Proposal for improvement of sunflower downy mildew race nomenclature

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ABSTRACT

- Present nomenclature for *Plasmopara halstedii* races was last defined in 2000, at the ISA Conference in Toulouse. It is based on the reaction of 9 differential lines classed in 3 triplets which made it possible to name races using 3 figures. However, its limits have appeared since, on one hand, new races which cannot be distinguished by these 9 differentials have appeared and, on the other hand, new sources of resistance are being used to provide efficient resistance against new, more virulent pathotypes. The aim of the study presented here was to update nomenclature without losing information gained and published during the last 10 years.
- Fifty one inbred lines considered to cover diversity available, and the 9 differential lines were tested with 17 downy mildew races in standard experimental conditions in individual confined growth chambers: Fourteen different resistance profiles were obtained which, taking into account particularities of symptoms which make some lines difficult to identify as resistant or susceptible, led to our proposition for new differentials and modified nomenclature.
- The line « GB » should replace « HA304 » and « QHP2 » should replace « QHP1 ». Six new genotypes should be added to make up 2 new triplets to cover present resistance profiles: « Y7Q », « PSC8 », « XA », « PSS2RM », « VAQ », and « RHA419 ».
- Race nomenclature would be based on 5 instead of 3 figures. The first 3 figures, from the first 3 triplets would not change, in order to keep all present knowledge. For example, race 100 would become race 10010, whilst the 2 forms of race 304 found in France in 2000 and 2002 would become 30410 and 30430 respectively.
- Eight genotypes were confirmed to be resistant to all French races and there are certainly other original sources are in course of study. It will thus be essential to check regularly the efficiency of new resistances to all the different races known and to new strains of downy mildew which may appear. It should be considered that new updates in nomenclature will be necessary every 10 years. However, this purely phenotypic approach requires quite large and expensive studies, with some uncertainties due to complex clustering of resistance genes. It would be of great interest to obtain detailed molecular characterisation of the genes involved in the differential lines, such that race nomenclature could be based on reaction with specific resistance genes.

Key words: *Plasmopara halstedii*, sunflower, downy mildew, nomenclature, differential lines

INTRODUCTION

Plasmopara halstedii, the Oomycete causing sunflower downy mildew (DM), shows considerable variation for virulence when confronted with sunflower lines carrying different major genes giving complete, dominant resistance. These differential lines are used by pathologists and geneticists to characterise the virulence profile of DM strains. Their reactions in seedling tests (Tourvieille de Labrouhe *et al.*, 2000) make it possible to group DM isolates according to their pathotype (all the strains with the same virulence pattern). To simplify and clarify nomenclature, Gulya *et al.* (1998) proposed use of the nomenclature system defined by Limpert and Muller (1994). Adopted in 2000, this nomenclature makes it possible to define the virulence of a DM strain on 9 sunflower lines using 3 digits (Tourvieille de Labrouhe *et al.*, 2000). The principal races that have been identified are presented in Table 1.

Table 1. Principal downy mildew races identified throughout the world [**W**] (Gulya, 2007) and in France [**F**] (J. Moinard, pers. com.) and reaction of 9 differentials [**D1** to **D9**] (Gulya *et al.*, 1998)

Races	**W**	**F**	**D1 HA304**	**D2 RHA265**	**D3 RHA274**	**D4 PMI3**	**D5 PM17**	**D6 803.1**	**D7 HAR4**	**D8 QHP1**	**D9 HA335**
100	X	X	S	R	R	R	R	R	R	R	R
300	X	X	S	S	R	R	R	R	R	R	R
304		X	S	S	R	R	R	R	R	R	S
307		X	S	S	R	R	R	R	S	S	S
314		X	S	S	R	S	R	R	R	R	S
330	X		S	S	R	S	S	R	R	R	R
334		X	S	S	R	S	S	R	R	R	S
700	X	X	S	S	S	R	R	R	R	R	R
703	X	X	S	S	S	R	R	R	S	S	R
704		X	S	S	S	R	R	R	R	R	S
707		X	S	S	S	R	R	R	S	S	S
710	X	X	S	S	S	S	R	R	R	R	R
714		X	S	S	S	S	R	R	R	R	S
717		X	S	S	S	S	R	R	S	S	S
730	X	X	S	S	S	S	S	R	R	R	R
770	X		S	S	S	S	S	S	R	R	R
774		X	S	S	S	S	S	S	R	R	S

One of the useful points of this nomenclature system is that it can be updated without losing information. Tests with a group of three new differential lines will give names with four digits (and then another group would give five digits), while retaining those first defined. Thus, a strain of the race 700, not virulent on 3 new differential lines making up a fourth triplet, would be called 7000 in the group "700x", together with 7001, 7003, 7004…, if they exist.

The rapid evolution of *P. halstedii* populations in the last 10 years (Gulya, 2007) means that changes in present nomenclature are now necessary. We have had two examples for which the present three digit names are not sufficient. The first concerns isolates of race 330, one from Spain, the other from the USA, to which the 9 differentials gave the same reaction, but which differed in their virulence on several INRA lines containing *Pl5* (data not shown). The second example was for a DM strain isolated in 2002 on a sunflower variety considered to be resistant to all French races. Tests on the 9 differential lines indicated that it belonged to race 304 although the variety on which it was collected was resistant to a 304 strain collected in 2000. Since then, other sunflower genotypes have shown the same reactions (data not shown). In addition, the differential line D9, « HA335 », which was resistant to all known races in 1998, is now susceptible to many races, denoted "xx4" or "xx7" whereas several sunflower genotypes with new, apparently original resistances to all known races are now available. A third reason for updating differential lines is that certain lines chosen in 1998 have shown defects, giving either symptoms which are difficult to interpret or problems of seed production. These genotypes need to be replaced by similar lines with the same resistance profile.

In order to make propositions concerning changes in International nomenclature, detailed analyses were made of the reactions of 51 inbred sunflower lines which appeared to cover the known variability of resistance patterns to 17 *P. halstedii* pathotypes.

MATERIAL AND METHODS

Sunflower genotypes:

The 9 differentials (8 lines and one population, « HAR4 ») and 50 inbred sunflower lines and one population (« HAR5 »), bred by either INRA, France or USDA, USA tested with 17 DM races are presented in Table 2. The inbred lines are maintained at INRA Clermont-Ferrand by selfing and the populations by sib-crosses.

Table 2. Sunflower genotypes tested for their reaction to 17 downy mildew races

Genotype	Origin	Genotype	Origin	Genotype	Origin	Genotype	Origin
361MTP	INRA	HAR4	USDA	PSS2	INRA	TX167	USDA
829MTP	INRA	HAR5	USDA	PSS2RM	INRA	VAQ	INRA
83HR4	INRA	OPA3	INRA	PST3	INRA	VHQ	INRA
83HR4RM	INRA	OPA3RM	INRA	PST3RM	INRA	VKQ	INRA
83RM	INRA	OQP7	INRA	PST5	INRA	X2Q	INRA
803.1	IFVC	OQP8	INRA	PST5RM	INRA	XA	INRA
GB	INRA	PAA1	INRA	QHP1	INRA	XJQ	INRA
HA304	USDA	PAC2	INRA	QHP2	INRA	XK	INRA
HA335	USDA	PBT4	INRA	QPR1	INRA	XPQ	INRA
HA336	USDA	PHU9	INRA	QPR2	INRA	XRQ	INRA
HA337	USDA	PMI3	INRA	RHA265	USDA	XUQ	INRA
HA338	USDA	PM17	USDA	RHA274	USDA	Y7Q	INRA
HA339	USDA	PRH3	INRA	RHA340	USDA	Y8Q	INRA
HA458	USDA	PSC88	INRA	RHA419	USDA	YSQ	INRA
HA459	USDA	PSC8RM	INRA	RHA428	USDA	YVQ	INRA

Plasmopara halstedii races:

The 17 races used are all maintained at INRA, Clermont-Ferrand. All were collected in France except race 330, provided by T. Gulya (USDA). Their virulence profiles are detailed in Table 1.

Resistance Test:

All the tests were carried out in small growth chambers (one race per chamber) with NS3 confinement in agreement with quarantine parasite legislation. Germinating sunflower seeds (radicle length about 5mm) were soaked in fresh zoosporangia suspensions and then, two weeks later, after 48 at 100%RH, seedlings were classed as resistant (no sporulation: "**RI**"; weak sporulation on cotyledons: "**RII**") or susceptible (sporulation on leaves: "**SI**"; or intense sporulation on cotyledons only: "**SII**") as defined by Tourvieille de Labrouhe *et al.* (2000). For each race, a minimum of 25 seedlings of each sunflower genotype were observed.

RESULTS

Some of the genotypes tested could not be defined as resistant or susceptible to certain races because either they showed intermediate reactions or were not completely homozygous or contained impurities: « HA304 », « HA338 », « HA339 », « HA459 », « HAR5 », « PBT4 », « PHU9 », « PRH3 », « PST3RM », « RHA428 », « TX167 », « QPR2 », « X2Q », « XRQ », « XUQ », and « Y8Q ». These genotypes were excluded from the choice of new differential lines. The other 44 lines presented 14 different resistance profiles with the 17 races tested (Table 3). It may be noted that, as always, since 1998, « QHP1 » and the population « HAR4 » have shown no difference in reaction.

However, even for some of the lines with the same resistance profile, differences in symptoms were observed. There were no problems in defining "Susceptibility", but "Resistance" was more difficult. To facilitate definition of downy mildew races, lines showing the least proportion of "RII" plants (with sporulation on cotyledons) appear preferable. Among present differentials, « HA304 », D1, gave problems of interpretation of resistance and susceptibility and « QHP1 » is very difficult to multiply, so we suggest they should be replaced by « GB » and « QHP2 » respectively.

Table 4 presents detailed results for these two lines and for the six lines we propose as new differentials to make up two new triplets. Only « VAQ » tested with race 710 and « XA » tested with race 300 show "RII" reactions. « XA » is the only lines for its resistance profile and « VAQ » has the least "RII" of its group.

Table 3. Reaction of 44 sunflower lines to 17 races of *Plasmopara halstedii*

Genotypes[(1)]	**100**	**300**	**304 (00)**	**304 (02)**	**307**	**314**	**330**	**334**	**700**	**703**	**704**	**707**	**710**	**714**	**717**	**730**	**774**
361MTP, 829MTP, HA458, OQP7, OQP8, PAA1, RHA340, RHA419	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
QPR1, *803-1*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
PSS2, PST5, OPA3, 83HR4, *RHA274*	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S
PMI3	R	R	R	R	R	S	S	S	R	R	R	R	S	S	S	S	S
XJQ, VAQ, XPQ,	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	S
PM17, YSQ	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	S	S
QHP2, *QHP1*, *HAR4*	R	R	R	R	S	R	R	R	R	S	R	S	R	R	S	R	R
83HR4RM, 83RM, OPA3RM, PSS2RM, PST5RM	R	R	R	R	S	R	R	S	R	R	S	S	R	S	S	R	S
XA	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S
PAC2, PSC8, PST3, XK	R	R	R	S	R	S	R	R	S	S	S	S	S	S	S	S	S
PSC8RM, Ha336, HA337, *HA335*, VHQ, VKQ	R	R	S	S	S	S	R	S	R	R	S	S	R	S	S	R	S
RHA265	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Y7Q, YVQ	S	S	S	S	S	S	R	S	R	R	S	S	R	S	S	R	S
GB,	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[(1)] Present differentials in italics

DISCUSSION

Characterisation of virulence profiles of *P. halstedii* strains is essential for the control of sunflower downy mildew since genetic control is the most environmentally favourable method and although major gene resistance shows poor durability, chemical control is no better. Quantitative, probably non race specific resistance exists and will be present in future sunflower varieties but major gene resistance is still required in areas with conditions favourable for downy mildew. Since *P. halstedii* appears to show rapid changes in virulence, it appears important to update nomenclature to permit identification of all the virulences that exist in sunflower fields. However, it must be possible to retain all the information already available. Our proposition is to retain the previous nomenclature for the first 3 digits and to retain 9 differentials giving these digits, even though « HAR4 » and « QHP1 » appear identical. The only modifications are firstly to replace « HA304 » by « GB », for the completely susceptible line since « HA304 » shows quite frequently little or no sporulation whereas this is never observed with « GB ». It may be that « HA304 » has some quantitative resistance. Secondly, « QHP1 », difficult to multiply, should be replaced by « QHP2 », a line from the same origin and with the same resistance profile and much better seed production.

Six additional lines make it possible to identify some different virulence profiles so we propose that they should forms fourth and fifth triplets (Table 5). This modification will make it possible to include a genotype, « RHA419 », resistant to all the races studied and to distinguish some the two strains not separated by the 9 old differentials, for example races 30410 and 30430. All the lines proposed are freely available for use as downy mildew race differentials.

Table 4. Observations of numbers of plants in each class of resistance and susceptibility for 8 sunflower genotypes tested with 17 *Plasmopara halstedii* races.

Race	Genotype	Number of plants				Race	Genotype	Number of plants				Race	Genotype	Number of plant			
100		SI	SII	RII	RI	300		SI	SII	RII	RI	304		SI	SII	RII	RI
	GB	50	0	0	0		GB	48	0	0	0	(00)	GB	50	0	0	0
	QHP2	0	0	0	28		QHP2	0	0	0	25		QHP2	0	0	0	57
	Y7Q	32	4	0	0		Y7Q	35	0	0	0		Y7Q	43	2	0	2
	PSC8	0	0	0	33		PSC8	0	0	0	27		PSC8	0	0	0	41
	XA	0	0	0	40		XA	0	0	21	35		XA	0	0	2	76
	PSS2RM	0	0	0	27		PSS2RM	0	0	0	41		PSS2RM	0	0	2	56
	VAQ	0	0	0	36		VAQ	0	0	0	25		VAQ	0	0	0	60
	RHA419	0	0	0	42		RHA419	0	0	0	26		RHA419	0	0	0	42
304		SI	SII	RII	RI	307		SI	SII	RII	RI	314		SI	SII	RII	RI
(02)	GB	51	0	0	0		GB	47	0	0	0		GB	50	0	0	0
	QHP2	0	0	0	25		QHP2	5	31	0	0		QHP2	0	0	0	31
	Y7Q	23	2	0	1		Y7Q	22	6	0	2		Y7Q	26	0	0	0
	PSC8	22	5	0	1		PSC8	0	0	0	72		PSC8	18	29	3	2
	XA	0	0	4	25		XA	5	18	2	6		XA	0	0	2	24
	PSS2RM	0	0	0	26		PSS2RM	25	11	4	1		PSS2RM	0	0	0	25
	VAQ	0	0	0	31		VAQ	0	0	0	46		VAQ	0	0	3	29
	RHA419	0	0	0	36		RHA419	0	0	0	41		RHA419	0	0	0	39
330		SI	SII	RII	RI	334		SI	SII	RII	RI	700		SI	SII	RII	RI
	GB	50	0	0	0		GB	48	0	0	0		GB	45	0	0	0
	QHP2	0	0	0	44		QHP2	0	0	0	69		QHP2	0	0	0	36
	Y7Q	0	0	0	53		Y7Q	36	1	0	0		Y7Q	0	0	6	27
	PSC8	0	0	0	39		PSC8	0	0	0	40		PSC8	36	0	0	0
	XA	38	0	0	0		XA	20	14	1	1		XA	32	0	0	0
	PSS2RM	0	0	0	44		PSS2RM	26	6	1	0		PSS2RM	0	0	0	30
	VAQ	32	3	0	0		VAQ	54	2	4	2		VAQ	0	0	0	30
	RHA419	0	0	6	25		RHA419	0	0	0	73		RHA419	0	0	0	31
703		SI	SII	RII	RI	704		SI	SII	RII	RI	707		SI	SII	RII	RI
	GB	50	0	0	0		GB	50	0	0	0		GB	50	0	0	0
	QHP2	48	0	0	0		QHP2	0	0	0	32		QHP2	6	19	0	0
	Y7Q	0	0	0	37		Y7Q	30	3	0	0		Y7Q	6	19	0	1
	PSC8	32	3	0	0		PSC8	45	18	6	1		PSC8	32	2	0	0
	XA	39	8	0	0		XA	21	18	0	0		XA	22	7	1	0
	PSS2RM	0	0	0	40		PSS2RM	14	14	1	0		PSS2RM	8	20	0	2
	VAQ	0	0	0	48		VAQ	0	0	0	27		VAQ	0	0	0	32
	RHA419	0	0	0	36		RHA419	0	0	0	25		RHA419	0	0	0	25
710		SI	SII	RII	RI	714		SI	SII	RII	RI	717		SI	SII	RII	RI
	GB	52	0	0	0		GB	50	0	0	0		GB	50	0	0	0
	QHP2	0	0	0	39		QHP2	0	0	0	25		QHP2	7	42	0	0
	Y7Q	0	0	0	42		Y7Q	28	0	0	0		Y7Q	39	2	0	0
	PSC8	88	15	0	1		PSC8	27	3	0	1		PSC8	34	1	0	3
	XA	41	23	3	0		XA	12	8	8	0		XA	17	15	6	0
	PSS2RM	0	1	0	98		PSS2RM	12	30	0	0		PSS2RM	42	0	0	0
	VAQ	0	0	30	0		VAQ	0	0	0	25		VAQ	0	0	0	37
	RHA419	0	0	0	99		RHA419	0	0	0	25		RHA419	0	0	0	42
730		SI	SII	RII	RI	774		SI	SII	RII	RI						
	GB	41	0	0	0		GB	45	0	0	0						
	QHP2	0	0	0	25		QHP2	0	0	0	38						
	Y7Q	0	0	0	25		Y7Q	5	26	1	1						
	PSC8	25	0	0	0		PSC8	32	0	0	0						
	XA	25	0	0	0		XA	29	0	0	0						
	PSS2RM	0	0	0	25		PSS2RM	4	20	1	0						
	VAQ	0	0	3	25		VAQ	15	10	5	4						
	RHA419	0	0	0	25		RHA419	0	0	0	34						

SI = sporulation on cotyledons and true leaves

SII = no sporulation on leaves, abundant sporulation on cotyledons

RII = weak sporulation on cotyledons

RI = no sporulation

The lines of the first group in Table 3 had, so far, appeared resistant to all known races, but T. Gulya (personal communication) suggests that « RHA419 » and "RHA340" may be susceptible to new

downy mildew strains in the USA. Some of the other lines confirmed for their resistance to all French races, together with further original sources in course of study, almost certainly contain different resistance genes but it will thus be essential to check regularly the efficiency of new resistances to all the different races known and to new strains of downy mildew which may appear. It should be considered that new updates in nomenclature will be necessary every 10 years. However, this purely phenotypic approach requires quite large and expensive studies, with some uncertainties due to complex clustering of resistance genes. It would be of great interest to obtain detailed molecular characterisation of the genes involved in the differential lines, such that race nomenclature could be based on reaction with specific resistance genes.

Table 5. Proposal of a new nomenclature for *Plasmopara halstedii* races

Races	**D1** GB	**D2** RHA265	**D3** RHA274	**D4** PMI3	**D5** PM.17	**D6** 803.1	**D7** HAR.4	**D8** QHP2	**D9** HA335	**D10** Y7Q	**D11** PSC8	**D12** XA	**D13** PSS2RM	**D14** VAQ	**D15** RHA419
100*10*	S	R	R	R	R	R	R	R	R	*S*	*R*	*R*	*R*	*R*	*R*
300*10*	S	S	R	R	R	R	R	R	R	*S*	*R*	*R*	*R*	*R*	*R*
304*10*	S	S	R	R	R	R	R	R	S	*S*	*R*	*R*	*R*	*R*	*R*
3043*0*	S	S	R	R	R	R	R	R	S	*S*	*S*	*R*	*R*	*R*	*R*
3075*1*	S	S	R	R	R	R	S	S	S	*S*	*R*	*S*	*S*	*R*	*R*
3143*0*	S	S	R	S	R	R	R	R	S	*S*	*S*	*R*	*R*	*R*	*R*
3304*2*	S	S	R	S	S	R	R	R	R	*R*	*R*	*S*	*R*	*S*	*R*
3345*3*	S	S	R	S	S	R	R	R	S	*S*	*R*	*S*	*S*	*S*	*R*
7006*0*	S	S	S	R	R	R	R	R	R	*R*	*S*	*S*	*R*	*R*	*R*
7036*0*	S	S	S	R	R	R	S	S	R	*R*	*S*	*S*	*R*	*R*	*R*
7047*1*	S	S	S	R	R	R	R	R	S	*S*	*S*	*S*	*S*	*R*	*R*
7077*1*	S	S	S	R	R	R	S	S	S	*S*	*S*	*S*	*S*	*R*	*R*
7106*0*	S	S	S	S	R	R	R	R	R	*R*	*S*	*S*	*R*	*R*	*R*
7147*1*	S	S	S	S	R	R	R	R	S	*S*	*S*	*S*	*S*	*R*	*R*
7177*1*	S	S	S	S	R	R	S	S	S	*S*	*S*	*S*	*S*	*R*	*R*
7306*0*	S	S	S	S	S	R	R	R	R	*R*	*S*	*S*	*R*	*R*	*R*
7747*3*	S	S	S	S	S	S	R	R	S	*S*	*S*	*S*	*S*	*S*	*R*

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