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SHORT COMMUNICATION / *COMUNICAÇÃO*

Loss of sensitivity of *Blumeria graminis* f. sp. *tritici* to triadimenol applied as seed treatment

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ABSTRACT

The objective of this work was to evaluate the loss of sensitivity of *Blumeria graminis* f. sp. *tritici* (Bgt) to the fungicide triadimenol as seed treatment. The experiments were carried out in a growth chamber with temperature and photoperiod partially controlled. A mixture of four isolates of Bgt was used and maintained on potted wheat plants in the same environment. The treatments consisted of triadimenol fungicide (150 Baytan SC®) applied at three concentrations of 150, 250, 350 mL/100 kg of seeds. The inoculation was performed by placing pots with wheat plants heavily colonized by Bgt among the experimental units. After the onset of pathogen symptoms/signs, the evaluations of foliar incidence and severity were performed. The fungicide's efficiency in controlling Bgt was lower than that reported in the literature. The sensitivity of Bgt to triadimenol was not dependent on the fungicide dose, and was not significant when compared to the control treatment with no fungicide. Maximum control was 18.1% for incidence and 31.8% for severity with no significant effect between fungicide concentrations. It can be concluded that the sensitivity to the fungicide triadimenol of the tested populations of *B. graminis* f. sp. *tritic*i was lost.
Key words: *Triticum aestivum*, chemical control, fungicide resistance, powdery mildew.

Wheat (*Triticum aestivum* L.) is an important winter crop in Southern Brazil. Brazilian annual production ranges between 5 and 6 million tons, with a consumption of around 10 million tons (CONAB, 2011). In this region, the weather is unstable (within the year and among years), especially in relation to rainfall and temperature. The occurrence of frequent rains during flowering, coupled with high temperature, favors the attack of several diseases, resulting in yield instability. The frequency of wetting periods and average temperatures above 15ºC during October and November meet the conditions required for the development of fungal diseases (Reis & Casa, 2007).

Powdery mildew of wheat, caused by *Blumeria* (sin. *Erysiphe*) *graminis* DC. Speer f. sp. *tritici* Marchal, is generally the first foliar disease that occurs in chronological order in the crop. The fungus can infect all green plant organs, but it is commonly present in leaves and basal leaf sheaths (Wiese, 1991). The fungal conidia do not require liquid water for the infectious process. The optimum temperature range for disease development is between 15 and 22ºC. Under favorable environmental conditions it is common to notice the signs of the pathogen in the first leaves, and later the infected tissues become chlorotic, dying and turning brown, similar to the appearance of leaf spot (Wiese, 1991).

In Brazil, 67 races of *B. graminis* f. sp. *tritici* (Bgt) have been identified. The disease has a widespread occurrence in Southern Brazil, with variable damage in grain yield, depending on the reaction of cultivar and the season. Reis et al. (1997) quantified damage up to 62%. The yield damage can be appraised by the equation Y = 1000 - 4 I, in which Y is the normalized grain yield for 1,000 kg/ha, and I is the foliar incidence (Reis et al., 1997).

The main strategy to control powdery mildew is by using resistant cultivars. However, resistance has been supplanted by new Bgt races produced with high frequency (Reis & Casa, 2007). The disease can also be controlled by using systemic fungicides as seed treatment, which implies an additional production cost. Demethylation inhibitor fungicides (DMIs), such as difenoconazole, flutriafol and triadimenol, are available on the market for controlling powdery mildew through seed treatment. The product most commonly used is triadimenol, which can protect wheat seedlings for up to 60 days after emergence (Marchioro & Franco, 2010).

Currently, an increase in the occurrence and severity of the disease has been observed, not only on wheat, but also on barley (*Hordeum vulgare* L.). The use of sub-doses of recommended fungicides both as seed dressing or applied in above-ground spraying, with disease intensity above the economic damage threshold, are some hypotheses to explain the occurrence of powdery mildew epidemics in recent seasons and the failure of control. Wheat farmers who had traditionally used triadimenol seed treatment in the control of wheat powdery mildew were satisfied with the control efficiency by protecting the plants for up to 60 days. However, from the 2008 growing season, many farmers and farm advisers complained about the failure to control powdery mildew in wheat by seed treatment with triadimenol. Thus, the objective of this work was to evaluate the loss of sensitivity of mixed Bgt populations to triadimenol fungicide as seed treatment.

The experiments were carried out at the Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo (RS), Brazil, in a growth chamber with temperature at 18 ± 2ºC. In the 2011 growing season, samples of wheat plants with symptoms/signs of Bgt were taken from four different fields in Passo Fundo, where wheat seeds had been treated with triadimenol. The biotrophic parasite inoculum was maintained and increased in potted wheat plants grown in the same environment. Polyethylene pots containing 2.0 kg Fertile® Peat substrate at pH = 5.8 ± 0.2 were used as experimental units.

Triadimenol (Baytan® 150 SC) [1-(4-chlorophenoxy)-1(1H-1, 2, 4-triazole)-3-methyl-2-butylalcohol - CAS nº 552; Bayer Co] fungicide at doses of 0, 150, 250 and 350 mL were applied per 100 kg seeds. The slurry preparation for seed treatment was performed in a beaker with a 100 mL capacity, in which the fungicide was added to water (1%, v:v) and shaken for 60 seconds to provide maximum homogeneity. After that the slurry was poured on the edges of a plastic bag containing 0.5 kg of seeds and shaken for 5 min to provide uniform seed coverage. Five seeds of the wheat cultivar Quartzo were sown per experimental unit. The experimental design was a completely randomized block with three replications.

Immediately after sowing, powdery mildew inoculation was provided by distributing wheat potted plants heavily colonized by the fungus among the experimental units. Inoculation was ensured by forced air circulation in the growth chamber.

The incidence (percentage of leaves with signs) and the estimated severity of powdery mildew (percentage of leaf surface covered by signs) on the first three leaves of five plants in each experimental unit were evaluated at four-day intervals. The efficiency of control, expressed in percentage, was calculated based on the proportion of incidence and severity for each treatment with that of the treatment without application of fungicide. Data were subjected to analysis of variance and means compared by Tukey's test (P = 0.05). The experiment was repeated twice.

Data from the two experiments were similar (Table 1). The powdery mildew signs were first evident seven days after seedling emergence regardless of treatment and fungicide concentrations. The signs were seen on the plumules of all plants and treatments. This result supports the evidence that the fungicide does not protect wheat seedlings from fungus infection as it had in previous seasons. In general, systemic fungicides applied in seed treatment are absorbed by the seedling roots and taken via xylem to above-ground organs, protecting them against powdery mildew and leaf rust. Having this property, triadimenol has been used for wheat and barley seed treatment, mainly to control powdery mildew, since 1980. When the treated seeds are sown, the chemical is diluted in the soil water and slowly taken up by the roots, providing foliar protection for up to 60 days (Reis et al., 2008). Reis et al. (1980) reported a protecting period from 50 to 80 days using triadimenol in the control of wheat powdery mildew at concentrations of 250 and 350 mL/100 kg seed, respectively. In our study, maximum control was 18.1%, considering leaf incidence, and 31.8%, considering leaf severity, with no significant effect between concentrations (Table 1). These control values are low when compared to those reported by Reis et. al. (1980).

Thus it was evident that there was a shift in Bgt sensitivity to triadimenol. This fungicide has been used for wheat seed treatment in Brazil to control powdery mildew for 28 years (Marchioro & Franco, 2010). This may have contributed to the selection of Bgt mutants with reduced sensitivity to the fungicide. In our work, the sensitivity of powdery mildew fungus to DMIs was not dependent on the fungicide dose, and was not

TABLE 1 - Effect of wheat seed treatment with triadimenol on the efficiency of control (%) of wheat powdery mildew

Fungicide rates (g/100 kg seeds)	**1st leaf**		**2nd leaf**		**3rd leaf**		**Mean**	
	Inc.[1]	**Sev.**	**Inc.**	**Sev.**	**Inc.**	**Sev.**	**Inc.**	**Sev.**
150	14.6	30.3	18.8	26.2	18.8	38.9	17.4 n.s.	31.8 n.s.
250	15.7	33.6	17.4	13.4	18.8	32.4	17.3	26.5
350	7.7	38.9	22.1	28.4	24.4	27.7	18.0	31.7
No fungicide	87.5	64.0	33.3	40.8	56.9	13.5	59.2	39.4
CV (%)	-		-		-		31.2	27.9

[1]Inc., Incidence (percentage of leaves with signs); Sev., Severity (percentage of leaf area covered with signs). n.s. = Non-significant by Tukey´s test at 0.05. Efficiency of control, expressed in percentage, was calculated based on the proportion of incidence and severity in each treatment compared to the treatment without application of fungicide (average of three replications). Evaluations were made at four-day intervals in three leaves of five plants (average of two experiments).

significant when compared to the control treatment with no fungicide for both incidence and severity (Table 1). However, an increase in fungicide concentration did not result in the expected control, which should be close to 80%, as reported earlier by Reis et al. (1980).

Qualitative sensitivity loss, or resistance, occurs abruptly by mutation in a single gene of the pathogen and results in inefficiency of the fungicide even at high concentrations, or when qualitative results from the interaction of several minor genes and control respond to the fungicide concentration (Georgopoulos 1982). Other authors reported that the fungal change in sensitivity to DMIs is slow and concentration-dependent (Ghini & Kimati, 2000). As Bgt sensitivity was not monitored over the seasons, we cannot explain the kind of sensitivity shift that has occurred.

The proven fact in our work is not a new finding. Change in sensitivity of *B. graminis* has been reported by other authors, such as for *B. graminis* f. sp. *hordei* (Heaney et al., 1984) and Bgt (Godet & Limpert, 1998) for DMI fungicides. Isolates from cucumber powdery mildew, *Sphaerotheca fuliginea*, showed sensitivity reduction to DMI fungicides with seven years of commercial use prior to the observation of resistance (McGrath, 2001).

Therefore, this study supports the hypothesis of Bgt loss of sensitivity to the fungicide triadimenol applied as seed treatment. It explains the control failure noticed by growers and farm advisers under field conditions in the last growing seasons in Southern Brazil.

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