Races of Isolates of *Plasmopara halstedii* from Spain and Studies on Their Virulence

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ABSTRACT

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Plants infected with downy mildew were collected from 1994 to 2000 in sunflower fields in Spain. The race of 102 bulk isolates of *Plasmopara halstedii* obtained from them was determined by inoculation of sunflower lines traditionally used as differentials for characterization of the pathogen. Nine different races of the fungus were determined. Although race 1 was most common and was the only one found in central Spain, races 4, 6, and 7 were widespread in southern Spain. The results allowed the identification of a new race of the pathogen, race 10, and of a race not previously reported in Europe, race 8. According to the proposal of a new system for characterization of the isolates of *P. halstedii* in the late 1990s, Coded Virulence Formulae (CVF) were assigned to bulk isolates and single-sporangium (*ss*) isolates obtained from them. The CVF of the bulk isolates (CVFi) did not always fit with the previous designation. Similarly, *ss* isolates from the same bulk isolate exhibited different CVF, not only among themselves, but also compared with the CVF of the source isolate. Although a revision of the differential lines used to perform the racial characterization of fungal isolates seems to be needed, the occurrence of a diversity of genotypes in field populations of *P. halstedii* and a high frequency of recombination and/or mutation of the fungal genome is also suggested.

Additional keywords: genetic resistance, *Helianthus annuus*

Downy mildew of sunflower (SDM) is a common disease in many areas of the world where sunflowers (*Helianthus annuus* L.) are grown. It is caused by the fungus *Plasmopara halstedii* (Farl.) Berl. & de Toni in Sacc. and infects young plants in spring when soil moisture is high and temperatures range from 15 to 18°C. The systemic infection of seedlings can be caused by both zoospores (asexual spores, mainly located on the underside of the leaves) and oospores (resting structures, mainly formed on the roots). Control of the disease is achieved primarily through genetic resistance.

The disease was first described in Spain in the 1970s, the European race (race 1 or 100) being the only race of the pathogen identified in the country (11,12). Race Red River (race 2 or 300) was first found in North Dakota and Minnesota. Resistance to these races is controlled by two genes: *Pl₁* and *Pl₂*, and both (together or independently) were incorporated into downy

mildew resistant sunflower hybrids (2,21, 22,26–28). During the last 20 years, seven new races of the pathogen have been identified in North America and several European countries (1,3–5,8,10,14,18,19,23,24). These new races are more virulent than the previous ones (race 100 in Europe and race 300 in North America) and have overcome the resistance genes *Pl₁* and *Pl₂*. Identification of SDM infections in resistant hybrids in Spain suggested that characterization of isolates of the fungus from Spain to race should be conducted. Previous research has been presented (15,16).

Different races of *P. halstedii* have been identified through the resistant/susceptible reactions of sunflower lines used as differentials. Nevertheless, this system has become too complex and confusing, since many isolates of different virulence are being continuously identified, thus requiring the use of many differential lines. Recently, the virulence of SDM isolates has been determined by means of a Coded Virulence Formula (CVF) obtained from the reactions of nine differential lines (9). The CVF has three digits, corresponding to the three sets of differentials tested. The first, second, and third differential of each set is assigned the values of 1, 2, and 4, respectively, only if the reaction is susceptible. All resistant reactions are assigned the value of 0. The sum of the values for the set equals the digit for that set (13). This nomenclature system is more dynamic

than race enumeration and is still compatible with former identifications.

The objectives of this research were: (i) to determine the race(s) of SDM bulk isolates collected in Spain, using both the traditional and the CVF systems, and (ii) to characterize the virulence of single-sporangium (*ss*) isolates using the CVF system, in order to compare it with the virulence of the corresponding SDM bulk isolate.

MATERIALS AND METHODS

Inoculum preparation. Field samples (110) of the pathogen were collected from commercial fields and breeding nurseries of sunflower in different locations in Spain from 1994 to 2000. The zoospores of the pathogen were recovered directly from individual plants that showed pathogen sporulation on leaves, or after 24 to 48 h of incubation in a humid chamber at 15°C in the dark. The inoculum was a zoosporangial suspension in deionized water filtered through sterile gauze. Its concentration was adjusted to 1.50×10^5 sporangia per ml with a hemacytometer.

To increase the initial inoculum, 30 seedlings of the susceptible cultivar Peredovik were inoculated with each SDM sample. Sunflower seeds were surface-sterilized by immersing them in 10% sodium hypochlorite for 5 to 10 min, then thoroughly rinsed in deionized water and incubated in the dark at saturation in a germinator at 24 to 28°C until radicles were 2 to 5 mm long. The seedlings were immersed in the zoosporangial suspension for 4 to 5 h at 15°C. Inoculated seedlings were sown in a sand:perlite mixture (2:3, vol/vol) and kept in a growth chamber (16 to 21°C and a 12-h photoperiod, with light irradiance of $100 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 10 to 12 days until expansion of the first pair of true leaves. Plants were incubated for 48 h at 100% relative humidity (RH) and the same conditions of temperature and light, in order to allow pathogen sporulation. Zoospores were used as inoculum for subsequent tests.

Racial characterization. Fifteen to 20 seedlings of each of the sunflower differentials were inoculated and incubated as described previously. The intense sporulation on cotyledons and/or first pair of true leaves was indicative of a susceptible reaction. Resistance was recorded by the absence of sporulation or when weak sporula-

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tion, often simultaneous with a hypersensitive reaction, was observed only on cotyledons. All the inoculations were performed twice, and when a resistant/susceptible reaction was doubtful, it was repeated a third time.

Characterization of virulence. The CVF of 19 bulk isolates of *P. halstedii* (CVFi) was determined after the inoculation of the three sets of sunflower lines used as differentials. Germination, inoculation, and growth of the seedlings were performed as for race characterization. Deionized water used for the preparation of inoculum suspensions was amended with calcium chloride (CaCl₂·2H₂O) to achieve a concentration of 140 ppm of calcium. Riboflavin was added to the inoculum suspension at 2.5×10^{-5} g/ml to induce activity of zoospores. For each isolate, 12 seedlings of each differential were incubated in the inoculum suspension in darkness at 15°C for 4 to 5 h before sowing in the sand:perlite mixture. Thereafter, plants were grown under greenhouse conditions (20 to 24°C and 12- to 16-h photoperiod, with average irradiance of 250 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). After 12 to 14 days, when the first true leaves were approximately 20 mm long, plants were placed in a chamber maintained at 16°C and 100% RH for 16 h and evaluated for resistant or susceptible reaction.

Since the R/S reactions exhibited by the 16 differentials constituted the traditional method to identify races of *P. halstedii*, this was the method we first used. Following the proposed use of the CVF method (9), we again characterized the virulence of our isolates. According to the traditional nomenclature, one value of CVF corresponds to each of the races, and that is why there is a CVF value for the new race 10. When

Table 2. Racial identification of isolates of *Plasmopara halstedii* from Spain and theoretical virulences (CVFi) associated with the races (1994 to 2000)

Year	Number of isolates	Location ^y	Race	CVFi ^z
1994	1	Cordoba, S	4	730
	1	Sevilla, S	6	310
	1	Sevilla, S	7	330
	2	Gerona, NE	10	—
1995	1	Cordoba, S	1	100
	2	Guadalajara, C	1	100
	2	Sevilla, S	1	100
	1	Sevilla, S	2	300
	2	Sevilla, S	4	730
	3	Sevilla, S	6	310
	7	Cordoba, S	7	330
	3	Gerona, NE	10	—
	7	Cadiz, S	1	100
1996	4	Cordoba, S	1	100
	3	Cuenca, C	1	100
	8	Guadalajara, C	1	100
	1	Sevilla, S	1	100
	1	Sevilla, S	4	730
	1	Cordoba, S	5	770
	1	Sevilla, S	6	310
	2	Cadiz, S	1	100
	2	Guadalajara, C	1	100
1997	1	Zaragoza, C	1	100
	3	Sevilla, S	4	730
	5	Sevilla, S	6	310
	2	Badajoz, C	7	330
	5	Sevilla, S	7	330
	2	Sevilla, S	1	100
	3	Cordoba, S	4	730
	3	Sevilla, S	7	330
	4	Cordoba, S	8	710
1998	4	Cordoba, S	1	100
	2	Sevilla, S	6	310
1999	1	Sevilla, S	2	300
	2	Cordoba, S	3	700
	5	Cordoba, S	6	310
	5	Sevilla, S	6	310
	3	Cordoba, S	7	330
2000	2	Sevilla, S	7	330
	1	Cordoba, S	8	710
	1	Gerona, NE	10	—

^y S = south, NE = northeast, C = center.

^z No value of CVFi could be assigned to isolates of race 10, since it is a new race.

Table 1. Reactions of differential sunflower lines to races of *Plasmopara halstedii*^w

Differential lines ^x	Races of <i>P. halstedii</i>									
	1	2	3	4	5	6	7	8	9	10 ^y
Peredovik	S ^z	S	S	S	S	S	S	S	S	S
IS003	S	S	S	S	S	S	S	S	S	S
HA-304	S	S	S	S	S	S	S	S	S	S
RHA-265	R	S	S	S	S	S	S	S	S	S
RHA-266	R	S	S	S	S	S	S	S	S	S
RHA-274	R	R	S	S	S	R	R	S	R	S
HA-61	R	R	S	S	S	R	R	S	R	S
DM-2	R	R	R	S	S	S	S	S	S	R
PM13	R	R	R	S	S	S	S	S	S	R
RHA-325	R	R	S	S	S	S	R	S	R	S
799-1	R	R	R	S	S	R	?	R	S	R
803-1	R	R	R	R	S	R	R	R	R	R
HA-R4	R	R	R	R	R	R	R	R	R	S
HA-R5	R	R	R	R	R	R	R	R	R	S
QHP1	R	R	R	R	R	R	R	R	R	S
RHA-340	R	R	R	R	R	R	R	R	R	R

^w Based on Gulya et al. (6,8).

^x Sunflower lines RHA-265 and RHA-266, and RHA-274 and HA-61, apparently have the same pattern of resistance to the fungus. We used RHA-266 and HA-61 for characterization of races, whereas RHA-265 and RHA-274 are used at USDA-ARS (Fargo, ND). Sunflower genotypes Peredovik, IS003, and HA-304 are susceptible to all known races. PM13 and DM-2 display the same susceptible/resistant pattern, and reactions of HA-R5 to different races are the same as those of QHP1.

^y The new susceptible/resistant pattern of reactions of the 16 differentials inoculated allowed us to use the number 10 to designate the race.

^z S = susceptible, R = resistant, ? = unknown reaction.

we inoculated the differentials proposed by Gulya et al. (9), the CVF of some bulk isolates did not show the expected value. Therefore, we assigned theoretical CVF values (CVFt) to those proposed by Gulya et al. (9) for each race, and isolate CVF (CVFi) to the value we obtained for each isolate.

After the comparison of the CVFt and CVFi obtained for the 19 SDM isolates, *ss* cultures were attempted for 14 isolates. Twelve to 24 pregerminated and dehulled seeds of susceptible cultivar IS003 (Interstate Payco Seed Co., West Fargo, ND) were infected with each of the *ss* cultures. Each seed was placed in a glass vial containing 5 ml of the calcium chloride solution amended with riboflavin as previously described, and a single sporangium was added. Individual sporangia were collected from a drop of diluted suspension ($<10^3$ sporangia per ml) of each isolate using capillary tubes under a stereoscope ($\times 20$).

After incubation in darkness at 15°C for 4 h, seeds were sown separately and grown for 12 to 14 days under the greenhouse conditions described above. When the pathogen sporulated, fungal structures from every plant were identified as different *ss* cultures from the same bulk isolate. Each *ss* isolate was increased on 'IS003' prior to virulence characterization (CVFss) as previously described. Eight *ss* isolates were collected using a dry collector (7) and kept at -80°C for 10 to 12 months before studying their virulence by thawing the inocula at room temperature before inoculating differentials.

RESULTS

Race characterization. The reactions of standard sunflower differential lines used for race characterization of *P. halstedii* are shown in Table 1. All of the differential lines exhibited some resistance to the fungus, except differentials Peredovik, IS003,

and HA-304, which are universally susceptible. The pathogenic characterization of 26 SDM isolates from 1994 and 1995 revealed that five (three from southern Spain and two from central Spain) collected in 1995 belonged to race 1 (Table 2). There was only one isolate of race 2 identified in southern Spain in 1995. Three isolates from this same area of the country were identified as race 4 (Table 2). Races 6 and 7 were also identified in both years: one isolate of each of the races in 1994, and three and seven isolates, respectively, in 1995 (Table 2). Five SDM isolates from northeastern Spain overcame resistance genes in RHA-266, HA-61, RHA-325, HA-R4, and HA-R5, but they did not induce sporulation on DM-2, 799-1, or 803-1 (Table 1). Therefore, they were referred to as race 10 (Table 2).

Most of the 26 SDM isolates from 1996 (88%), including all the isolates from central Spain as well as some from southern Spain, were characterized as race 1, as they did not overcome any of the resistance genes of the differentials (Tables 1 and 2). The remaining three SDM isolates from southern Spain were identified as races 4, 5, and 6 (Table 2).

Racial characterization was performed on 32 SDM isolates from 1997 and 1998. Race 7 was recovered most frequently (31% of isolates) (Table 2). Races 1 and 4 were also identified in seven and six isolates, respectively (Table 2). Race 6 was only observed in 1997 (five isolates from southern Spain) (Table 2). Four SDM isolates from 1998 were characterized as race 8, because they were pathogenic to all the differentials except 799-1 through RHA-340 (Table 1).

Race 1 was most common among the isolates of 1999 (66%), while the remaining isolates were identified as race 6 (Table 2). This race was also identified in 50% of the isolates in 2000. One isolate of each of the races 2, 8, and 10 was also identified, as well as five SDM isolates of race 7 (Table 2). The remaining two isolates induced symptoms on Peredovik through HA-61

Table 3. Racial determination and Coded Virulence Formulae (CVF) of isolates of *Plasmopara halstedii* collected during 1994 to 1996 in different areas of Spain

Isolate of <i>P. halstedii</i>	Location and year	Racial group ^x	CVFt ^y	CVFi ^z
R1094	Gerona, 1994	10	—	703
FA195	Fuentes de Andalucía, Sevilla, 1995	1	100	330
LC195	La Carlota, Cordoba, 1995	1	100	310
FA695	Fuentes de Andalucía, Sevilla, 1995	6	310	330
BA1095	Bañolas, Gerona, 1995	10	—	703
CA1095	Castello, Gerona, 1995	10	—	703
2AR196	Aranzueque, Guadalajara, 1996	1	100	100
4AR196	Aranzueque, Guadalajara, 1996	1	100	100
6AR196	Aranzueque, Guadalajara, 1996	1	100	310
8AR196	Aranzueque, Guadalajara, 1996	1	100	310
CA196	Carrascosa, Cuenca, 1996	1	100	753
CO196	Montellano, Sevilla, 1996	1	100	100
MO196	Cordoba, 1996	1	100	100
PA196	Paterna, Cadiz, 1996	1	100	100
1SA196	Saelices, Cuenca, 1996	1	100	100
2SA196	Saelices, Cuenca, 1996	1	100	710
SE196	Sevilla, 1996	1	100	100
EC496	Ecija, Sevilla, 1996	4	730	310
EC696	Ecija, Sevilla, 1996	6	310	310

^x Determined according to previous results of differentials.

^y Theoretical CVF corresponding to the inoculation of three sets of differentials with *P. halstedii* isolates of the race listed in the previous column, except in the case of isolates of race 10 (new race).

^z CVF obtained after the inoculation of the three sets of differentials with the given isolates.

Table 4. Reactions of differential sunflower lines to races of *Plasmopara halstedii* and the corresponding theoretical Coded Virulence Formulae (CVFt)^z

Differential line	Reaction to races of <i>P. halstedii</i>								
	1	2	3	4	5	6	7	8	9
SET 1									
HA-304	S	S	S	S	S	S	S	S	S
RHA-265	R	S	S	S	S	S	S	S	S
RHA-274	R	R	S	S	S	R	R	S	R
SET 2									
PM13	R	R	R	S	S	S	S	S	S
PM17	R	R	R	S	S	R	S	R	S
803-1	R	R	R	R	S	R	R	R	R
SET 3									
HA-R4	R	R	R	R	R	R	R	R	R
QHP1	R	R	R	R	R	R	R	R	R
HA-335	R	R	R	R	R	R	R	R	R
CVF	100	300	700	730	770	310	330	710	330

^z Based on Gulya et al. (9).

and RHA-325 (Table 1); they were assigned to race 3 (Table 2).

Characterization of virulence. CVFi values obtained for each bulk isolate of *P. halstedii* differed from the corresponding CVFt values expected according to the previous racial characterization in 42% of the cases (Table 3). Six out of the 13 isolates previously identified as race 1 had a virulence higher than expected (Table 3). The isolate EC496, characterized as race 4, showed a CVFi lower than 730, because it did not cause infection on RHA-274 or PM17 (Tables 3 and 4). Isolate FA695 caused symptoms on PM17 (CVFi 330) while resistance was expected (CVFt 310) (Tables 3 and 4). The virulence values obtained for the three isolates of race 10 tested showed CVFi values of 703.

The attempts to obtain *ss* isolates resulted in 0 to 8% of inoculated seedlings showing sporulation (data not shown). The highest number of *ss* isolates obtained corresponded to the bulk isolate CA1095, while no *ss* isolate was developed from four of the 14 bulk isolates used. Thus, these four bulk isolates are omitted in Table 5, which shows CVF corresponding to each of the *ss* isolates evaluated (CVFss).

The race designation of the bulk isolate MO196 corresponded with the CVFi. However, none of the *ss* isolates derived from it or from CA1095 had a CVF identical to the CVFi (values 100 and 703, respectively) (Tables 3 and 5). All the *ss* isolates from CA1095, except two, showed CVF lower than expected, while the virulence of *ss* isolates from MO196 was higher than 100 (Table 5). Two isolates of race 1 (FA195 and LC195) and isolates EC496 (race 4) and FA695 (race 6) yielded *ss* isolates that had CVF equal to the CVF of the original bulk isolates (values 310, 310, and 330, respectively) (Table 5). The virulence of *ss* isolates from the bulk isolates of race 1 other than FA195, LC195, and MO196, was lower than the corresponding CVFi (Table 5).

The virulence of the *ss* isolates was altered by freezing at -80°C . Stable CVF were obtained for some *ss* isolates from LC195, FA695, MO196, and CA1095 (Table 5). In general, the virulence of the rest of the *ss* isolates was higher after the inoculum was frozen (Table 5).

DISCUSSION

Racial diversity of *P. halstedii* was not found in Spain until 1994 (15). In our study, different isolates belonging to races 2, 4, 6, 7, and 8 (from southern Spain), and to race 10 (northeast Spain) were identified from field samples of SDM collected between 1994 and 1998. An isolate of race 5 was also obtained from one sample that was collected at a greenhouse in Cordoba. Races 2, 4, 6, and 7 have been previously isolated in other European countries (8,23–25), and isolates belonging to race 8 have been identified in North America (3,18),

but this is the first report of the occurrence of race 8 in Europe and of races 2 to 8 in Spain. Race 5, one of the most virulent races of the pathogen, was first described in North America as a greenhouse mutation (14), and there was no reference of its occurrence elsewhere until Spring et al. (19) reported it in fields in Germany. Our results confirm the occurrence of race 5 in Europe, apparently restricted to one greenhouse in Spain. While races 6 and 7 seem to be widespread in different areas of southern Spain, race 8 was restricted to two fields in Cordoba in 1997 and 2000. SDM isolates from northeastern Spain collected in 1994, 1995, and 2000 induced a pattern of resistant/susceptible reactions on the differentials that led to the identification of a new race of the pathogen: race 10. However, race 10 from Spain seems to be similar to race B reported in France (17,20), and it is apparently restricted to

the northeastern area of the country close to the French border. This race is only distinguished from race 3 by the susceptible reactions exhibited by HAR-4, HAR-5, and QHP1.

Despite the identification of races of *P. halstedii* different from race 1 during the 7 years of study, the latter seems to be the only race identified in the central and northern sunflower-growing areas of Spain, and in some locations in southern Spain. Because the climatic conditions in spring of some of the years were not very suitable for plant infection and disease development, important epidemics of SDM might happen under more favorable conditions, as commercial hybrids grown in Spain apparently do not carry any source of resistance to races 3, 4, 5, 8, and 10 of *P. halstedii* (J. Domínguez, personal communication).

Differential line 799-1 consistently showed susceptible or resistant reactions to

Table 5. Virulence of bulk isolates and single-sporangium (*ss*) isolates of *Plasmopara halstedii* compared to the theoretical Coded Virulence Formula (CVFt) expected

Isolate of <i>P. halstedii</i>	CVFt ^v	CVFi ^w	Single-sporangium isolate	CVFss ^{x,y}	CVFss ^{*y,z}
FA195	100	310	<i>ss A</i>	310	730
			<i>ss B</i>	310	330
			<i>ss C</i>	310	730
			<i>ss D</i>	310	330
			<i>ss E</i>	310	710
			<i>ss F</i>	310	730
			<i>ss G</i>	310	330
LC195	100	310	<i>ss A</i>	310	330
			<i>ss B</i>	310	–
			<i>ss C</i>	310	330
			<i>ss D</i>	310	310
			<i>ss A</i>	330	330
			<i>ss B</i>	330	330
			<i>ss A</i>	–	100
6AR196	100	310	<i>ss A</i>	–	110
8AR196	100	310	<i>ss B</i>	–	710
CA196	100	753	<i>ss A</i>	710	–
			<i>ss B</i>	–	710
			<i>ss D</i>	–	700
			<i>ss E</i>	–	100
			<i>ss A</i>	700	700
			<i>ss B</i>	730	710
			<i>ss A</i>	–	700
2SA196	100	710	<i>ss B</i>	–	770
			<i>ss A</i>	310	130
EC496	730	310	<i>ss A</i>	730	730
CA1095	–	703	<i>ss B</i>	330	330
			<i>ss C</i>	330	330
			<i>ss D</i>	730	730
			<i>ss E</i>	330	330
			<i>ss F</i>	310	330
			<i>ss G</i>	310	–
			<i>ss H</i>	310	–
			<i>ss I</i>	310	730
			<i>ss J</i>	330	730
			<i>ss K</i>	310	330
			<i>ss L</i>	330	731

^v Coded Virulence Formula expected for each bulk isolate referred, according to its racial group, except for CA1095, isolate of the new race 10.

^w Coded Virulence Formula obtained for each of the bulk isolates evaluated.

^x Coded Virulence Formula obtained for each *ss* isolate, after increase on 'IS003' susceptible seed and before storage at -80°C .

^y Dashes correspond to missing values of CVFss or CVFss* due to the unavailability of the corresponding *ss* isolate.

^z Coded Virulence Formula obtained for each *ss* isolate after the increase of inoculum on susceptible seed 'IS003' and storage at -80°C .

isolates belonging to races 4 and 8, respectively. However, the resistant reaction of 799-1 to race 7 mentioned by Gulya et al. (6,8) was not homogeneous, since after inoculation with some isolates belonging to race 7, sporulating and stunted plants were observed simultaneously with apparently resistant asymptomatic plants.

In the case of isolates MO196 and CA1095, the CVFss were not identical to the CVFt or the CVFi (only CVFi in the case of CA1095, since it is a new race). The frequent changes from 1 to 3 or 7 in the first digit of the CVF shows the inconsistency of the reaction to the pathogen exhibited by sunflower lines RHA-265 and RHA-266 as well as RHA-274 and HA-61. Similarly, the observed changes in the second digit of the CVF could be apparently attributed to differences in the reactions of line PM17 compared with the reactions of 799-1. Therefore, a revision of the differential lines used to perform the racial characterization of *P. halstedii* is needed. It is commonly assumed that RHA-274 and HA-61 carry the same source of resistance to the pathogen. However, and perhaps due to the incorporation of resistance from HA-61 to the Spanish resistant hybrids, this line shows a more distinct reaction (either resistant or susceptible) than RHA-274.

On the other hand, and similar to previous results (18), the diversity observed in the virulence of different *ss* isolates from bulk isolates of *P. halstedii* shows the genetic heterogeneity within each bulk isolate, suggesting a diversity of genotypes existing in the field populations of the pathogen, as well as a high frequency of recombination and/or mutation of the fungus. This hypothesis is also enhanced by the diversity of virulences of *ss* isolates before and after storage at -80°C . Nevertheless, a more detailed study on the virulence of *ss* isolates compared with that of the original bulk isolate, as well as on the effect of temperature of storage on the virulence, should be conducted.

The results of this study show the diversity of races of *P. halstedii* present in Spain. Although race 1 or 100 is still the most frequent race in the country, races 2 to 8 and 10 are widespread, and they can cause disease on genotypes carrying resistance to race 1. Genetic heterogeneity in field populations of the pathogen (bulk

isolates) has been found, suggesting the need for further studies on the mechanisms driving fungal diversity.

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