Field incidence of sunflower downy mildew using seed dressing with metalaxyl-M

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ABSTRACT

- The phenylamide metalaxyl-M is applied extensively as a seed dressing against the soilborne oomycete *Plasmopara halstedii* Farl. Berl. and de Toni (sunflower downy mildew, [SDM]). Previous works have shown the lack of control of *P. halstedii* populations by metalaxyl-M when it is applied at the recommended dose (1 g a.i. / kg seed) and the plants are grown under controlled conditions, suggesting that the pathogen has developed biological resistance to the chemical compound. The objectives of this work were to assess, under field conditions, the control of SDM achieved by means of sunflower seed dressing with metalaxyl-M and to determine the in situ disease levels when different doses of the phenylamide were used.
- An experiment was conducted in naturally infested fields in 2011. Three experiment sites were established in fields where SDM infections had previously occurred. Two fields were located in Seville province and one in Cordoba province (southern Spain). In each site, the experiment was designed as a randomised complete block with four replications. The same sunflower hybrid, genetically susceptible to all races of *P. halstedii*, was treated with metalaxyl-M and afterwards sown in the three fields in March. Two seed sizes (regular and small) and three doses (0, 1 and 2 g a.i. / kg seed) were used, the six treatment levels being the combinations seed size x dose of metalaxyl-M. The experimental plots consisted of four 10 m-long rows, 0.7 m apart, and plants being 0.2 m apart within the row. The incidence of SDM (percentage of symptomatic sunflower plants) was weekly recorded from April to June and sequential values were used to calculate the area under the disease progress curve (AUDPC) by trapezoidal integration method. Disease variables analysed were the AUDPC, the final SDM incidence (FSDMI) and the incidence of dead plants (DPI, expressed as the percentage of sunflower plants that died during the experiment). The effect of treatments was analyzed by means of analyses of variance, after transformation of data, and Fisher's protected LSD tests.
- Seed dressing with metalaxyl-M had a significant effect on the disease development (AUDPC) as well as on the final incidence of symptoms (FSDMI) in the three fields. No significant effects of treatment were obtained for the DPI. Significantly highest values of AUDPC and FSDMI were associated to untreated seed (both sizes) in the three fields, and no differences of FSDMI were obtained for metalaxyl-M treated seed irrespective of size and dose. Regarding the AUDPC in plants from dressed seed, a significant effect of size was observed with moderate and high infection of the crop. Plants from small seed size had more AUDPC than those from regular seed. Under low sunflower infection and in plants from dressed seed, no significant differences of AUDPC were obtained among the four levels seed size x dose.
- The occurrence of SDM in plants from dressed seed shows that metalaxyl-M does not guarantee an efficient control of the disease under field conditions. Significantly lower disease values in plants from dressed seed as compared to those in plants from undressed seed could be related to the heterogeneity (mixture of sensitive and resistant components) of populations of the pathogen. On the other hand, and since no response of *P. halstedii* to dose was obtained, the highest AUDPC in plants from dressed small seed could be associated to a low dose per seed when it was small, and therefore a shorter protection period, or to a harmful effect of the chemical on the early vigour and/or development of the plant.
- This is the first report of a low control of SDM by metalaxyl-M under field conditions worldwide, as well as the first time its partial efficacy is quantified in terms of disease control. The partial effectiveness of the compound can be related to the co-existence, in field populations of *P. halstedii*, of components holding inherent resistance and sensitiveness or to the intermediate reaction of the pathogen to it, as reported for other oomycetes. Future research aims at studying the genetic composition of these field populations of *P. halstedii*.

Key words: chemical control – mefenoxam – oomycetes – phenylamides – *Plasmopara halstedii* – soilborne pathogens.

INTRODUCTION

Sunflower downy mildew (SDM) is the second production-limiting disease in Spain after broomrape. Symptomatic plants in infested fields are usually 5-10% of the total, but when environmental conditions are favorable shortly before planting and along the following two or three weeks (temperatures between 10 and 20ºC and high water content in soil) diseased plants can reach 50 or even 80% (Gulya *et al*, 1997). High disease incidences force farmers to replant the affected area and therefore they considerably increase the operating costs of the farms. The chemical control of SDM with the systemic phenylamide metalaxyl-M has been widely used in Spain during the last decade. Currently, susceptible sunflower hybrids are seed dressed with metalaxyl-M (MARM, 2011).

As for the 90's, the resistance of *P. halstedii* to the phenylamide metalaxyl, very similar in its composition to metalaxyl-M, has been reported in Spain as well as in other countries where sunflowers are grown (Albourie *et al*, 1998; Gulya *et al*, 1999; Molinero-Ruiz *et al*, 2003). Moreover, when populations of the pathogen collected between 2002 and 2006 were artificially inoculated to seed dressed with metalaxyl-M at the commercial dose (1 g a.i./ kg seed), one third caused symptoms in the plants, suggesting that, somehow, previous treatment with metalaxyl in the 80's could have favored the selection of isolates that being resistant to metalaxyl were also resistant to metalaxyl-M (Molinero-Ruiz *et al*., 2008). The frequent occurrence of SDM in fields sown with chemically dressed seed in Spain in the last years could also be related to the resistance of the pathogen to the compound.

Thus, the objectives of this work were to assess, under field conditions, the control of SDM achieved by means of sunflower seed dressing with metalaxyl-M and to determine the *in situ* disease levels when different doses of the phenylamide were used.

MATERIALS AND METHODS

An experiment was conducted in naturally infested fields of southern Spain in 2011. Three experiment sites were established in fields where SDM infections had previously occurred. Two fields were located in Seville province (La Palmera and Pernía) and one in Cordoba province (El Alcaparro). In each site, the experiment was designed as a randomised complete block with four replications. The same sunflower hybrid, genetically susceptible to all races of *P. halstedii*, was sown in the three locations. Seed was dressed with metalaxyl-M and afterwards sown in the three fields in March. Two seed sizes (regular and small) and three doses (0, 1 and 2 g a.i. / kg seed) were used, the six treatment levels being the combinations seed size x dose of metalaxyl-M. The average seed weights were 0.1 and 0.06 g / seed for regular and small sizes respectively.

The experimental plots consisted of four 10 m-long rows, 0.7 m apart, and plants being 0.2 m apart within the row. Incidence of SDM (percentage of symptomatic sunflower plants) was weekly recorded from April to June and sequential values were used to calculate the area under the disease progress curve (AUDPC) by trapezoidal integration method (Campbell and Madden, 1990). Disease variables analysed were the AUDPC, the final SDM incidence (FSDMI, expressed as percentage of symptomatic plants) and the incidence of dead plants (IDP, expressed as the percentage of sunflower plants that died during the experiment). All disease records were taken on the four rows of each experimental plot. The effect of treatments on the incidence of disease symptoms was analyzed by means of analyses of variance (ANOVA) and Fisher's protected LSD tests. Prior to ANOVA, logarithmic and square root transformations were applied to AUDPC and percentage data respectively.

RESULTS AND DISCUSSION

Average values of FSDMI in the control treatments (RT0 and ST0) in the three fields ranged from low (5% in La Palmera) to high (53% in Pernía) (Figure 1 A, B and C). Besides, seed dressing with metalaxyl-M had a significant effect on the disease development (AUDPC) as well as on the final incidence of symptoms (FSDMI) in the three fields ($P \leq 0.0020$ and $P \leq 0.0154$ respectively). Significantly highest values of AUDPC and FSDMI were associated to untreated seed (both sizes) in the three fields. Under low disease incidence (La Palmera) the phenylamide provided a control of 46% (averages 5 and 3% FSDMI for untreated and treated seed respectively) (Fig. 1A). When field incidence was moderate (El Alcaparro), plants from treated seed had 45% less FSDMI than those from untreated seed (averages 31 and 17% respectively) (Fig. 1B). Finally, when the infection was severe (Pernía), the chemical treatment still provided a control of 43% (averages 53 and 30% FSDMI for the treated and the untreated seed respectively) (Fig. 1C).

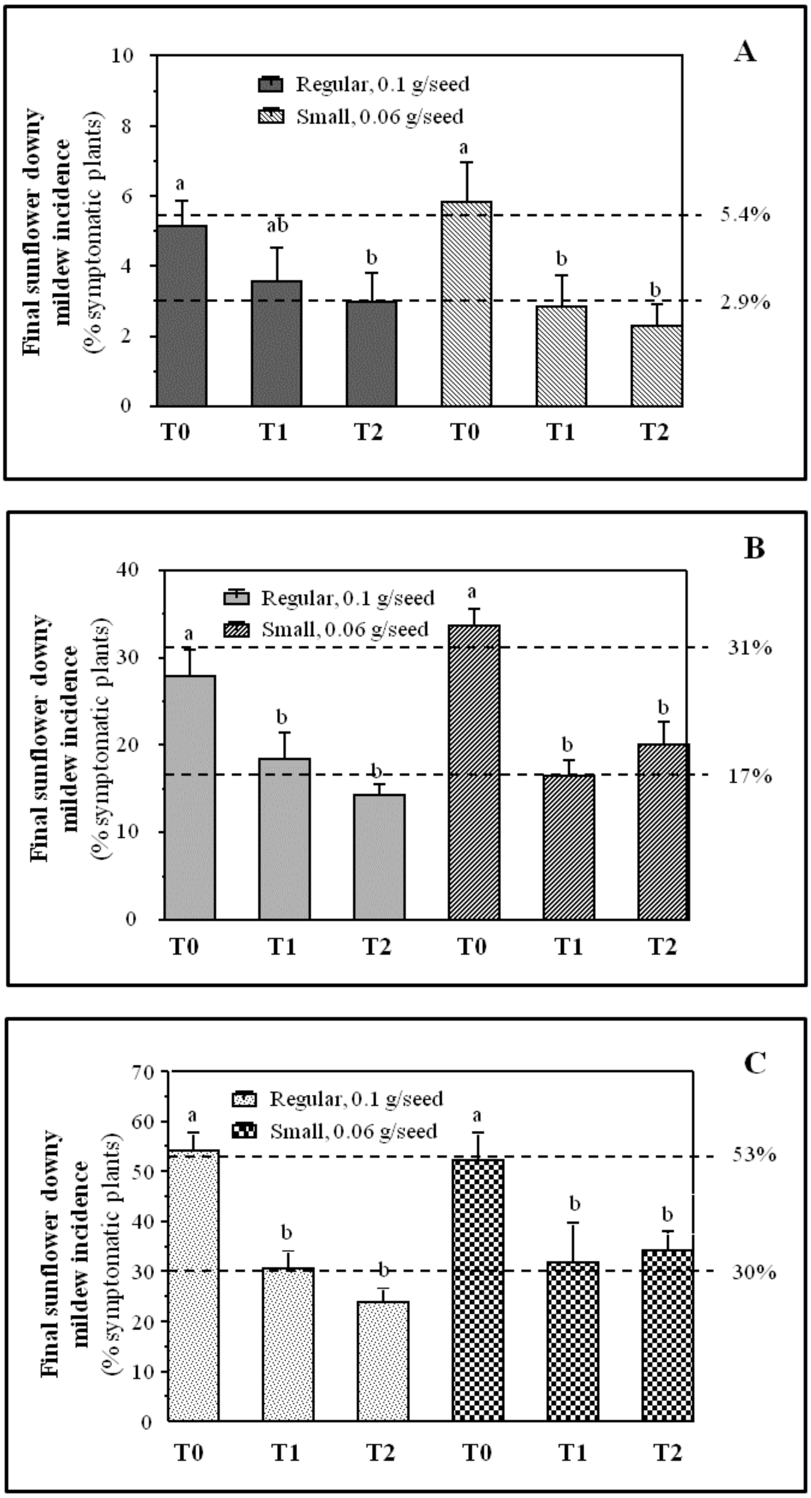

Figure 1. Final downy mildew incidence in sunflower plants from two seed sizes (regular and small) dressed with metalaxyl-M at the doses of 0 (T0), 1 (T1) and 2 (T2) g a.i./kg seed. Field experiment was conducted in La Palmera (low disease incidence) (A), El Alcaparro (moderate disease incidence) (B) and Pernía (severe disease incidence) (C).

No significant effects of treatment were obtained for IDP, which varied from averages of 1 to 11% in La Palmera and El Alcaparro respectively (Table 1).

Table 1. Natural infection of sunflower plants by *P. halstedii*, expressed as incidence of dead plants, when metalaxyl-M dressed seed (R = regular, S = small) (T0 = 0, T1 = 1 and T2 = 2 g a.i. /kg seed) was sown

	Incidence of dead plants (%)		
	La Palmera	El Alcaparro	Pernía
R-T0	2.3	14.8	9.7
R-T1	1.7	8.8	11.4
R-T2	0.6	7.4	7.4
S-T0	0.6	11.4	12.9
S-T1	1.9	10.1	12.0
S-T2	0.3	12.2	8.5
Average	1.2	10.8	10.3

Statistical analyses were performed on the Transformed IDP (Arcsin [IDP/100]).

Although previous research identified the resistance of *P. halstedii* to metalaxyl-M under controlled conditions (Molinero-Ruiz et al, 2008), this is the first report of a low control of SDM by metalaxyl-M under field conditions worldwide. Most importantly, this is the first time the partial efficacy of sunflower seed dressing with metalaxyl-M is quantified in terms of disease control in the field and under different levels of natural infection. The partial effectiveness of the compound: 3 to 30% FSDMI in dressed seed as compared to 5 to 53% FSDMI in undressed seed could be explained by the co-existence of resistant and sensitive components within field populations of the pathogen. The resistant plus sensitive components could be responsible for significantly highest disease levels in untreated seed, while only the resistant ones would be causing disease in plants from treated seed. Other hypothesis for the partial effectiveness of metalaxyl-M could be the intermediate reaction of the pathogen, as reported in *Phytophthora capsici* and other *Phytophthora* spp. (Lamour and Hausbeck, 2000; Hu et al., 2010; Olson and Benson, 2011). In order to find evidence that may explain the partial effectiveness of metalaxyl-M against *P. halstedii*, future research aims at studying the genetic composition of these field populations of the oomycete at the molecular level.

No differences of FSDMI were obtained for metalaxyl-M treated seed irrespective of size and dose. Regarding the AUDPC in plants from dressed seed, a significant effect of size was observed with moderate and high infection of the crop. In both El Alcaparro (moderate infection) and Pernía (high infection) small dressed seed had more AUDPC as compared to regular dressed seed (Table 2). Significant differences of AUDPC but not of FSDMI between both seed sizes seem to be related to an early development of the disease in small size treated seed. Since doses of the chemical are adjusted as g a.i. / g seed, a higher amount of metalaxyl-M would be dressing each regular seed (0.1 g weight) as compared to individual small seed (0.06 g weight). This high amount of metalaxyl-M would provide longer protection to the plant and a delay in the first appearance of symptoms. However, a harmful effect of the chemical on the early vigour and/or development of the seedling and therefore an increased sensibility of the plant to *P. halstedii* infection could also be possible. The effect of size was already reported to be potentially responsible for higher disease incidence in confectionary hybrids than in oilseed hybrids (Molinero Ruiz et al, 2005).

Table 2. Significant effect of metalaxyl-M on the natural infection of sunflower plants by *P. halstedii*, expressed as the area under the disease progress curve, when dressed seed (R = regular, S = small) (T0 = 0, T1 = 1050 and T2 = 2100 g a.i. /kg seed) was sown

	AUDPC		
	La Palmera	El Alcaparro	Pernía
R-T0	6.87 A	77.25 B	79.25 A
R-T1	3.12 B	55.81 C	32.47 BC
R-T2	3.66 B	39.97 C	24.81 C
S-T0	9.44 A	134.50 A	85.16 A
S-T1	3.00 B	53.78 BC	39.72 BC
S-T2	3.19 B	59.25 BC	39.87 B

Statistical analyses were performed on the Transformed AUDPC (Log[AUDPC+0.5]).
Values followed by the same letter within a column did not differ significantly (P = 0.001) as determined by ANOVA and the Fisher's protected LSD test.

Our results show the partial effectiveness of metalaxyl-M for the control of SDM in the field, and point to the need of new control measures that are effective, friendly to the environment and complementary to the current genetic and chemical measures.

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