Build-up of resistance to triadimefon for isolates of *Erysiphe graminis* f. sp. *tritici* from Nova Scotia, Canada

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Sensitivity to triadimefon, a triazole demethylation inhibitor (DMI), of *Erysiphe graminis* f. sp. *tritici* populations collected in 1992 from fields of the wheat cultivar Absolvent in the Annapolis Valley and Colchester County of Nova Scotia, Canada, was studied *in vitro*. *E. g. f. sp. tritici* isolates were collected before, as well as at two and six weeks after triadimefon application. Exposing powdery mildew fungal populations from the Annapolis Valley to the selection pressure of triadimefon resulted in an increase in the $\log_{10}$ transformed mean value of EC_{50} from 0.30 µg/mL for population of isolates collected before triadimefon application, to 0.85 µg/mL for population of isolates collected six weeks after triadimefon application. In the case of Colchester County, the $\log_{10}$ transformed mean value of EC_{50} increased from 0.34 µg/mL to 1.04 µg/mL for isolates collected before, and six weeks after triadimefon application, respectively. The frequency distribution of EC_{50} values within each population of fungal isolates collected at each sampling time was lognormal. The findings suggest that isolates of *E. g. f. sp. tritici* with reduced sensitivity to triadimefon had existed in winter wheat fields before the fungicide was applied. After exposing fungal populations to triadimefon more isolates with reduced sensitivity were built-up, and by the end of the season they dominated the populations.

Can. Plant Dis. Surv. 76:1, 9-14, 1996.

La sensibilité de triadiméfon, inhibiteur de déméthilation du triazole, provenant des populations de *Erysiphe graminis* f. sp. *tritici* recueillies en 1992 dans des champs de cultivars de blé Absolvent dans la Vallée d'Annapolis, dans le comté de Colchester, en Nouvelle-Écosse, au Canada, a été étudié *in vitro*. Des isolats *e. g. f. sp. tritici* avaient déjà été recueillis avant, ainsi qu'à la 2e et 6e semaines après l'application de triadiméfon. Le fait d'exposer des populations de champignons infectées par le blanc, dans la vallée d'Annapolis, à la pression sélective de triadiméfon a abouti à l'augmentation du $\log_{10}$ de la valeur moyenne transformée de EC_{50} à partir de 0,30 µg/mL pour la population d'isolats recueillis avant l'application de triadiméfon, jusqu'à 0,85 µg/mL pour la population d'isolats recueillies 6 semaines après l'application de triadiméfon. Pour ce qui est du comté de Colchester, la moyenne transformée du $\log_{10}$ de EC_{50} a augmenté de 0,34 µg/mL à 1,04 µg/mL pour les isolats trouvés auparavant, et 6 semaines après l'application de triadiméfon, respectivement. La distribution de fréquence des valeurs de EC_{50} dans chaque population d'isolats fongiques prélevée à chaque temps d'échantillonage était lognormal. Les résultats semblent indiquer que les isolats de *E. g. f. sp. tritici* dont la sensibilité au triadiméfon était réduite avaient existé dans les champs de blé d'hiver avant l'application du fongicide. Après l'exposition des populations fongiques au triadiméfon un plus grand nombre d'isolats présentant une sensibilité réduite sont apparus, et à la fin de la saison ils dominaient les populations.

Introduction

Powdery mildew, caused by *Erysiphe graminis* DC. f. sp. *tritici* E. Marchal (syn. *Blumeria graminis* DC. f.sp. *tritici* E. Marchal), is one of several foliar diseases which occur throughout western Europe (Large and Doling, 1962; Wolfe and Barrett, 1977), in North America (Al-Mughrabi and Gray, 1995a; Jørgensen, 1988; McFadden, 1989) and other areas of the world (Moseman, 1973; Smith and Smith, 1974) where wheat (*Triticum aestivum* L. em Thell) is grown. Estimated yield losses up to 30% (Kasper and Kolbe, 1971) can occur when infection occurs early during crop development and conditions remain favourable for disease progress (Prescott *et al.*, 1986).

Triadimefon, a systematic triazole fungicide, has both curative and eradicant effects against powdery mildew and other fungi (Clark *et al.*, 1978). Triadimefon and other triazoles are members of the demethylation inhibitor (DMI) group of compounds, which inhibit the C-14 demethylation step in the synthesis of ergosterol (Dekker, 1985). Powdery mildew control in wheat in Nova Scotia often includes one or two foliar spray applications of fungicides which inhibit ergosterol biosynthesis.

In a fungal population that is originally sensitive to a particular fungicide, resistant forms may arise, or be present

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Accepted for publication January 2, 1996.

at low frequency (Georgopoulos, 1977; Wolfe, 1975). Growth and reproduction of these resistant forms is favoured by the selection pressure of the fungicide, so that eventually the pathogen population may become resistant (Dekker, 1985; Wolfe, 1975).

In cereal powdery mildew, shifts in sensitivity to DMIs have been recorded but correlation between poor control and reduced sensitivity has not been established (Fletcher and Wolfe, 1981). For example, the pyrimidine fungicide ethirimol continued to be effective and has given yield increase for a number of years even though resistant isolates of barley powdery mildew have been obtained from ethirimol-treated crops (Shephard *et al.*, 1975; Wolfe, 1971).

The purpose of this research was to examine whether resistant isolates of *E. g.* f. sp. *tritici* were present in 'Absolvent' wheat fields before triadimefon application, and whether such isolates became dominant in the population after triadimefon was applied.

Materials and methods

Collection and transport of samples

Al-Mughrabi and Gray (1995b) found that the winter wheat cultivar Absolvent was more favourable for the development of resistant isolates of *E. g.* f. sp. *tritici*, in the sense that higher mean $\log_{10}$ (EC_{50}) values, and higher frequencies of resistant isolates to the fungicide triadimefon, were found for samples collected from Absolvent compared to other wheat cultivars such as 'Borden.'

Samples were collected from 2-4 Absolvent wheat fields in the Annapolis Valley (AV) and Colchester County (CC). Fields were seeded with untreated seed. Thirty individual isolates were collected randomly three times from each location; before triadimefon was applied, and two and six weeks after application (250 g/ha). Leaves with sporulating pustules of *E. g.* f. sp. *tritici* were placed in plastic bags and brought to the laboratory and either transferred immediately to host plants growing in test tubes or kept at 4°C until the next day.

Host plants

Test plants were grown in glass test tubes (25 x 250 mm) with plastic closures containing perlite (20 cc) and Hoagland's solution (10 mL) (Dhingra and Sinclair, 1985). Fungicide-free Absolvent wheat seeds were surface disinfested with 0.6% NaClO for 10 min, rinsed in sterile distilled water for 10 min, and placed in sterile distilled water for 12 h. Five pregerminated seeds were transferred to the perlite surface in each tube. The tubes were then incubated in a growth chamber at 18°C and 12 h day light at 293-390 $\mu E/m^2/sec$. At night, the temperature was lowered to 15°C. Plants were inoculated as soon as the first leaf was fully expanded; usually after 7 days of incubation. The powdery mildew isolates were maintained in a growth camber on Absolvent seedlings grown in test tubes by sub-culturing every 2-3 weeks.

Preparation of inoculum

For each population of the fungus, the inoculum was prepared by inoculating 30 host plants in test tubes. Small sections of leaf, each bearing a single pustule, and selected at random, were cut from the seedlings and added to each tube. The tubes were plugged, shaken on a rotary shaker to disseminate the available conidia, and then incubated in the growth chamber. The inoculum was ready to be used in a sensitivity test as soon as sporulating pustules were formed on the seedlings, approximately 14 days after inoculation. Old spores were dislodged from the leaves by shaking the tubes one day before inoculating the sensitivity test. Fresh spores were normally produced within 24 h (Schein *et al.*, 1984).

Fungicide preparation and application

Foliar spray tests were carried out with the formulated product Bayleton® 50% WP (a.i. triadimefon). Bayleton suspensions (1.0 mg a.i./mL) were freshly prepared in Hoagland's solution and diluted with a suspension of blank formulation of Bayleton (*i.e.*, formulated product without active ingredient), so that each concentration, including the control, contained the same amount of the blank formulation. The concentration range adopted for concentration-response tests was 0.0, 0.1, 1.0, 10.0, and 100.0 µg a.i./mL. The fungicide was sprayed on the first fully expanded leaf with an atomizer, and the seedlings were kept for 24 h to dry. Infected wheat seedlings from the tubes were cut into 3-5 cm leaf sections. Two leaf sections, each with 3-5 similar-sized mildew pustules, were added to each tube of 7-day-old seedlings from the growth chamber. For one test series with the concentrations 0.0, 0.1, 1.0, 10.0, and 100.0 µg a.i./mL (5 tubes x 5 seedling x 2 replicates), two test tubes of single pustule-derived inoculum were needed for inoculation. After inoculation, tubes were capped, shaken and incubated in the growth chamber.

Data collection and statistical analyses

For each isolate, the mean percent of leaf area covered with powdery mildew on the primary leaf of five seedlings per test tube (2 replicates/isolate) treated with various concentrations of triadimefon was estimated using standard area diagrams (James, 1971). Values estimated for treatments were expressed as percentages of the control. Average percentages of two replicates for each isolate were transformed to probits, and EC_{50} values were calculated by linear regression (*i.e.* probit analysis) from the concentration-

response curves (Finney, 1971). Analyses were made with the Statistical Analysis System (SAS Institute Inc., 1983). Frequency distribution of $\log_{10}$ transformed EC_{50} values determined for each individual field population were analyzed using the univariate procedure (SAS Institute Inc., 1983). The differences in sensitivity of powdery mildew populations from the two locations collected at three sampling times were then compared based on the EC_{50} values. The experiment was 2 x 3 factorial arrangement in a complete-randomised design (2 locations x 3 sampling times). Analysis was performed using the analysis of variance (ANOVA) procedure on SAS (SAS Institute Inc., 1983). A $\log_{10}$-transformation step was applied to all EC_{50} values prior to statistical analysis. Least significant difference (LSD) test was applied to separate experimental means (Chew, 1976; Peterson, 1977).

Results and discussion

Results demonstrated that, within each population of *E. g.* f. sp. *tritici* isolates collected before and after triadimefon application, isolates fell into a wide range of EC_{50} values with lognormal distribution according to Shapiro-Wilk test ($0.1<p<5.4$) (Figure 1). There was a highly significant interaction between locations and sampling times ($p= 0.0019$) for the $\log_{10}$ transformed mean values of EC_{50} (Table 1). In the AV, the $\log_{10}$ transformed mean value of EC_{50} increased from 0.30 ± 0.12 µg/mL to 0.85 ± 0.12 µg/mL for populations of isolates that were collected before, and six weeks after triadimefon application, respectively (Table 2). Similar trend was found for population of isolates collected from CC, where the $\log_{10}$ transformed mean value of EC_{50} increased from 0.34 ± 0.12 µg/mL to 1.04 ± 0.12 µg/mL for population of isolates that were collected before, and six weeks after triadimefon application, respectively.

The highly significant difference among sampling times within locations, in terms of the $\log_{10}$ transformed mean values of EC_{50} suggests that triadimefon application has resulted in an increase in the resistance of populations when it was assessed two and six weeks, respectively, after treatment. These results also suggested that the resistant isolates were present in the field before triadimefon application. Exposing the fungal populations to the selection pressure of triadimefon might have eliminated a proportion of the sensitive isolates allowing the resistant isolates to increase and dominate the fungal populations. Our results are in agreement with those of Fletcher *et al.* (1987) who found that two triadimefon sprays resulted in a large decrease in the sensitivity of the *E. g.* f. sp. *tritici* population when it was assessed shortly after the second spray. The mean EC_{50} values of the whole population increased from 22.5 µg/mL to 32.7 µg/mL after the second spray, and to 408.3 µg/mL at the fourth spray.

Results (Table 2 and Figure 1) revealed that in the AV populations the fungus built-up a resistance to triadimefon two weeks after it was applied. Meanwhile, six weeks after treatment, a very slow, but not significant increase in resistance was observed. This indicates that there was a change in the distribution of isolates of powdery mildew fungus within the range of EC_{50} values. However, it was clear that the fungicide application has caused the resistant isolates to dominate the sensitive ones. Since the powdery mildew fungus has a short life cycle, resistant isolates are expected to propagate and produce new generations of resistant spores in the same growing season. This process might have contributed to the increase in the resistant isolates after the fungicide application. For populations of the fungus from the CC, the $\log_{10}$ transformed mean values of EC_{50} for isolates collected before and two weeks after triadimefon application were not significantly different. However, these values were significantly different from those for isolates collected six weeks after treatment. These results suggest that in CC, since the fungus is not well established compared to the AV, and since the favourable environmental conditions do not exist until later in the season, the build-up of resistance was very slow until six weeks after treatment. Dekker (1986) indicated that environmental conditions that increase the severity of the disease may also speed the development of resistance. It is uncertain whether there is a relationship between the build-up of resistance and powdery mildew severity in winter wheat fields of Nova Scotia.

Acknowledgement

This research was supported by a grant from the Canada/Nova Scotia Livestock Feed Agreement. We thank O. Brian Allen and William Matthes-Sears of the University of Guelph for their advice on statistical treatment of the data.

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Table 1. Analysis of Variance (ANOVA) for $\log_{10}$ (EC_{50}) values of *Erysiphe graminis* f. sp. *tritici* isolates collected in 1992 from two locations (the Annapolis Valley & Colchester County) on three sampling times (before, and two and six weeks after triadimefon application).

Source of Variation	DF	Mean Square	Pr > F
Location	1	0.76	0.1782
Sampling time	2	6.31	0.0001
Location x Sampling time	2	2.68	0.0019
Error	174	0.41	

Table 2. Sensitivity distribution and mean values of log_{10} transformed EC_{50} for isolates of *Erysiphe graminis* f. sp. *tritici* collected before, and two and six weeks after triadimefon application.

Location	Sampling time*	Population	LOG_{10} EC_{50} (µg/mL)	
			Range	Mean**
Annapolis Valley (AV)	BT	AV-BT	-0.70 to 1.80	0.30a***
	WK+2	AV-WK+2	-0.52 to 1.99	0.80b
	WK+6	AV-WK+6	-0.70 to 2.00	0.85b
Colchester County (CC)	BT	CC-BT	-0.70 to 1.50	0.34a
	WK+2	CC-WK+2	-1.00 to 1.87	0.19a
	WK+6	CC-WK+6	0.08 to 1.95	1.04b

* BT= before treatment; WK+2 and WK+6= two and six weeks after triadimefon application, respectively.

** Mean value of log_{10} transformed EC_{50} of 30 individual isolates of *E. graminis* f. sp. *tritici* per sampling time per location. Each individual isolate was replicated twice.

*** Means followed by the same letter within each location are not significantly different from each other at p= 0.05 according to LSD test. Standard error (SE)= 0.12.

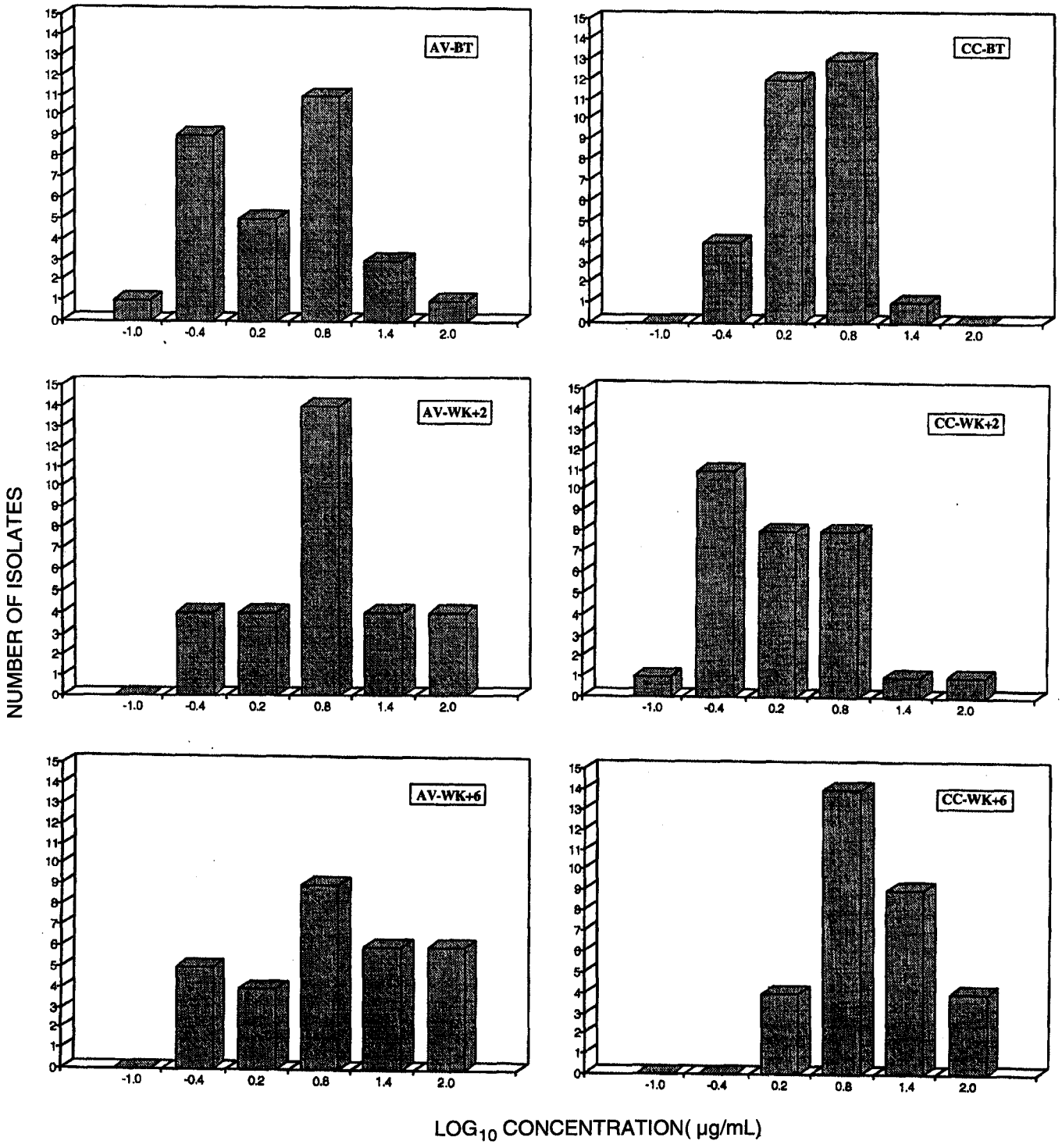

Figure 1. Frequency distribution of EC_{50} values to triadimefon for isolates of *Erysiphe graminis* f. sp. *tritici* collected from Absolvent wheat fields in the Annapolis Valley (AV) and Colchester County (CC) of Nova Scotia before (BT), and two (WK+2) and six (WK+6) weeks after triadimefon application.