TOLERANCE TO METALAXYL IN SPANISH ISOLATES OF *Plasmopara halstedii*

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SUMMARY

Incidence of downy mildew on metalaxyl-treated sunflower plants of hybrid PRO9103 depended upon the isolate of *P. halstedii* inoculated. Seven out of eleven bulk isolates were highly tolerant to the fungicide, whereas two of them showed very low tolerance. The inoculation of metalaxyl-treated sunflower plants of hybrid CAR270 with nine bulk isolates of *P. halstedii* indicated that two of them were highly tolerant, in contrast to three of them, which were very sensitive to metalaxyl. There was no relationship between tolerance and race of *P. halstedii*.

The tolerance of isolates to metalaxyl seemed not to be related to the formulation nor to the rates of application of the fungicide. The american bulk isolate RAM8 confirmed its high tolerance to the fungicide. The lower sporulation on the confectionery hybrid RH3701 treated with metalaxyl when inoculated with RAM8, as compared to the other two oil hybrids, suggested a dependency on the amount of fungicide applied to each seed, infection being less when a high rate of metalaxyl was used.

INTRODUCTION

Since 1980, sunflower downy mildew caused by *Plasmopara halstedii* is being controlled with the use of metalaxyl, mainly applied as seed dressing (7, 8). In different Peronosporales, the appearance of isolates tolerant to metalaxyl after continous and repeated foliar applications of the fungicide to several crops has been widely documented (2, 3, 4, 12, 13). The single application of metalaxyl to sunflower seeds in one season reduces the probability of increasing tolerance to this fungicide in *P. halstedii*. Nevertheless, the occurrence of tolerant isolates of the pathogen under greenhouse conditions has been reported (10). Tolerance was also found when dosages of metalaxyl lower than recommended were applied in Turkey (5) and Hungary (11). Recently, isolates of *P. halstedii* tolerant to commercial dosages of metalaxyl have been found in France and the USA (1, 6) but the recommended dosage of metalaxyl for sunflower seed treatment in the latter is 15% of that used in France.

MATERIALS AND METHODS

Two experiments were conducted on artificial inoculation of sunflower seedlings with 22 bulk isolates of *P. halstedii*. The experimental unit consisted of one tray (25x25x6 cm) filled with a sand: perlite (2:3, v) mixture in which 40 seeds of a downy mildew susceptible oilseed hybrid (PRO9103 and CAR270, for the first and second experiment respectively) were sown. Three days after planting, when seeds had germinated but had not emerged yet, inoculation was performed by the irrigation of the flats with 100 ml of a suspension of zoosporangia in distilled water (20 x 10^3/ml). Afterwards, the flats were kept in a greenhouse at 24-31C and with 16 h photoperiod of 350 µmol. $m^{-2}.s^{-1}$, and watering was done daily for 10-12 additional days until first pair of leaves were 1-2 cm long. Then, flats were transferred to a dark room at 16C and 100% RH for 14-16 h to enable pathogen sporulation. Susceptibility was recorded by the incidence of seedlings that showed sporulation on cotyledons and/or leaves.

In the first experiment, two flats were inoculated with each of the 14 bulk isolates of *P. halstedii* studied. One was sown with untreated seed and the other with sunflower seed treated with Apron-XL 32% at the dosage of 0.3 g a.i./kg seed. Three flats with seed similarly treated and three of the untreated check were sown and inoculated with each of the nine bulk isolates of *P. halstedii* used in the second experiment. Only one isolate of the pathogen was inoculated in both experiments.

Inoculation with isolates of the first experiment was repeated when disease incidence for metalaxyl-treated seed was lower than 50% of the corresponding untreated control. Thus, the possible low tolerance of these isolates was checked. Disease incidence values in seedlings from treated seeds inoculated with the different isolates tested were corrected according to the values obtained for untreated controls. Disease incidence values of the three replications of each isolate tested in the second experiment were corrected similarly, and these corrected values were averaged for each isolate. Then, analysis of variance was performed with transformed values [(% corrected disease incidence + $0.5)^{0.5}$], and Fisher´s protected LSD test (P = 0.05) was used for the comparison of means for the nine isolates tested.

Two other experiments, with two bulk isolates, MS596 and RAM8, of moderate and very high tolerance to metalaxyl respectively, were conducted with two sunflower oil hybrids

(PRO9103 and CAR270) and one confectionery-type hybrid (RH3701), all of them susceptible to downy mildew, were tested. Seed was treated with two different formulations of metalaxyl: Allegiance 28% LS, currently used in the USA, and Apron-XL 32% LS, recently registered, and containing only the most water-soluble steroisomer of phenylalanine. Dosages of 2 g a.i./kg seed and 0.3 g a.i./kg seed, currently used in Europe and the USA respectively, were compared for each hybrid-formulation combination.

A complete randomized factorial design was set up for the experiments, with hybrids, fungicide formulation and dosages as the factors to be analyzed. There were four replications of each treatment combination, the experimental unit being one flat with 40 seeds.

Seed treatment, as well as subsequent inoculation and evaluation procedures were as reported in the previous experiments. Angular transformation was applied to incidence values of sporulated seedlings in each flat before analysis of variance was performed. When significant differences were shown, mean values were compared according to the Fisher's protected LSD test (*P*=0.05), and ortogonal contrasts were made to analyze the interaction between factors.

RESULTS

Eleven out of the 14 bulk isolates of *P. halstedii* tested in the first experiment determined disease incidences in inoculated non-treated plants higher than 64%. The low incidence values for the three other isolates prevented their consideration for evaluation of tolerance to the fungicide. After correction of the incidence of disease of plants from treated seed according to the corresponding disease incidence in the untreated controls, three isolates (one of each of the races 4, 6 and 7) had a very high tolerance to metalaxyl, since disease incidence was 100%, four other isolates (races 1, 6, 8 and 10) were highly tolerant (disease incidence over 90%), two isolates (races 4 and 5) moderately tolerant (disease incidence between 50 and 75% respectively), and other two (races 2 and 6) showed very low tolerance to metalaxyl (disease incidence under 20%), although the reaction of these two isolates to the fungicide should be confirmed.

In the second experiment, two of the bulk isolates (both of race 1) showed high tolerance, with disease incidence in plants from treated seed higher than 90%, and four bulk isolates (three of race 1 and one of race 10) were of moderate tolerance (50-60% disease incidence), whereas the three remainder bulk isolates showed to be sensitive (disease incidences lower than 10%).

There was no significant effect of the formulation of the fungicide nor of the rates of application on the plant symptoms when inoculated with bulk isolates of *P. halstedii* showing different degrees of tolerance to metalaxyl. However, the higher tolerance of isolate RAM8 was confirmed, since mean disease incidence was 92-95% for the two rates of fungicide tested, whereas that of the isolate MS596 was 53-56%. Oilseed hybrids PRO9103 and CAR270 showed, respectively, the highest and the lowest incidence of disease when inoculated with the latter isolate of *P. halstedii*, while the confectionery-type hybrid sunflower RH3701 was significantly the least susceptible to the highly tolerant isolate RAM8 when the dosage of 2 g a.i./kg seed, regardless of the formulation, was applied.

DISCUSSION

The occurrence of different levels of tolerance to metalaxyl in Spanish bulk isolates of downy mildew shown in the first experiment, ranging from 0 to 100%, widens the conclusions recently obtained in France (1) and USA (6) to this regard. The only bulk isolate inoculated in both experiments showed to be highly tolerant in both of them, and it was the only Spanish isolate previously referred as tolerant to the fungicide when applied to a much higher rate of application (2 g a.i./kg seed) (9). Thus, the fungicide dosage used in the present work (0.3 g a.i./kg seed) seems to be involved in the occurrence of a range of levels of tolerance to metalaxyl, suggesting that the threshold of fungicide that affects the ability of the bulk isolates of *P. halstedii* to induce disease in treated plants ranges between 0.3 and 2 g a.i./kg seed. The frequent occurrence of tolerant isolates of *P. halstedii* when low dosage of metalaxyl is applied to sunflower seeds, also reported by Gulya *et al*. (6), suggests a risk of selection for tolerant isolates that would not be controlled by the fungicide applied at a higher rate. No relationship between tolerance to metalaxyl and race of *P. halstedii* was observed in the set of Spanish bulk isolates of the pathogen tested. This agrees with previous studies in other countries (6).

The results from the two experiments on the effects of formulation and dosage of metalaxyl applied to different hybrids on the fungicide tolerance of two isolates of *P. halstedii* indicated a similar protection of sunflower seed when either of the formulations or dosages were used. The differences between hybrids could be related to their different rates of germination and growth, that would result in different degrees of susceptibility at the time of inoculation, because root systems had different lengths. The lower incidence of disease observed in the confectionery hybrid RH3701 treated with the high dosage of metalaxyl when inoculated with RAM8, could be related to the higher amount of fungicide applied to each individual seed. Since this hybrid has larger seed than the two others, the corresponding increase in fungicide amount at the highest dosage of treatment enables a control of the pathogen. However, when the low dosage of metalaxyl was applied, the amount of fungicide was not high enough to control the infection in either of the hybrids.

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