Effectiveness of the genetic resistance to *Plasmopara halstedii* under natural conditions and diversity of the pathogen within sunflower fields

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ABSTRACT

Genetic and chemical strategies are effective ways for controlling the downy mildew caused by *Plasmopara halstedii* in sunflower. Although genetic resistance is very frequent in sunflower hybrids, new races of the pathogen that overcome the genes of resistance appear. The chemical treatment with the systemic fungicide metalaxyl-M is mandatory in Spain for sowing susceptible sunflower, but resistance of *P. halstedii* to metalaxyl-M has been reported. Four experiments were conducted under dryland conditions in four locations of Andalusia. Ten resistant hybrids were grown in three of the experiments with the objective of assessing the effectiveness of the genetic resistance against natural infections by *P. halstedii*. Values of downy mildew incidence and also sunflower production depended significantly on the cultivar in the three fields. However, lowest productions did not correspond to the most susceptible hybrids, showing the efficiency of the genetic resistance. Aiming at assessing the frequency of resistance of *P. halstedii* to metalaxyl-M within one sunflower field and the race of the resistant isolates, metalaxyl-M treated seeds of 19 commercial sunflower hybrids were sown in the fourth experiment. Thirty four isolates with resistance to metalaxyl-M were recovered from plants of different hybrids and different positions within the field, and all of them were characterized as race 310. These results show that isolates with resistance to the fungicide can be widely spread within the field and that resistance to the fungicide can happen in races with a high virulence.

Key words: downy mildew – genetic control – *Helianthus annuus* L. – natural infections – resistant sunflower hybrids.

INTRODUCTION

Sunflower downy mildew is a disease caused by the Oomycete *Plasmopara halstedii* Farl. Berl. & de Toni, that was reported for the first time in Spain in the 1970's. Favourable conditions for disease development are temperatures of 14-16ºC and high moisture in the soil during crop emergence (Gulya et al., 1997). Dwarfing of the plants and chlorosis of the leaves are typical symptoms in sunflower. An effective method of controlling the disease is the incorporation of genetic resistance into the host, but more virulent races of the pathogen that overcome the genes of resistance can appear (Molinero-Ruiz et al., 2002). The treatment with the systemic fungicide metalaxyl-M (or mefenoxam) is being widely used in Spain as a chemical way of controlling the pathogen. The fungicide is applied as a seed dressing because of the early infection of sunflower. However, this method of control may not be effective, since resistance of *P. halstedii* to metalaxyl-M has been recently reported in this country (Molinero-Ruiz et al., 2008). In this work, we assessed the efficacy of the incorporation of genetic resistance into sunflower hybrids when natural infections by *P. halstedii* occur and we also studied the diversity of the pathogen in sunflower fields as far as races and reaction to metalaxyl-M are concerned.

MATERIALS AND METHODS

Aiming at assessing the performance of genetically resistant sunflower cultivars and of metalaxyl-M treated sunflower cultivars under natural infections by *P. halstedii*, four experiments were conducted under dryland conditions in different locations of Andalusia: two in Ecija, Sevilla (Casilla Tejada and La Palmera), one in Carmona, Sevilla (Tomejil) and one in Santa Cruz, Córdoba (El Alcaparro). Plants were sown in March, 2007, in fields where there were previous records of infections by downy mildew. In each location the experiment was designed as a randomised complete block with four replications. Experimental unit consisted of four 10-m-long rows 0.7 m apart. In three of the experiments, those in La Palmera, Tomejil and El Alcaparro, 12 sunflower genotypes were sown: the susceptible control (the open pollinated variety Peredovik), 10 hybrids commercialized by different seed companies as genetically resistant to *P. halstedii*, and two resistant controls (two hybrids whose seed was treated with metalaxyl-M). The fourth experiment was conducted in Casilla Tejada, a field where the existence of populations of *P. halstedii* resistant to metalaxyl-M was suspected. Nineteen commercial varieties from the Andalusian Network of Agricultural Trials (RAEA) and seed treated with metalaxyl-M at the commercial dose of 2 g a.i./kg seed were tested.

Downy mildew symptoms considered were chlorosis of the leaves and/or dwarfing of the plants. Evaluation of symptoms and harvest were performed on the two central rows of each experimental unit. Disease incidence (percentage of symptomatic plants), and yield (kg of seed per hectare) were calculated and analyzed by means of ANOVA and Tukey comparisons ($P = 0.05$).

Samples from diseased plants were collected in the four fields. In each of them, samples were independently collected from different genotypes in order to determine the diversity of *P. halstedii* as far as races and reaction to metalaxyl-M (sensitivity or resistance) were concerned. Sixty-two samples were processed: 7 from Tomejil, 3 from El Alcaparro, 18 from La Palmera and 34 from Casilla Tejada. Also, 62 isolates of *P. halstedii* were recovered after incubation of samples in a humid chamber kept in darkness. The race of each of the isolates was determined with the methodology internationally used for racial characterization of sunflower downy mildew (Gulya et al., 1998; Molinero-Ruiz et al., 2002). Each isolate was inoculated to nine sunflower lines (differentials) that were grown in a chamber under controlled conditions of temperature (15-18ºC) and photoperiod (14 h of light). After two weeks, sporulation of the pathogen in the plants was induced by means of incubation at 100% relative humidity. Resistant or susceptible reactions were noted and considered to determine the race (numeric code) of the isolates. Similarly to race characterization, the reaction of each isolate of *P. halstedii* to the fungicide metalaxyl-M was determined after its inoculation to 40 treated (2 g a.i./kg seed) and 40 non treated Peredovik seeds. After inoculation, growth, and induction of symptoms, as explained, sensitive or resistant reaction of the pathogen to the fungicide was noted as the percentage of sporulated treated plants. When resistance was observed, the inoculation was repeated in order to verify the results.

RESULTS AND DISCUSSION

The incidence of the disease in the susceptible control Peredovik (DMI), ranged between 2.4 and 18.7% in Santa Cruz and Tomejil respectively, and depended significantly on the sunflower cultivar in the three fields ($p \leq 0.005$). Peredovik and Midi were the most susceptible varieties, with DMI values of between 2.5 and 18.7% and 1.8 and 9.9% respectively (Fig. 1). The remaining varieties, with the exception of Leila, showed DMI values not significantly different from zero in the three fields. Leila showed an intermediate DMI (2.1%) in Tomejil (Fig. 1). First downy mildew infections were observed between 6 and 7 weeks after sowing and the incidence of the disease reached its highest values between 9 and 12 weeks after sowing (Fig. 1). All the isolates of *P. halstedii* recovered from Tomejil, Santa Cruz and La Palmera were race 310 (Table 1). Although Molinero-Ruiz et al. (2002) suggested that a diversity of races of *P. halstedii* can exist in one sunflower field, we only found one race.

The disease in the trial of commercial varieties also depended significantly on the sunflower variety ($p = 0.0049$) and the DMI ranged between 0.4% (Kardan) and 17.2% (F-101) (Table 2). Amira was the only variety which did not show symptoms of downy mildew, what could be due to genetic resistance of the hybrid. Since high incidences of disease happened, no records on yield were obtained. Thirty-four populations of *P. halstedii* were recovered from this field. All of them were characterized as race 310 and all of them were resistant to metalaxyl-M at the commercial dose tested (Table 2). The resistance to metalaxyl-M has already been reported in Spain (Molinero-Ruiz et al.*,* 2008), as well as in USA and France.

Yield was only analyzed in the three fields of genetically resistant cultivars and it depended significantly on the cultivar in the three cases ($p \leq 0.0005$) (Fig. 2). Peredovik was the only cultivar with a significantly lower production in Tomejil, with a little more than one third of the average production of the rest of the varieties (Fig. 2). It showed the highest DMI, but it is also an open pollination variety and not a hybrid, and, consequently, its potential of production may be lower. On the other hand, both resistant controls PR64A14 and Olimpia were highly productive, and their yields did not differ from those of the most productive varieties of each of the experiments (Fig. 2). Fig. 2 shows that although the highest DMI was observed in Tomejil, the average yield in this field was twice times higher than those in Santa Cruz and in La Palmera. These differences were due to very high infections by broomrape (*Orobanche cumana* Wallr.) in these two fields compared to those in Tomejil (Table 3). The highest yields not significantly different to those of the resistant controls in the case of Es Isabella and Leila in La Palmera seem to be due to the good production potential of the hybrids, since incidence of downy mildew was recorded in both of them.

Our results show that the effectiveness of the genetic resistance to downy mildew depends on the race of *P. halstedii* that is present in the field, since races not controlled by the genes of resistance may easily exist. Therefore, a good knowledge of the genetic resistance in sunflower hybrids is advisable. On the other hand, this work also shows that when resistance of *P. halstedii* to metalaxyl-M happens, treatment with the fungicide at the commercial dose is ineffective, and infections can result in a complete loss of sunflower production. As a conclusion, it seems important to analyze the advantages and disadvantages of both the genetic and the chemical strategies for the control of sunflower downy mildew.

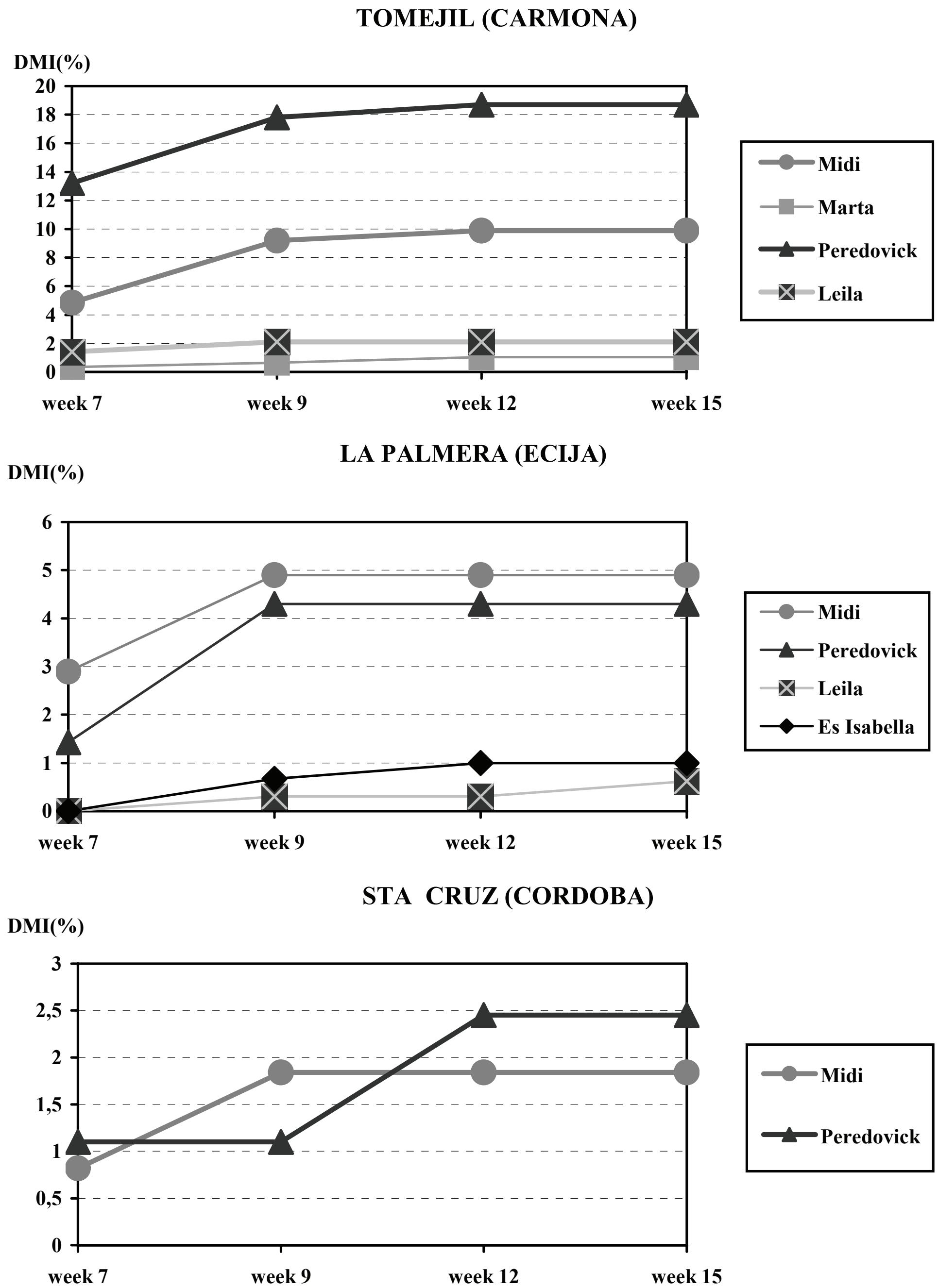

Fig. 1. Downy mildew incidence (DMI) in susceptible sunflower cultivars grown in three different fields of Seville and Córdoba in 2007.

Table 1. Race of 28 isolates of *Plasmopara halstedii* recovered onto different cultivars with resistance to the disease collected in three different sunflower fields

Field	Cultivar	Race
Tomejil	Marta (1) [a]	310
Tomejil	Leila (2)	310
Tomejil	Midi (2)	310
Tomejil	Peredovik (2)	310
Santa Cruz	Midi (1)	310
Santa Cruz	Peredovik (2)	310
La Palmera	Leila (1)	310
La Palmera	Midi (10)	310
La Palmera	Peredovik (5)	310
La Palmera	Es Isabella (2)	310

[a] Parentheses show the number of isolates from each cultivar that were recovered and characterized.

Table 2. Race and reaction to metalaxyl-M showed by 34 isolates of *Plasmopara halstedii* recovered in Casilla Tejada from different commercial sunflower hybrids

Cultivar	Incidence (%)	Race	metalaxyl-M reaction
NX 35607	6.8	310 (1)[a]	R [b]
Quisol	6	310 (1)	R
F-103	4.6	310 (2)	R
PR64A71	9	310 (1)	R
F-104	3.8	310 (1)	--
Voraz	7.5	310 (1)	R
Imigen	5.4	310 (3)	R
F-101	17.2	310 (2)	R
Es AMIRA	0	-- [c]	--
PR64A14	4.1	310 (2)	R
Masoli	3.2	310 (1)	R
PR63A76	10.5	310 (2)	R
Solnet	7.6	310 (2)	R
Transol	6.2	310 (5)	R
Kardan	0.4	310 (6)	R
Olimpia	6	310 (4)	R

[a] Parentheses show the number of isolates from each cultivar that were recovered and characterized.
[b] R= resistant.
[c] -- Not characterized.

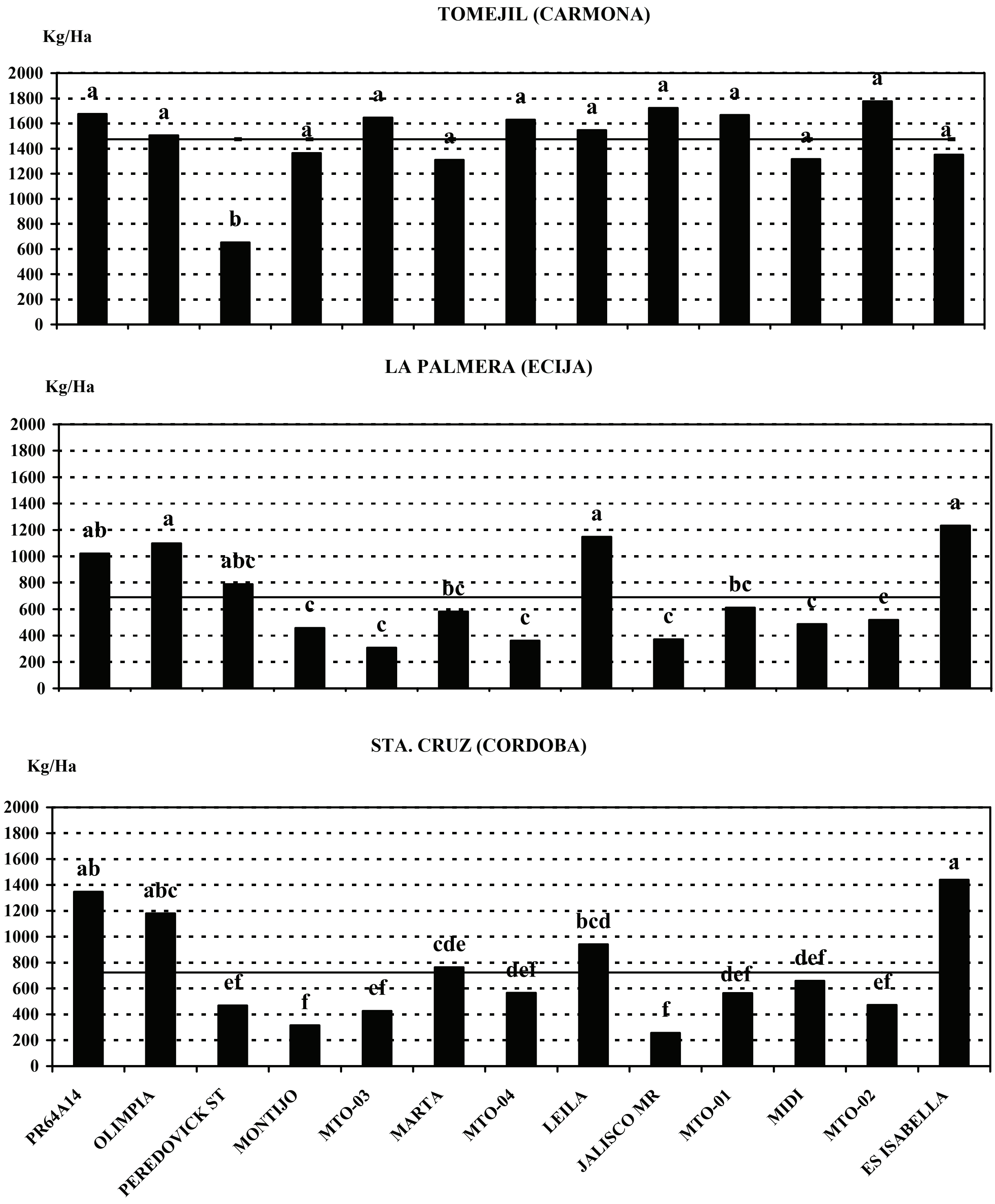

Fig. 2. Yields of 12 sunflower hybrids genetically resistant to sunflower downy mildew and one susceptible control (Peredovik) obtained in three different fields of Córdoba and Seville.

Table 3. Incidence of broomrape in downy mildew experiments in three different locations of Andalusia in 2007

Cultivar	Incidence of broomrape (%)		
	La Palmera	Santa Cruz	Tomejil
Montijo	100	98.7	13.5
MTO-03	100	100	32.25
Marta	100	93	4.25
MTO-04	100	100	25.75
Leila	100	46.6	1
Jalisco MR	35.25	100	20.5
MTO-01	100	100	22.25
Midi	100	96.3	3.75
MTO-02	100	95	24.25
Peredovik	93.75	89.2	3.5
Es Isabella	9.5	11.2	0
PR64A14	71.5	66.4	1
Olimpia	18.75	20.4	0.75

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