two *P. halstedii* isolates was presented by SAKR (2011b).

Table 2. Aggressiveness within pathogen isolate for ten *Plasmopara halstedii* single zoosporangium isolates and among two *Plasmopara halstedii* isolates (five replications per pathogen isolate that correspond to five single zoosporangium isolates) measured on the sunflower inbred line `FU`

Single zoosporangium isolates	Race	Percentage infection	Latent period	Sporulation density	Hypocotyl length	Index of aggressiveness
		Mean (%)	Mean (days)	Mean ($\times 10^5$ zoosporangia per cotyledon)	Mean (mm)	
MIL001 M2	100	95.0 ab	9.09	19.68	32.4 b	5.5
MIL001 M3	100	100.0 a	9.30	12.70	28.7 c	3.4
MIL001 M4	100	95.6 ab	10.20	11.97	28.3 c	3.3
MIL001 M5	100	97.2 ab	8.93	13.16	27.9 c	3.1
MIL001 M6	100	92.1 b	8.58	14.11	36.8 a	4.1
		p = 0.0	p = 0.3 ns	p= 0.12 ns	p = 0.0	
		vc= 4.61%	vc = 7.76%	vc = 16.13%	vc = 2.05%	
MIL002 M1	710	96.0	10.35	7.44	28.3 b	2.1
MIL002 M2	710	95.6	11.76	5.45	32.8 a	2.0
MIL002 M3	710	95.6	11.07	8.45	29.6 b	2.5
MIL002 M4	710	95.6	10.32	8.25	26.1 c	2.1
MIL002 M5	710	94.4	10.48	5.56	27.4 b	1.5
		p = 0.5 ns	p = 0.6 ns	p= 0.5 ns	p = 0.0	
		vc = 7.06%	vc = 5.52%	vc = 29.68%	vc = 4.42%	
MIL 001 Mean	100	96.1	9.22 b	14.32 a	30.8	3.9
Standard deviation		2.6	0.54	2.77	3.4	0.8
MIL 002 Mean	710	93.2	10.80 a	7.03 b	28.3	2.0
Standard deviation		2.6	0.55	1.29	2.3	0.3
Probability		0.3747 ns	0.0033	0.0014	0.3616 ns	0.0043
F isolates		0.884	17.094	22.835	0.936	15.53

The method described to measure aggressiveness in *P. halstedii* isolates was presented by Sakr (2011 a,b,c), according to the Newman-Keuls test, means followed by the same letter are not significantly different at $p > 0.05$, ns = not significant, Probability (p), Variation Coefficient (vc), F-tests ($p > 0.05$), index of aggressiveness = (percentage infection × sporulation density) / (latent period × dwarfing).

to 96%, with the lowest mean level (93%). The latent period ranged from 8.58 d for isolate MIL001 M6 to 11.76 d for isolate MIL002 M2. Table 2 shows that the two pathogen isolates MIL 001 and MIL 002 did not reveal significant variability. Sporulation density varied four fold: 5.45 $\times 10^5$ zoosporangia were produced by cotyledons for isolate MIL002 M2 and 19.68 $\times 10^5$ for isolate MIL001 M2. The two pathogen isolates MIL 001 and MIL 002 were not significantly variable (Table 2). Diseased plants had hypocotyls with only one third the mean lengths of *P. halstedii*-free sunflower inbred line FU (30.85 ± 0.6 mm and 90.0 ± 2.3 mm respectively) whatever the tested isolate of *P. halstedii*. Hypocotyl length varied from 26.1 mm for isolate MIL002 M4 to 36.8 mm for isolate MIL001 M6. The index of aggressiveness ranged between 1.5 and 2.5 for tested isolates of race 710 and it varied between 3.1 and 5.5 for tested isolates of race 100 (Table 2). Indeed, the index of aggressiveness revealed the presence of significant differences (P=0.0043, F isolates=15.530) between the two tested isolates of races 100 and 710 (Table 2).

Analysis of the relation between sporulation percentage based on incubation period (Figure1) showed differences in behaviour among the *P. halstedii* isolates. There were two main groups after 9 d of inoculation: tested isolates of race 100 sporulated faster than tested isolates of race 710. All plants infected with isolates of race 100 showed more than 80% sporulation 9 d after incubation, whereas isolates of race 710 needed 11 d after incubation to reach the same level of sporulation. Figure 2 shows that the quantities of zoosporangia produced increased with time. There were two main groups on day 9 of incubation: tested isolates of race 100 produced more zoosporangia than tested isolates of race 710 (Figure 2). The quantity of zoosporangia produced was at a maximum 12 d after incubation.

Morphology of zoosporangia and sporangiophores. The two most observed forms were oval and round (Figure 3). Table 3 showed that the proportion of oval form varied from 37 to 94% and the zoosporangia size from 315.8 to 918.6 µm². The proportion of oval zoosporangia varied within the two races for which five single zoosporangium were available, for example for race 100 it varied from 87% to 94% and for race 710 it ranged from 37% to 92%. Zoosporangia size also varied considerably within and between the two races 100 and 710, with no relation to form. The sporangiophore length ranged between 325.9 and 715.9 µm (Table 3). Mean sporangiophore width was largest in MIL 001 M3 isolate. The sporangiophore width varied from 6.6 µm to 15.1 µm. The dimensions of sporangiophores ranged significantly within and between the two isolates MIL 001 and MIL 002. No aggressiveness criterion was correlated with form or size of zoosporangia (r = 0.055 and r = -0.567 for form and size, respectively, for percentage infection, r = -0.207 and r = 0.495 for latent period, r = 0.405 and r = 0.366 for sporulation density and r = 0.526 and r = 0.495 for hypocotyl length), and length or width of sporangiophore (r = 0.153 and r = 0.510 for length and width, respectively, for percentage infection, r = 0.178 and r = -0.241 for latent period, r = -0.293 and r = 0.280 for sporulation density and r = -0.270 and r = -0.138 for hypocotyl length).

Molecular analysis. The combination of 12 EST-derived markers revealed two multilocus genotypes (MLG) among the ten *P. halstedii* isolates (Table 4). There was no *intra-isolate* genetic variation for the two isolates MIL 001 and MIL 002 (Table 4). The tested isolates of MIL 001 and MIL 002 were different for all genomic markers excepting Pha54.

DISCUSSION

Knowledge of the interaction between parasite and its host plant would help to understand the co-evolution of pathogen populations that use their pathogenicity to better improve adaptation to their host, and particularly processes that generate genetic diversity (BROWN & TELLIER 2011). With this in mind, morphological, pathogenic and genetic variation was studied in two *P. halstedii* predominant races 100 and 710 in Europe (GULYA 2007, IOCIC *et al.* 2012). Studying these aspects could help to establish the diagnostic value of these characters in population studies of *P. halstedii*. As tools for analyzing the obligate parasitic Peronosporacea are very limited (SPRING & THINES 2004), it appears desirable to research new methods.

The two *P. halstedii* isolates of races 100 and 710 used were uncloned. Consequently, they represent a mixture of different races which may show the same race phenotype. To diminish this problem, analyses were performed with single zoosporangium isolates (SAKR *et al.* 2007). These may give rise to genetically homogenous isolates if only a single karyon entered the zoosporangium or when the parental mycelium was homokaryotic. Furthermore, we confirmed that single zoosporangium isolates were of the same race as the pathogen isolates (Tables 1 and 2). Indeed, in the same pathosystem, Molinero-Ruiz *et al.* (2002) found that some single zoosporangium isolates obtained from one parental isolate showed differences in the virulence profile as compared with the parental one. However, there is a possibility of genetic inhomogeneity of single zoosporangium isolates (in contrast to single zoospore isolates) because indications now exist (SPRING & ZIPPER 2006) that two or even more nuclei immigrate into a single zoosporangium; these nuclei may then mitotically divide there. This may cause that in a heterokaryotic mycelium genetically inhomogeneic zoospores can develop in a single zoosporangium.

Differences in aggressiveness of *P. halstedii* pathotypes are indicated when single zoosporangium isolates vary in the amount of damage that they cause in sunflower plants

Table 3. Morphological characters of zoosporangia obtained on sunflower inbred line FU for ten single zoosporangium isolates of *Plasmopara halstedii*

Isolates of *P. halstedii*	Race	% of oval zoosporangia [a]	Size of zoosporangia in µm² [a]	Sporangiophore length (µm) [b]	Sporangiophore width (µm) [b]
MIL001 M2	100	87	315.8	325.9	12.3
MIL001 M3	100	94	434.9	550.2	15.1
MIL001 M4	100	88	392.9	715.9	10.9
MIL001 M5	100	91	418.7	660.2	8.7
MIL001 M6	100	90	432.2	489.3	6.9
MIL002 M1	710	82	463.5	459.3	7.8
MIL002 M2	710	92	513.3	569.3	6.6
MIL002 M3	710	90	918.6	553.6	8.9
MIL002 M4	710	37	352.9	440.4	12.0
MIL002 M5	710	53	419.4	5[illegible]3	11.9
Probability		0.0001	0.0001	0.0001	0.0001
F isolates		23.35*	595.744*	[illegible]35*	5.04*
Number replications		2	2	2	2

[a]50 zoosporangia per replication, [b]50 sporangiophores per replication, F-tests ($p > 0.05$).

Table 4. Multilocus genotypes (MLG) characterized using 12 EST-derived genomic markers on ten single zoosporangium isolates of *Plasmopara halstedii*

Isolate	EST-derived markers											
	Pha6	*Pha39*	*Pha42*	*Pha43*	*Pha54*	*Pha56*	*Pha74*	*Pha79*	*Pha82*	*Pha99*	*Pha106*	*Pha120*
MIL001M2	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001M3	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001M4	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001M5	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL001M6	2/2	2/2	1/1	1/1	1/1	1/1	1/1	3/3	2/2	2/2	1/1	2/2
MIL002 M1	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	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	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	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	1/1	1/1	2/2	2/2	1/1	2/2	2/2	1/1	1/1	1/1	2/2	1/1

SAKR 2009, 2011a,b,c, 2012). Index of aggressiveness was calculated for each isolate and revealed the presence of significant differences in and between single zoosporangium isolates of two races 100 and 710 (Table 2). Aggressiveness for one representative zoosporangium could be multiplied and become dominant in the tested isolates for the two races. Consequently, *intra*-isolate variation may be due to the multiplication and domination of aggressiveness for one zoosporangium. The frequency of sporulated plants based on incubation period reflected the speed of appearance of symptoms on the plants (Figure 1) (latent period), and the number of zoosporangia produced by cotyledons reflected the level of invasion of infected tissues (sporulation density) (Figure 2).

The two pathogen isolates MIL 001 and MIL 002 revealed significant variability for all aggressiveness criteria (Table 2). It is possible that variation among

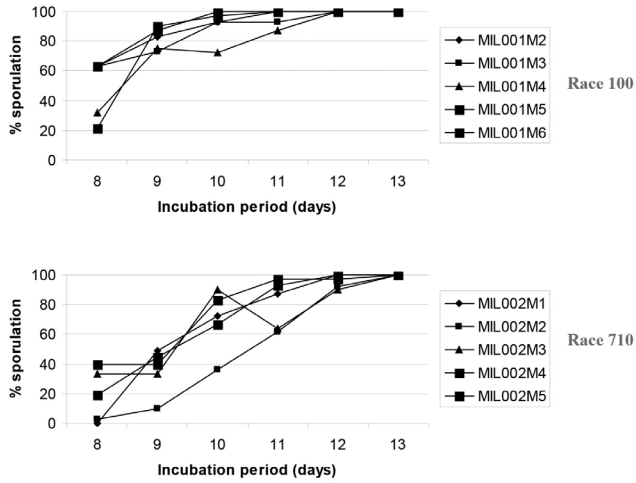

Figure 1. Sporulation of ten single zoosporangium *Plasmopara halstedii* isolates of two races 100 and 710 on the sunflower inbred line `FU`, based on incubation period

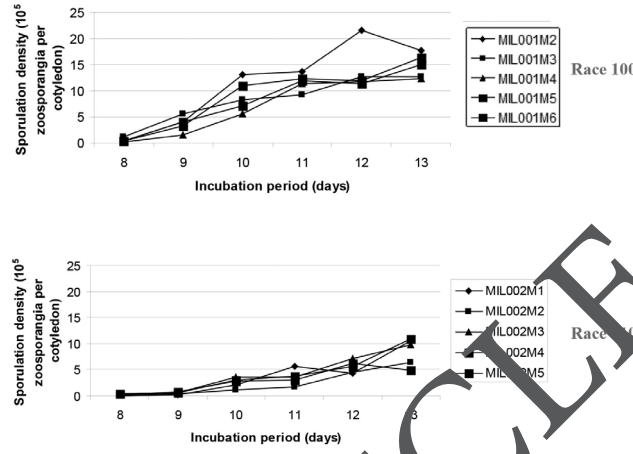

Figure 2. Sporulation density of ten single zoosporangium *Plasmopara halstedii* isolates of two races 100 and 710 on sunflower inbred line `FU`, based on incubation period

isolates of races 100 and 710 is due to the origin of pathogen isolates used in this study (Table 1). The isolates MIL 001 and MIL 002 belong to two races 100 and 710 and may be found to be an effect of additional virulence genes in *P. halstedii* isolates as observed for other pathogens (MONTARRY *et al.* 2010; DE VALLAVIEILLE-POPE *et al.* 2012). High percentage infection, short latent period, high sporulation density, and significant reduction in the length of the hypocotyl represent high aggressiveness (SAKR 2009, 2011a,b,c, 2012). Our results are in accordance with previous analyses showing that race 100 is more aggressive than race 710 (SAKR 2009, 2011a,b,c). Single zoosporangium isolates of race 710 were more virulent than those of race 100 (Table 1) as defined by Tourvieille de Labrouhe *et al.* (2000) and SAKR (2009, 2011a,b,c). It seems that *P. halstedii* single zoosporangium isolates may be divided into two pathogenic groups as more virulent and less aggressive single zoosporangium isolates of race 710 and less virulent and more aggressive single zoosporangium isolates of race 100. The finding that both virulence and aggressiveness groups are identical has also been demonstrated for *P. halstedii* (SAKR 2011a) and some other pathogens (DE WET *et al.* 2003; BONDE *et al.* 2006; MONTARRY *et al.* 2006).

Table 2 showed that there were significant morphological differences amongst the ten pathogen single zoosporangium isolates of two isolates MIL 001 and MIL 002. The proportion of zoosporangia of different forms and their sizes and the morphology of sporangiophores do not appear to be useable to differentiate the ten single zoosporangium isolates of two races 100 and 710 (Tables 2 and 3) as defined by Tourvieille de Labrouhe *et al.* (2000). The results also showed that zoosporangia morphology did not distinguish single zoosporangium isolates according to their aggressiveness (Tables 2 and 3).

Our results provided evidence that no relation between morphology of zoosporangia and sporangiophores race virulence profiles or aggressiveness criteria may be established in *P. halstedii*. However, for the same pathosystem, SAKR (2011c) found a relationship between another phenotypic character (viability of zoosporangia) and aggressiveness in *P. halstedii*. In accordance with our results, ISLAM *et al.* (2004) found no relationship between groups of isolates characterized for their morphological patterns of *Phytophthora capsici* and their pathogenicity traits. This is in contrast with the results of DE WET *et al.* (2003) who observed morphological differences between strains of *Sphaeropsis sapinea*, which divided them into 3 morphotypes (A, B and C) that presented differences in pathogenicity (virulence and aggressiveness).

Our results (Table 4) demonstrated genetic distances between the two races 100 and 710, which differed for all genomic markers except Pha54, in agreement with previous studies (DELMOTTE *et al.* 2008; AS-SADI *et al.* 2011; SAKR 2011b,d). Absence of *intra*-isolate genetic variation may be because the molecular markers used in the present study were non-specific and insufficiently polymorphic within *P. halstedii* to detect differences among single zoosporangium isolates of the same race. A correlation was detected between pathogenicity traits (Table 2) and EST genotypes (Table 4). Indeed, for *P. halstedii*, SAKR (2011b,d) found no correlation between aggressiveness traits and EST genotypes. In accordance with our results, DE WET *et al.* (2003) found that aggressiveness groups A, B and C were separated into three differential clades in *Sphaeropsis sapinea*. However, MONTARRY *et al.* (2006) found no clear correlation between pathogenicity phenotypes and genotypes based on AFLP markers for *P. infestans*. No correlation was detected between EST genotypes (Table 4) and

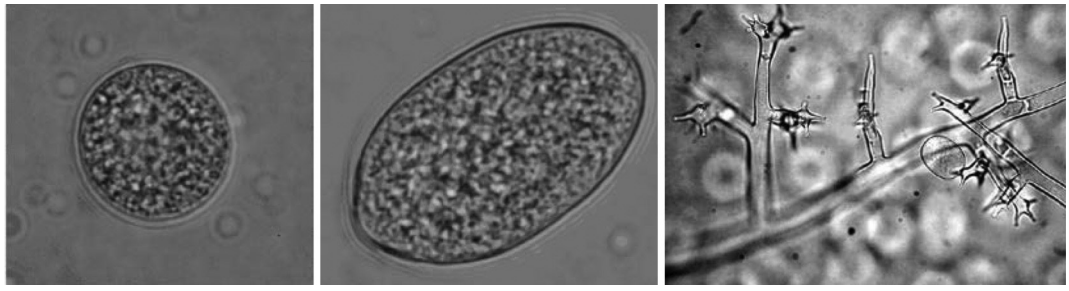

Figure 3. *Plasmopara halstedii* zoosporangia forms and sporangiophores observed on sunflower inbred line `FU`: round (left), oval (center) and sporangiophore (right)

morphological characteristics (Table 3). In accordance with our results, MAHDIZADEH *et al.* (2011) reported no correlation between genetic diversity based on Inter Simple Sequence Repeat (ISSR) and morphological characteristics for *Macrophomina phaseolina*.

CONCLUSION

Although major progress has been made towards understanding the complex interaction of *P. halstedii* and sunflower, as well as the mechanisms of pathogenicity evolution, a number of questions have remained unanswered. This paper presents fundamental data about significant differences between the two European predominant races 100 and 710. The most important characteristics which distinguished the single zoosporangium isolates for two races were pathogenic (aggressiveness and virulence) and genetic criteria. To improve our knowledge regarding the pathology of sunflower downy mildew populations, it will be necessary to identify the morphological, pathogenic and genetic groups in a large collection of *P. halstedii* isolates with different races from several parts of the world to provide a better insight into interactions between this obligate parasite and its host.

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REZIME

Variranje *intra-* i *inter-isolata* kod dve predominantne rase suncokretove plamenjače *Plasmopara halstedii* u Evropi

Nachaat SAKR

U ovom radu proučavana je morfološko, patogeno i genetičko variranje kod dve rase *Plasmopara halstedii* (suncokretova plamenjača) i to rase 100 i 710 uz pomoć izolacije pet zoosporangijuma po patogenu.
Kriterijum agresivnosti analiziran je na jednoj liniji suncokreta preko eksprimiranja kvantitativne rezistencije. Morfološke analize vršene su na zoosporangijama i sporangioforama. Genetički odnosi utvrdjivani su na osnovu 12 EST markera.
Indeks agresivnosti utvrdjivan je na osnovu značajne razilike kod izolata dve rase 100 i 710. Utvrdjena je značajna morfološka razlika kod različitih patogenih izolata zoosporangijuma. Nije nadjena veza izmedju morfologije zoosporangija i sporangiofora i rase ili agresivnosti.
Nije utvrdjena genetička razlika izmedju *intra*-izolata dve patogene rase, ali je utvrdjena značajna genetička varijabilnost medju pojedinačnim izolatima kod obe istraživane rase.
Utvrdjena je korelacija izmedju patogenosti i EST genotipova.

Ključne reči: *Plasmopara halstedii*, suncokretova plamenjača ,EST- markeri, morfologija zoosporangija i sporangiofora, agresivnost, virulentnost, *Helianthus annuus*