Pathogenic Characterization of *Plasmopara halstedii* Isolates from Spain

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Introduction

Plasmopara halstedii (Farl.) Berl. and de Toni, is a biotrophic fungus in the Class Oomycetes, that causes downy mildew in sunflower. Plants are affected at the first stages of development with systemic infections produced in the host, which becomes stunted and can be dead. Like the crop, sunflower downy mildew has its origin in America. It has spread from there to all the areas over the world where sunflower is cropped.

Until 1980, only some races of *P. halstedii* had been identified: race 1 (European race) (in Europe and Canada), race 2 (in ND and MN) and races Odessa and Fundulea (in Russia and Rumania respectively). The selection pressure associated to the widespread use of resistant commercial sunflower hybrids or varieties has brought about the appearance of new more virulent races, and racial characterization of *P. halstedii* has become more complex. In 1990 races 5, 7 and 8 were identificated in the USA and in 1992 races 3, 4, 6 and 7 had been identificated in Europe.

Conventionally, ordinal numbers have been successively assigned to new races when these resulted virulent on differential lines previously referred as resistant lines. However, the continuous appraisal of new races makes necessary the search for new resistant differentials and the progressive use of a higher number of them in order to perform the racial characterization.

Only race 1 of *P. halstedii* had been described in Spain before 1980. Commercial resistant sunflower hybrids of the Spanish Catalog only carry the resistance gene $P1_2$, which confers resistance to races 1 and 2. Between 1994 and 1997, pathogenic characterization of different isolates from Spanish sunflower fields were performed, and races 2, 3, 4, 6 and 7 were identified. It was also observed that race 3 seemed to be located only in Northeastern areas of the country, whereas race 1 was the only pathogenic race found in Central Spain. On the other hand, the introgression of new resistance genes effective against new races of *P. halstedii* to sunflower lines of agronomic interest also provides resistance to the previous, less virulent races. This, often makes the discrimination of different races from the same field sample impossible by just collecting the old races of the pathogen from differential lines resistant to the new ones.

The objectives of our work were : a) getting the virulence codes for the Spanish isolates previously identified as races 1, 3, 4 or 6, and b) obtaining the single sporic cultures of the isolates for which mixtures of races were suspected and their corresponding virulence codes.

Materials and Methods

Fifteen Spanish isolates of P. halstedii were used, for which racial identification had been previously determined. These and the geographical origin are shown in Table 1.

In order to obtain the correspondent Coded Virulence Formulas (CVF), the isolates were inoculated in four sets of differential lines. For the digits of CVF, additive values of 1, 2 and 4 were assigned when susceptible reactions were observed on the first, second and third differentials of each set. The differential lines used were the nine USDA lines by Gulya (1995) plus Rha 295, Rha 325 and Rha 340. All the inoculations were made according to the WSI method with one modification : calcium was added to the water used to prepare the zoosporangial suspension (3.44 ml $CaCl_2.2H_2O$ (2M) / l water).

Table 1 : Location and races of *P. halstedii* isolates from Spain. 1994-96

Reference	Location	Race
FA I / 95	Sevilla	1
LC II / 95	Cordoba	1
SE I / 96	Sevilla	1
PA I / 96	Huelva	1
MO I / 96	Cordoba	1
SA I / 96	Cuenca	1
MT I / 96	Cordoba	1
AR II / 96	Guadalajara	1
AR IV / 96	Guadalajara	1
GE I / 94	Gerona	3
CA I / 95	Gerona	3
BA I / 95	Gerona	3
EC II / 96	Sevilla	4
EC I / 96	Sevilla	6
FA II / 95	Sevilla	6

When values of CVF determined for the isolates differed from those corresponding to the race previously identified, single sporic cultures of the samples were tried, except for three isolates previously identified as belonging to race 3, which were now identified as race 10. For each sample, 24 dehulled seeds of IS003 were inoculated. Riboflavin was added to the calcium solution at the rate of 2.5 10^{-5} gr / ml, and each seed was placed in a small glass vial containing 5 ml of this solution. To infect the seeds, a diluted zoosporangial suspension was prepared. Using stretched capillar tubes and a stereoscopic microscope with transparency light, one zoospore was added to each vial. After 4 h of incubation in the dark at 15C, seeds were planted individually and were grown according to the standardized method. After 12-14 days, flats containing the plants were transferred to a humid chamber (16C and 100 % HR in darkness) were they were kept for 16 h to allow sporulation. Sporangia were collected from each plant separately, then named as different single sporic cultures from the same isolate and increased on IS003. When there was enough quantity of inoculum available, differential lines were inoculated again, with the aim to obtain the reactions to each single sporic culture.

Results and Discussion

CVF corresponding to the isolates and the single sporic cultures obtained from some of them, are presented in Table 2.

Seven out of ten isolates belonging to race 1, showed a CVFi 1000. Isolate EC II / 96 had a CVFi 3103, different than the one associated to race 4, while CVFi 7033 was obtained for all the three isolates of race 10. Isolate EC I / 96 had the CVFi 3103 corresponding to race 6. FA II / 95 showed a CVFi 3300 and not 3103 as expected, but its two single sporic cultures kept the same reaction. When racial identifications of the isolates were made in Spain, some of the differentials used were not the same as the lines used in the USDA lab. This could be a reason for the unexpected values of CVFi obtained for the ten isolates identified as race 1.

Table 2 : Coded Virulence Formulas associated to *Plasmopara halstedii* isolates from Spain, and reactions of single sporic cultures obtained from them. 1994-96

Reference	Race (1)	CVFi (2)	Single Sporic Culture	CVFssp (3)
FA I / 95	1	3300	spA spB spC spD spE spF spG	3100 3100 3100 3100 3100 3100 3100
LC II / 95	1	3100	spA spB spC spD	? 3100 ? 3100
SE I / 96	1	1000	-	-
PA I / 96	1	1000	-	-
MO I / 96	1	1000	spA spB	7003 7003
SA I / 96	1	1000	-	-
MT I / 96	1	1000	-	-
AR II / 96	1	1000	-	-
AR III / 96	1	7133	spA spB	? 7033
AR IV / 96	1	1000	-	-
EC II / 96	4	3103	spA	3100
FA II / 95	6	3300	spA spB	3300 3300
EC I / 96	6	3103	-	-
GE I / 94	10	7033	-	-
CA I / 95	10	7033	spA spB spC spD spE spF	? ? 3100 ? ? ?
BA I / 95	10	7033	-	-

(1) Coded Virulence Formulas corresponding to *P. halstedii* isolates.

(2) revious racial identification was performed the year the samples were collected, except for three isolates, whose race was determined before the beginning of this Experiment.

(3) Coded Virulence Formulas corresponding to the single sporic cultures from each *P. halstedii* isolate.

When single sporic cultures were made for the isolates MO I / 96 and CA I / 95, in which CVFi and race coincided, their CVF ssp differed from the CVFi (7003 and 7303 vs 1000 and 3100 vs 7033, respectively). Conversely, only one of the isolates of race 1, LC II / 95, and one of the isolates of race 6, FA II / 95, maintained identical CVFi and the corresponding CVFssp. CVFi associated to EC II / 96 was also different to the single CVFssp obtained. The fact that, in general, CVFssp values are not the ones expected from their original CVFi, might be associated to the presence of a mixture of spores in the isolates, even when those had been collected from selected differentials (susceptible just to a few cluster of races) and, in some way, this could explain the apparent loss of virulence (FA I / 95, AR III / 96, EC II / 96 and CA I / 95). On the contrary, an increase of virulence seems to occur with isolate MO I / 96. One reasonable answer to this behaviour might be the ease of *P. halstedii* to withstand spontaneous changes at mutation frecuencies higher than usual.

In all the isolates from which more than one single sporic culture were obtained, CVFssp were the same between different spores, except for isolate MO I / 96. More CVFssp should be compared within downy mildew isolates to determine whether CVFssp keep consistent. If so, results corresponding to MO I / 96 should be checked.

In general, unexpected values of CVFi were found in isolates collected from fields where mixtures of fungal races had been detected every year since 1995. This can lead us to consider that many more pathotypes than initially thought can be present, and that we are dealing with a genetically heterogeneous complex of *P. halstedii*.

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