

EVALUATION OF SPANISH ISOLATES OF *Plasmopara halstedii* FOR TOLERANCE TO METALAXYL

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SUMMARY

One out of 42 Spanish isolates of *P.halstedii* ascribed to races 1, 2, 4, 6, 7 or 10 showed consistent tolerance to metalaxyl when seeds of sunflower 'Peredovik', treated with this fungicide at 2 g.a.i./kg, were inoculated by whole-seedling dipping. When this metalaxyl-tolerant isolate of *P.halstedii* was inoculated to sunflower seedlings by this method, the fungicide seemed to affect neither zoosporangia nor zoospores. In contrast, the incidence of seedlings with high sporulation on cotyledons after 10-12-day incubation was significantly decreased with increasing dosages of metalaxyl applied to the seeds of sunflower. EC₅₀ of this tolerant isolate of *P.halstedii* was estimated at 3.5-5 g.a.i./kg.

Key words: downy mildew, fungicide tolerance, sunflower

INTRODUCTION

Sunflower downy mildew (*Plasmopara halstedii* Farl. Berl. and de Toni), was first described in Spain in 1972 (Jiménez Díaz, 1973). It affects the sunflower (*Helianthus annuus* L.) crop every growing season, providing that favorable conditions of moisture in the soil and cool temperatures occur (Jiménez Díaz *et al.*, 1980).

The introduction of genetic resistance into commercial sunflower hybrids is the most effective method of control, but the appraisal of new physiological races of the pathogen that overcome the genetic resistance of the host makes chemical control by seed treatment with metalaxyl widely used (Gulya *et al.*, 1997). This fungicide was found to control downy mildews since the late seventies, showing to be very effective against sunflower downy mildew when used as a seed treatment (Iliescu,

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1981; Melero-Vara *et al.*, 1982). However, tolerant strains of other downy mildews appeared early after the repeated use of metalaxyl (Wiglesworth *et al.*, 1988).

Incidence of *P.halstedii* isolates tolerant to the fungicide when used at commercial rates has not been reported until recently (Penaud *et al.*, 1997). This suggested the necessity of evaluating the sensitivity/tolerance of the Spanish isolates of *P.halstedii*.

MATERIAL AND METHODS

Experiment 1

Seeds of sunflower 'Peredovik' were treated with metalaxyl (Apron 20% LS) at 2 g.a.i./kg. Forty-two Spanish isolates of *P.halstedii*, which were collected from 1994-1997, showed to belong to races 1, 2, 4, 6, 7 or 10. After these were inoculated to treated and untreated sunflower seeds by whole seedling dipping in zoosporangial suspension ($30\text{-}300 \times 10^3$ z/ml) for 3-4 h, seedlings were incubated in a growth chamber at 16-24°C during 10-11 days. Afterwards, disease incidence was evaluated by the frequency of seedlings with high (> 10% surface of cotyledons) or high + low sporulation.

Experiment 2

Sunflower seeds of 'Peredovik' were treated with metalaxyl (Apron 20% LS) at 0.5, 2, 3.5 and 5 g.a.i./kg. Pregerminated seeds were inoculated by whole seedling dipping in zoosporangial suspension (20×10^3 z/ml) of the metalaxyl-tolerant isolate JER197.

The experiment was arranged as a completely randomized design with 4 replications of 10 seeds for each treatment.

Zoospore release and zoosporangia degeneration were recorded by microscopic observations of inoculating suspensions at 30', 2^h30' and 4^h30' after seedling dipping. Comparison among treatments was made after analysis of variance of transformed data on intact, empty, and degenerated zoosporangia.

Incidence of sporulated plants and plants highly sporulated on cotyledons and first pair of leaves were evaluated after 10-12-day incubation of inoculated seedlings in growth chamber conditions as in the Experiment 1.

The analysis of variance of angular-transformed data and comparison of means, were performed after that.

RESULTS

Experiment 1

All the isolates of *P.halstedii* tested were pathogenic, with variable disease incidence in plants from untreated seeds, most of the plants showing high fungal

sporulation. Disease incidence and sporulation level were not related to inoculum concentration. Plants from metalaxyl-treated seed showed no sporulation on cotyledons/first two leaves, except when isolate JER197 was inoculated.

Inoculation of treated seedlings of sunflower 'Peredovik' with isolate JER197 was repeated three times, and the tolerance of this isolate to metalaxyl was consistently shown. There was a low (6.2%) incidence of plants with high sporulation as compared with that of the untreated seed (86.4%), when inoculum concentration was as low as 39×10^3 zoosporangia/ml.

Experiment 2

Regardless of the treatment, a very low percentage (<20%) of zoosporangia had liberated their zoospores (observed as empty zoosporangia) 30' after pregerminated sunflower seeds were dipped into the inoculating suspension. Significantly higher germination of zoosporangia was observed after 2^h30' seedling dipping, with slight increases in zoospore liberation after 2 additional hours of immersion. Zoosporangia degeneration averaged 35%, but there were no significant differences between treatments nor between the time they were in contact with the fungicide (Table 1).

Table 1: Number of intact, empty and degenerated zoosporangia of *P.halstedii* (JER197) observed in a suspension, in which non-treated or metalaxyl-treated seeds of sunflower 'Peredovik' were dipped

Sampling time	Metalaxyl rate (ml/100g)	% zoosporangia		
		Intact	Empty	Degenerated
30'	0	82.5	0.0	23.3
	0.5	61.3	1.6	37.1
	2	56.8	3.3	39.9
	3.5	47.2	19.9	32.9
	5	51.1	4.3	44.6
2 ^h 30'	0	21.4	47.9	30.6
	0.5	15.8	54.2	30.0
	2	3.1	55.5	41.1
	3.5	4.6	56.5	38.9
	5	7.6	55.6	36.8
4 ^h 30'	0	3.9	56.4	39.8
	0.5	0.0	66.4	33.6
	2	2.4	70.1	27.5
	3.5	2.3	64.6	33.1
	5	0.0	59.1	40.9

Significantly higher incidence of sunflower plants with high sporulation of *P.halstedii* was observed in untreated plants and those with seed treatment at the lowest dosage (0.5 g.a.i./kg). In contrast, seed treatment at 5 g.a.i./kg determined nil incidence of highly sporulated plants (Figure 1).

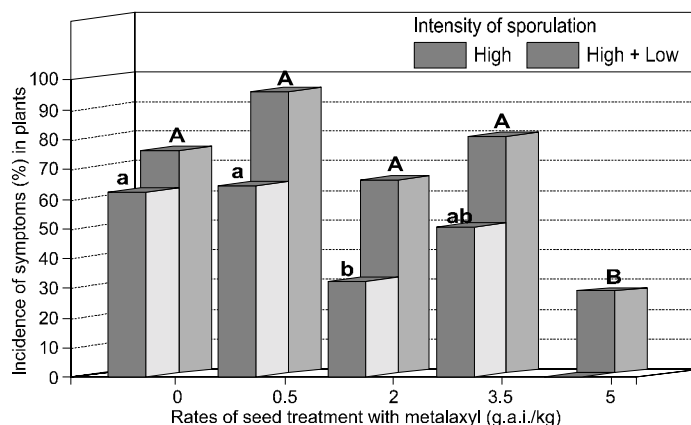


Figure 1: Effect of dosage of metalaxyl applied to sunflower seeds of 'Peredovik' on the incidence of high or total sporulation of *P.halstedii* on cotyledons

Incidence of fungal sporulation (high+low intensity) was significantly lower only when the highest dosage of the fungicide (5 g.a.i./kg) was used, nevertheless reaching the value of 27.5%.

The effective concentration of fungicide required for 50% inhibition of infection (EC_{50}) in isolate JER197 was 3.5-5 g.a.i./kg.

DISCUSSION

After the discovery of isolates of *P.halstedii* tolerant to metalaxyl in France (Albourie *et al.*, 1998), one isolate out of 42 tested was also found to be tolerant to the fungicide when used as seed treatment at the dose recommended in Europe.

The unfrequent use of metalaxyl-treated seed in Spain as compared with the generalized use in France, where a higher frequency of epidemics of downy mildew occur, in part because of the more suitable conditions for infection, suggest that the risks of appearance of metalaxyl-tolerant isolates in Spain are lower.

The metalaxyl-tolerant isolate JER197 was found in a sunflower nursery where metalaxyl-treated seed is currently planted to avoid infections of the female lines, which are usually susceptible to downy mildew.

Single sporangial isolates from the bulk isolate JER197 are needed to determine whether this is a mixture of metalaxyl-tolerant and metalaxyl-susceptible zoosporangia.

The similar values of degenerated zoosporangia in the inoculum suspension, regardless of the fungicide dosage used and the time of contact, and the similar trends in zoosporangia germination for the different treatments and the untreated seed, suggest that metalaxyl is not effective against free-living forms of the fungus. It only affects the host-dependent stages of *P.halstedii*, in coincidence with previous results (Virányi and Oros, 1991).

The higher EC₅₀ values found for an isolate tolerant to metalaxyl in France (Albourie *et al.*, 1998) as compared with the Spanish isolate JER197 suggest that a lower degree of metalaxyl tolerance occurs in the latter.

Increasing fungicide concentration to 250% of the recommended dose seems not to reduce the risk of development of tolerant strains of the pathogen, since low-intensity sporulation affected to more than 25% of the plants treated with that high concentration of metalaxyl.

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EVALUACIÓN DE AISLADOS ESPAÑOLES DE *Plasmopara halstedii* POR SU TOLERANCIA A METALAXYL

RESUMEN

De 42 aislados españoles de *P.halstedii* evaluados, adscritos a las razas 1, 2, 4, 6, 7 ó 10, sólo uno mostró consistentemente tolerancia a metalaxyl cuando se inocularon semillas pregerminadas de girasol 'Peredovik', previamente tratadas con el fungicida a razón de 2 g.m.a./kg., por inmersión en suspensión de zoosporangios del patógeno. La inoculación, con este aislado, de semillas pregerminadas de girasol 'Peredovik' que habían sido tratadas a diferentes dosis del fungicida mostró la ineficacia del metalaxyl sobre los esporangios y zoosporas del hongo, en tanto que la incidencia de plántulas esporuladas en cotiledones tras 10-12 días de incubación disminuyó significativamente con las dosis crecientes de metalaxyl en los tratamientos de las semillas. Se estimó la EC₅₀ de este aislado de *P.halstedii* tolerante a metalaxyl en 3.5-5.0 g.m.a./kg.

**ÉVALUATION DES ISOLATS DU CHAMPIGNON
Plasmopara halstedii D'ORIGINE ESPAGNOLE D'APRÈS
LEURS RÉACTIONS AU MÉTALAXYL**

RÉSUMÉ

L'un des 42 isolats espagnols du champignon *P.halstedii*, qui appartient aux races 1, 2, 4, 6, 7 ou 10, a montré une tolérance constante au métalaxyl quand les semences de la sorte 'Peredovik' étaient traitées à l'aide de ce fongicide en quantité de 2 g.i.a./kg, par la méthode d'immersion du germe complet. Quand l'isolat *P.halstedii* tolérant au métalaxyl a été utilisé pour l'infection artificielle des germes de tournesol à l'aide la même méthode, le fongicide n'a eu d'effet ni sur les zoosporanges, ni sur les champignons zoospores. D'un autre côté, la sporulation massive sur les cotylédons après 10 à 12 jours d'incubation a été significativement diminuée par l'augmentation des doses de métalaxyl sur les graines de tournesol. EC_{50} de cet isolat tolérant du champignon *P.halstedii* a été évalué de 3.5 à 5. g.i.a./kg.