NOTAS CIENTÍFICAS

Sensitivity reduction in *Blumeria graminis* f. sp. *hordei* to triadimenol fungicide applied as barley seed treatment

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Data de chegada: 26/06/2012. Aceito para publicação em: 01/10/2013.

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ABSTRACT

Reis, E. M.; Zanatta, M.; Brustolin, F.; Danelli, A.L.D. Sensitivity reduction of *Blumeria graminis* f. sp. *hordei* to triadimenol fungicide applied in barley seed treatment. *Summa Phytopathologica,* v.39, n.4, p.276-280, 2013.

Experiments were carried out in a growth chamber with controlled temperature and photoperiod to test two populations of *Blumeria graminis* f. sp. *hordei* from Guarapuava, Paraná State, and Passo Fundo, Rio Grande do Sul State, Brazil. Treatments consisted in application of the fungicide triadimenol (Baytan 150 SC®) at three rates of its commercial formulation: 150, 250, 350 mL/100 Kg barley seeds. The experiments were conducted separately in a growth chamber for each population, adopting the same temperature and photoperiod. For inoculation, pots containing barley seedlings colonized by the fungus were placed among the plots. After emergence of the first symptoms, the disease severity was assessed at two-day intervals. The experiments were repeated twice for each fungus population. Data were expressed as area under the disease progress curve and as powdery mildew control by comparing the severity after the fungicide treatments to that of control. Data were subjected to analysis of variance and regression analysis; the area under the disease progress curve was also calculated. Comparing the data obtained in the present study with those reported in the literature and the control, the maximum value of 26.1% is considered insufficient to prevent the damages caused by the disease. The control response to the fungicide rate was significant. We can conclude that there was a reduction in the sensitivity of both *B. graminis* f.sp. *hordei* populations to the fungicide triadimenol, which explains the control failure observed in barley farms.

Additional keywords: Barley powdery mildew, chemical control, fungicide resistance, *Hordeum vulgare*.

RESUMO

Reis, E. M.; Zanatta, M.; Brustolin, F.; Danelli, A.L.D. Redução da sensibilidade de *Blumeria graminis* f. sp. *hordei* ao fungicida triadimenol aplicado em tratamento de sementes. *Summa Phytopathologica,* v.39, n.4, p.276-280, 2013.

Em experimentos conduzidos em câmara de crescimento com temperatura e fotoperíodo controlados foram testadas duas populações de *Blumeria graminis* f.sp. *hordei* oriundas de Guarapuava, PR e de Passo Fundo, RS. Os tratamentos constaram da aplicação do fungicida triadimenol (Baytan 150 SC®) em três doses da formulação comercial: 150; 250 e 350 mL/100 Kg de sementes de cevada. Os experimentos foram conduzidos separadamente com cada população em câmara de crescimento com o mesmo fotoperíodo e temperatura. Na inoculação posicionou-se entre as parcelas, vasos contendo plântulas de cevada colonizadas pelo fungo. Após o início do aparecimento dos sintomas, procederam-se as avaliações da severidade estimada da doença realizadas em intervalo de dois dias. Os experimentos foram repetidos duas vezes individualmente com cada população do fungo. Os dados foram expressos com curvas de progresso da severidade e como controle do oídio pela comparação da severidade nos tratamentos com fungicida em relação à testemunha. Os dados foram submetidos à análise da variância, regressão e calculada a área abaixo da curva de progresso da severidade. Comparado os dados de controle com os citados na literatura, o máximo obtido neste trabalho de 26,1%, foi considerado insuficiente para evitar os danos da doença. A resposta do controle às doses foi significativa. Pode-se concluir que houve redução da sensibilidade de *B. graminis* f.sp. *hordei* ao fungicida triadimenol aos dois isolados o que explica a falha de controle observada em lavouras.

Palavras-chave adicionais: Controle químico, *Hordeum vulgare*, oídio da cevada, resistência fungicida.

Barley (*Hordeum vulgare* L.) is an important winter crop in southern Brazil. In 2011 growing season, it was grown in an acreage of around 90,000 hectares, showing average yield of 2,287 kg/ha and total production of 205 thousand tons (4).

The main fungal diseases affecting barley are brown spot [caused by *Bipolaris sorokiniana* (Sacc.) Shoem.], net blotch [*Drechslera teres* (Sacc.) Shoem], stripe blotch [*Drechslera graminea* (Rabe.) Shoem.], smut [*Ustilago hordei* (Pers) Lagerh] and powdery mildew (*Blumeria graminis* f. sp. *hordei* In. Marchal) (10, 12).

The major control strategy against powdery mildew is the use of resistant cultivars. However, resistance has been ''broken'' by new races which are originated at a very high frequency (10). Fungicides are traditionally applied to the aerial organs of plants. However, some molecules that move through the xylem can be used for seed treatment, such as triadimenol. Systemic fungicides for seed treatment cost approximately $30.00/ha. Currently, the most frequently used chemical is the fungicide triadimenol which, when applied to susceptible cultivars, protects the seedlings for up to 60 days after emergence. Another option is to spray systemic fungicides on the foliage (12).

Chemotherapy has been adopted as an emergency measure to control fungi associated with above-ground plant parts, but it is often used indiscriminately, resulting in several applications of the same active ingredient in one crop cycle and use of sub-doses. These procedures can contribute to the occurrence of fungal resistance to fungicides (1, 2, 4).

To minimize the damage caused by barley powdery mildew, different management techniques can be employed, including seed treatment with systemic fungicides (12).

In recent seasons, farmers from Campos Gerais, Paraná State, noted that powdery mildew control by applying the fungicide triadimenol for seed treatment has failed. However, in the previous seasons control was satisfactory.

We hypothesized that there was a shift in *B. graminis hordei* (*Bgh*) sensitivity to the fungicide. Thus, the aim of this study was to clarify *Bgh* population control failure when barley seeds were treated with triadimenol.

In 2008 growing season, barley plant samples showing symptoms/signs of *Bgh* were collected in Passo Fundo, Rio Grande do Sul State, and Guarapuava, Paraná State, Brazil. The biotrophic parasite inoculum was maintained and multiplied on potted living plants of barley cultivar BRS 195 in separate growth chambers at 20 ± 2 °C and 12h photoperiod.

Experimental units consisted of polyethylene pots containing 2.0 kg Fertile® Peat substrate, pH = 5.8 ± 0.2, electrical conductivity (EC) = 0.7 ± 0.2 mS/cm, density dry (kg/m^3) = 330, maximum humidity (% w/w) = 55, and H_2O retention capacity (%) = 102. Five seeds of barley susceptible cultivar BRS 195 were sown per pot.

Triadimenol (Baytan ® 150 SC), a demethylation inhibitor (DMI) fungicide, was used for seed treatment at the levels 150, 250, 350 mL/100 kg seeds. A micropipette was employed to measure the fungicide and the water, which were mixed in a beaker (100 mL) by agitation for 60 s to provide maximum homogeneity. Then, the fungicide slurry was poured onto the inner walls of a plastic bag containing 0.5 kg seeds. The bag was shaken during five min for complete and uniform coverage of seed surface.

Infected plants with abundant fungus sporulation were placed among the experimental units during seedling emergence. Inoculation was ensured by the air circulation system inside the growth chamber.

Experimental design was in randomized blocks with five replicates and 3x2 factorial arrangement (three fungicide levels x two powdery mildew isolates).

Experiments were conducted in separate chambers under the same environmental conditions and repeated twice for each population.

Pathometric determinations were performed at two-day intervals and the first three leaves of five plants of each experimental unit were evaluated as they emerged and developed. The percentage of leaf surface covered by signs, or the estimated severity, was used to quantify the disease. The evaluator was previously trained by using the Distrain program (13).

Data from the two experiments were analyzed together and subjected to analysis of variance. The means of severity control were compared according to Tukey's test at 5% probability (Tables 1 and 2). The area under the severity progress curve (AUSPC) was calculated by using the data from all assessments according to Campbell &

Table 1. Effect of seed treatment with triadimenol on the severity control (%) of barley powdery mildew (Experiment I)

Treatment	Level [mL/100 kg seeds (CF)]	Population sample		
		Passo Fundo, RS.	Guarapuava, PR.	Mean
Triadimenol	150	11.4	9.5	10.5 b
Triadimenol	250	16.6	18.3	17.5 a
Triadimenol	350	22.0	22.6	22.3 a
	Mean	16.7	16.8 ns	
	CV (%)	27.19		

CF = comercial formulation.
Means followed by the same letter in the column do not differ according to Tukey's test at 5%. Control values, mean of four evaluations at two-day intervals on three leaves of five plants, and five replicates.

Table 2. Effect of seed treatment with triadimenol on the severity control (%) for barley powdery mildew (Experiment II)

Treatment	Rate [mL/100 kg seeds (CF)]	Population sample		
		Passo Fundo, RS.	Guarapuava, PR.	Mean
Triadimenol	150	15.1	8.0	11.5b
Triadimenol	250	20.6	10.5	15.5ab
Triadimenol	350	26.1	10.5	18.3a
	Mean	A 20.6	B 9.7	
	CV (%)	32,51		

CF = comercial formulation.
Means followed by the same letter in the column do not differ according to Tukey's test at 5%. Control values, mean of four evaluations at two-day intervals on three leaves of five plants, and five replicates.

Madden (3) .

The disease severity progress lines, the means of two experiments, are shown in Figures 1 and 2. These values were integrated in the average AUSPC and compared according to Tukey's test (Figs. 3 and 4). There was a significant effect of fungicide level on AUSPC when the highest level was compared to control (Figs. 3 and 4).

The fungicide performance on barley powdery mildew control was similar in both experiments repeated with the same inoculum sample. Powdery mildew signs were seen at 7-9 days after seedling emergence on plumules of all plants of all treatments, regardless of the fungicide level. It was evident that the fungicide's protection period was not the same as in the previous seasons. Reis et al. (8) reported a protection period of up to 80 days when triadimenol was used in wheat seed treatment to control *B. gramins* f.sp. *tritici*. Later, Reis et al. (11) reported 51 days of protection period at the highest triadimenol level. By the end of this period, control was 100%, which was not observed either for farms showing control failure or for those of our study.

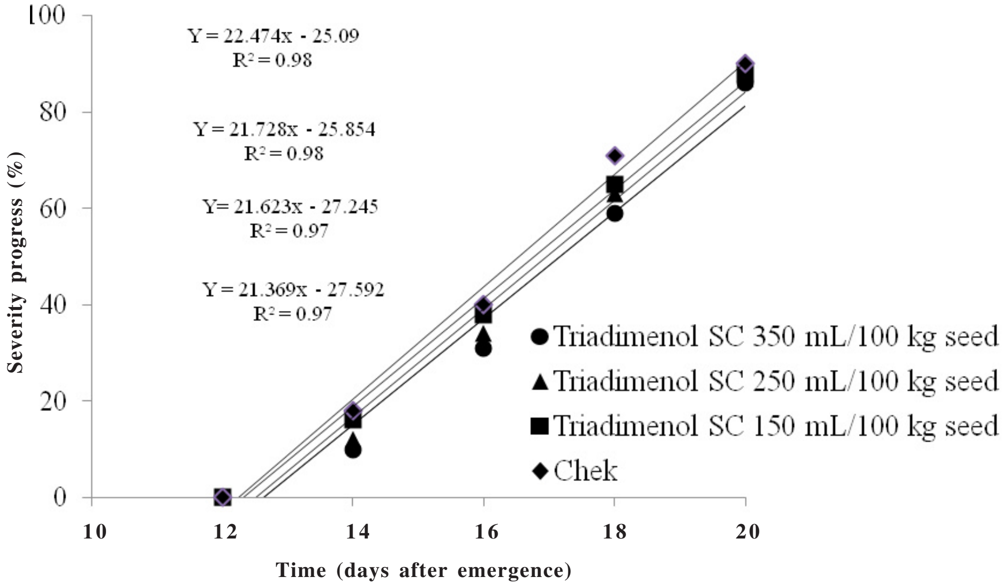

Figure 1. Severity progress lines of barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) in barley cultivar BRS 195, in function of triadimenol (150 SC) levels applied as seed treatment. Sample from Guarapuava, PR. Means of two experiments, three leaves per plant (plumule, second and third leaves), four evaluations per leaf, five replicates with five plants per plot.

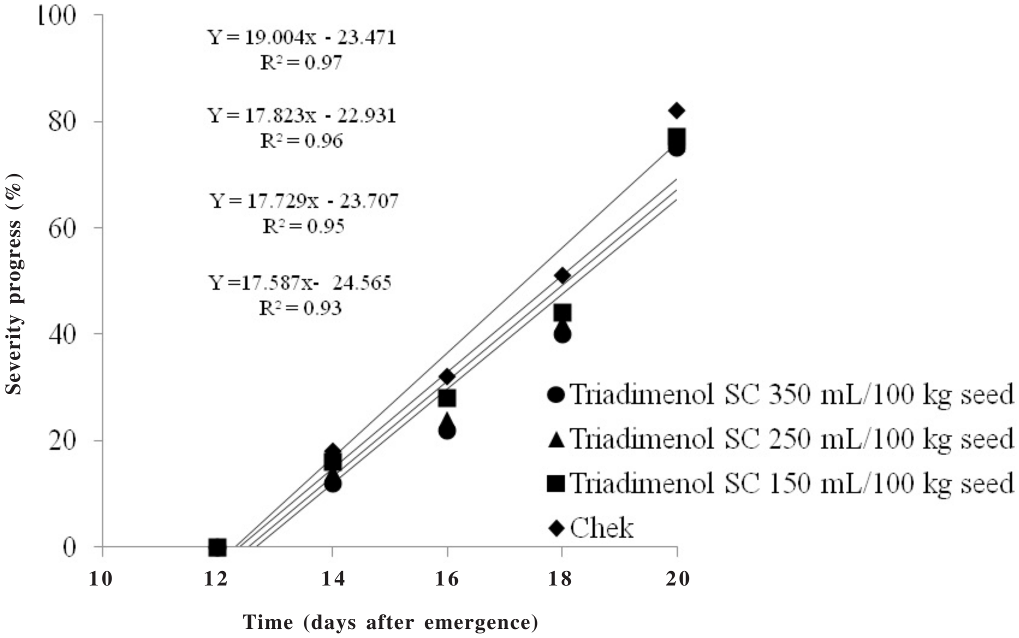

Figure 2. Severity progress lines of barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) in barley cultivar BRS 195, in function of triadimenol (150 SC) levels applied asseed treatment. Sample from Passo Fundo, RS. Means of two experiments, three leaves per plant (plumule, second and third leaves), four evaluations per leaf, five replicates with five plants per plot.

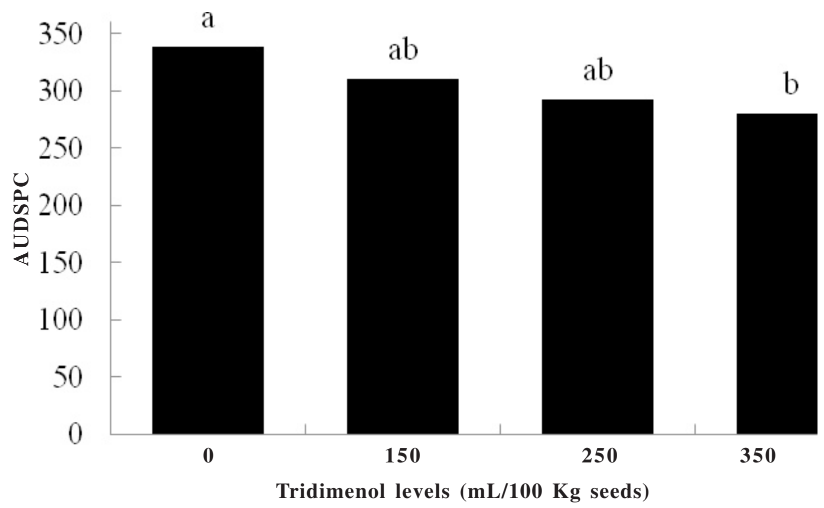

Figure 3. Area under the disease severity progress curve for barley powdery mildew cultivar BRS 195, in function of triadimenol (150 SC) levels applied as seed treatment. Means of two experiments, sample from Guarapuva, PR. Means followed by the same letter in the columns do not differ according to Tukey's test at 5%.

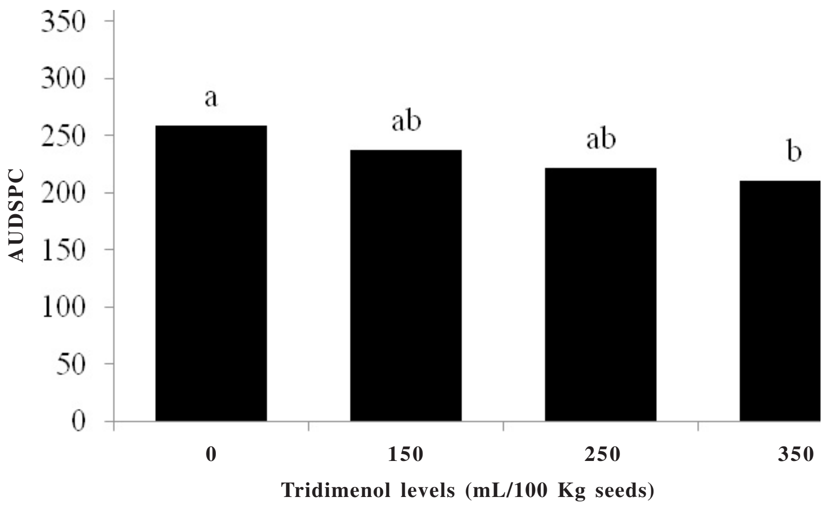

Figure 4. Area under the disease severity progress curve for barley powdery mildew cultivar BRS 195, in function of triadimenol (150 SC) levels applied as seed treatment. Means of two experiments, sample from Passo Fundo, RS. Means followed by the same letter in the columns do not differ according to Tukey's test at 5%.

The low control values, e.g., a maximum value of 26.1%, can be attributed to *Bgh* sensitivity reduction (resistance) to triadimenol (Table 2). This fungicide has been used in barley seed treatment to control powdery mildew for 19 years. Its continued use for so long may have contributed to the selection of mutants with reduced sensitivity to the fungicide, as reported by Brent (1) and Brent & Hollomon (2). In addition, other DMI fungicides, including tebuconazole, have been recommended by researchers and used by farmers on above-ground barley plant parts for disease control (9).

The sensitivity of both isolates was similar, although the isolate from Passo Fundo showed 16.7 and 20.6% control, more sensitive than that from Guarapuava, which had only 9.5 and 8.0% control (Tables 1 and 2). Even the value of 20.6% control is not satisfactory since it shows a reduction in the sensitivity of both isolates to the tested fungicide.

Fungal sensitivity to DMI fungicides is governed by several genes and therefore responds to different fungicide concentrations (5). As shown in Figures 3 and 4, there was a significant response in the AUSPC when the highest fungicide level was compared to the control treatment.

Regarding the AUSPC (Figs. 1 and 2), there was a significant difference only between the highest level and the control treatment; the lowest and intermediate levels did not differ.

The fact shown in our study is not new. Change in *B. graminis* sensitivity has already been reported by other authors. For *Bgh* it was reported by Heaney et al. (7) and for *B. graminis* (DC) Speer f.sp. *tritici* Em. Marchal, by Godet & Limpert (6), considering DMI fungicides. In 1982, isolates of cucumber and barley powdery mildew, *Sphaerotheca fuliginea* (Schlecht.) Pollacco and *Bgh*, had reduced sensitivity to DMI fungicides after seven years of commercial use detected by Brent (1) and Brent & Hollomon (2).

Therefore, our study confirms the hypothesis that the observed control failure for barley crop in Guarapuava can be attributed to *Bgh* reduced sensitivity to the fungicide triadimenol applied as seed treatment. The population from Passo Fundo also showed sensitivity reduction. Sensitivity varied with the level, which confirms that the mechanism of action of DMI fungicides is governed by several genes (1, 2, 5).

If the phenomenon of sensitivity reduction occurs throughout the barley grown area, technical assistance should be informed that the powdery mildew control by triadimenol has shown reduction in its protection period.

Recently, Reis et al. (9) showed a reduction in *B. graminis* f.sp. *tritici* sensitivity to triadimenol, also applied as seed treatment for wheat powdery mildew control.

All fungicides used on small grains are a mixture of two compounds with the same mechanisms of action: DMI and QoI. DMI was most effective in controlling powdery mildew. With the *Bgh* and *B. graminis tritici* reduction in sensitivity to triadimenol, a DMI fungicide, there is no other available active ingredient that results in effective control of powdery mildew in the Brazilian market.

Therefore, this study supports the hypothesis that *Bgh* had a reduction in sensitivity to the fungicide triadimenol applied as seed treatment. It explains the control failure noticed by farmers and advisers for the fields in the last growing seasons in southern Brazil.

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