Competition Between Triadimefon-Sensitive and Triadimefon-Resistant Isolates of *Erysiphe graminis* f. sp. tritici

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**ABSTRACT**


The competitive abilities of randomly selected triadimefon-sensitive (S) and triadimefon-resistant (R) single-pustule derived isolates of *Erysiphe graminis* f. sp. tritici obtained from triadimefon-treated and -untreated fields of the Annapolis Valley (AV), Nova Scotia, were tested. In 1991, mixed inoculations of S/R isolates in 75:25, 50:50, and 25:75 ratios were studied in vitro in the absence of triadimefon on wheat cultivar Absolvent. In 1992, a 50:50 (S/R) inoculation ratio was used to study the competition between R and S isolates collected from triadimefon-treated fields in the AV. Results indicated that in the absence of triadimefon the mean EC50 values of mixtures of various S/R ratios tested in 1991 and of the 50:50 ratio tested in 1992 decreased significantly (*P < 0.05*) after five generation cycles. Resistant isolates were less fit (less competitive) than sensitive ones in the absence of triadimefon.

Additional keywords: Bayleton, demethylation inhibitor, fitness of resistant strains, triazole

Powdery mildew, caused by *Erysiphe graminis* DC. f. sp. tritici Ém. Marchal, is a foliar disease that occurs throughout the world (7,21,24,25,33). Yield losses of up to 30% (23) may result if infection occurs early in the growing season and conditions remain favorable for disease development (28).

Triadimefon, a triazole fungicide, is reputed to be a good protectant and eradicant effective against powdery mildews (9). Triazoles are members of the demethylation inhibitor (DMI) group of compounds, which inhibit the C-14 demethylation step in the synthesis of ergosterol (11). Powdery mildew control in wheat in Nova Scotia may include one or two foliar spray applications of DMI fungicides.

In the absence of the fungicide, some resistant strains are less fit than sensitive ones (5,6,10,18,19,26). This may have consequences for commercial practices, as it may slow down the build-up of a resistant pathogen population in the field by favoring its decline in the absence of the fungicide. Fitness of resistant strains can be estimated by growing mixtures of resistant and sensitive isolates for several generations while monitoring the change in sensitivity (21,34). Resistance to DMI fungicides is often accompanied by poor conidial germination and reduced mycelial sporulation, which contribute to a lack of fitness and poor viability of the fungus (16).

Competition experiments have been conducted on various pathogen cultures to test the relative ability of resistant strains, compared with sensitive ones, to infect plants in the absence of a fungicide (1,5,18,22,31). Such experiments may determine whether resistant strains will become less fit and then disappear as a result of competition with sensitive strains in the absence of the fungicide (36). Two reports indicated that the competitive abilities of DMI-resistant *Erysiphe graminis* f. sp. tritici and *Erysiphe graminis* DC. f. sp. hordei Ém. Marchal isolates were inferior to those of sensitive ones in absence of fungicides (5,6). The degree of resistance in the mixture of sensitive and resistant isolates (50:50 ratio) to the triazole fungicides decreased from 50 to 32% after five transfers. Buchenauer et al. (5) found that triadimefon-resistant isolates of barley powdery mildew fungi were more sensitive than triadimefon-sensitive ones to ethephon. The results of their competition experiments of a mixture of triadimefon-resistant and -sensitive isolates (50:50) of *E. g. f. sp. hordei* showed that in the absence of triadimefon, the portion of resistant isolates in the population decreased after five passages. Ethirimol- and tridemorph-resistant isolates of barley powdery mildew (19,37) also showed reduced competitive abilities compared with those of sensitive pathogens in the absence of the fungicides.

Measuring fitness in the field is a difficult task because of the migration of conidia within the field and from outside sources. For this reason, a test tube method was adopted to test the competitive ability of triadimefon-resistant and -sensitive isolates of *E. g. f. sp. tritici* in mixed-isolate inoculations of the fungus.

**MATERIALS AND METHODS**

1991 experiment. Collection and transport of samples. Thirty individual isolates of *E. g. f. sp. tritici* were randomly collected during the fall of 1991 from both triadimefon-treated and -untreated fields of the winter wheat cultivar Absolvent in the Annapolis Valley. Plants and leaves with freshly sporulating pustules of *E. g. f. sp. tritici* were collected 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 Absolvent plants were grown in 25 x 250 mm glass test tubes with plastic closures. Each test tube was filled with 20 ml of perlite and 10 ml of Hoagland's solution (13). Fungicide-free wheat seeds were surface disinfested by submerging in 0.6% NaClO for 10 min and then rinsing in sterile distilled water for 10 min. After 12 h pregermination in sterile distilled water, five seeds per tube were placed on the surface of the perlite. The tubes were then incubated in a growth chamber at 18°C and 12 h daylight at 293 to 390 μE·m−2·s−1. At night, the temperature was lowered to 15°C. Plants were inoculated when the first leaf was fully expanded (about 7 days).

Preparation of inoculum. For each field population of 30 isolates of the fungus, 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 seedlings and added to each tube. The tubes were capped and shaken on a rotary shaker to disseminate the available conidia. Test tubes were then incubated as described in the section on host plants.

The inoculum was ready to be used in a sensitivity test as soon as sporulating pustules occurred on seedlings (approximately 14 days after inoculation). Old spores were dislodged from leaves by shaking the tubes 1 day before inoculating plants in the sensitivity test. Fresh sporulation normally occurred within 24 h (28).

Maintenance of isolates. Powdery mildew isolates were maintained on disease-free wheat seedlings (cv. Absolvent) in test tubes as described previously. Subculturing of isolates onto disease-free seedlings was carried out every 2 to 3 weeks. To increase the amount of inoculum produced by single pustules, each isolate was subcultured onto host plants in two test tubes.

Fungicide preparation. Foliar spray

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tests were carried out with the formulated product Bayleton 50% WP (a.i. triadimefon). Bayleton suspensions (1 mg a.i. per 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. per ml.

Application of the fungicide. After the first leaf was fully expanded in the test tubes (7 days), the fungicide was atomized with compressed air. Plants were then left to dry for 24 h before inoculation with the fungus.

Shaner (30) found that powdery mildew pustules of similar size produced approximately the same number of conidia. Therefore, infected wheat seedlings were cut into a 3- to 5-cm leaf sections. Two leaf sections, each with three to five similar-sized mildew pustules, were added to each tube of the host seedlings grown as described previously. For one test series with the concentrations (5 tubes with 5 seedlings and 2 replicates), two test tubes of single-pustule-derived inoculum were needed for inoculation. After inoculation, tubes were capped, shaken, and incubated under the conditions described previously.

Based on the responses of these isolates to various concentrations of triadimefon, isolates that showed growth at 10 μg triadimefon per ml were considered resistant (R), and those that did not grow were considered sensitive (S).

Preparation of mixtures of R and S isolates. For isolates from each treated and untreated field, two R and two S isolates were randomly selected and used in this study. Disease-free wheat seedlings were inoculated with various mixtures of pustules of S and R isolates to produce ratios of 25, 50, and 75% of resistant isolates (two replicates per ratio). After sporulation, the mixed cultures were used to inoculate untreated disease-free seedlings. After 14 days, the resulting conidia were used to inoculate fresh untreated disease-free seedlings (two replicates per S/R mixture). This procedure was repeated four times (i.e., five generation cycles), and the change in sensitivity of resistant and sensitive isolates (expressed as EC₅₀ value) was tested for one, three, and five generations on triadimefon-treated (0.0, 0.1, 1.0, 10.0, and 100.0 μg triadimefon per ml) wheat seedlings. The experiment was repeated twice. Fungicide preparation and application, and inoculation of treatments with the various S/R mixtures was done as described previously.

Data collection and statistical analyses. For each isolate mixture, mean percentage of surface area covered with powdery mildew on the primary leaf of five seedlings per test tube (2 tubes per isolate) treated with various concentrations of triadimefon was estimated using standard area diagrams (20). Values estimated for treatments were expressed as percentages of the control. Percentages were transformed to probits, and EC₅₀ values were calculated by linear regression (i.e., probit analysis) from the concentration-response curves (14). Analyses were made with the Statistical Analysis System (SAS) (29). The change in sensitivity of S/R isolates grown in the absence of the fungicide was tested after one, three, and five generation cycles (two replicates per S/R mixture) in the absence of triadimefon and averaged for the two experiments. The two replicates were not averaged for each mixture.

The experiment was a split-split-plot design, with the whole plot in a complete randomized design arrangement. Treatments (treated and untreated fields) were considered as the main plot factor. Isolates were nested within treatments and were tested as an error term for the main plot factor. Mixtures (three mixtures) were considered as the split-plot factor. The isolates × mixture interaction was tested within treatment and was tested as an error term for the split-split plot factor (mixture) and for the treatment × mixture interaction. Generations (three generation cycles) were considered as the split-split-plot factor. The overall error was tested as an error term for the split-split-plot factor and its interactions. All factors and their interactions were considered fixed effects, while error terms were considered random effects. Analyses were done using the General Linear Model (GLM) procedure on SAS (29). Prior to analysis, all EC₅₀ values (two replicates per mixture) were transformed to log(EC₅₀) values using the natural logarithm. A least significant difference (LSD) test was applied to separate experimental means (8,27).

1992 experiment. Based on the results of the competition experiment of 1991, no significant difference was found between treated and untreated fields in terms of fitness of isolates collected from each of these fields (Table 1). Therefore, isolates in this study were collected from treated fields. Thirty individual isolates of E. g. f. sp. tritici were collected during the summer of 1992 from treated fields of Absolvent in the Annapolis Valley. Materials and methods followed in the 1991 experiment were used in this experiment to test the response of isolates to triadimefon.

Based on the results of the 1991 competition experiment, all mixtures of ratios of S/R isolates showed a decline in fitness of the R isolate when kept for five generation cycles in the absence of triadimefon. Therefore, the 50:50 ratio was chosen to study the competition between R and S isolates in 1992. Five R and five S isolates were randomly selected and used in this study. Five isolate mixtures were made by mixing five different R and S isolates (50:50 ratio). Each isolate mixture received only one R and one S isolate. The effect of keeping mixtures of R and S isolates in the absence of triadimefon over three generation cycles (G1, G3, and G5) on the EC₅₀ values was compared in a randomized complete block experiment, with generation cycles considered as treatments and isolates (5 isolates × 2 replicates) considered as blocks; the two replicates were averaged for each isolate. The

Table 1. Analysis of variance for log(EC₅₀) values of mixtures of ratios of triadimefon-resistant and -sensitive isolates of Erysiphe graminis f. sp. tritici selected from populations collected in 1991 from triadimefon-treated and -untreated fields of wheat cultivar Absolvent in the Annapolis Valley, Nova Scotia, and tested in three generation cycles in the absence of triadimefon

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d*</th>
<th>Mean square</th>
<th>P &gt; F</th>
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<tbody>
<tr>
<td>Treatment</td>
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<td>11.651</td>
<td>0.114</td>
</tr>
<tr>
<td>Isolate (treatment)</td>
<td>2</td>
<td>1.552</td>
<td>0.4301</td>
</tr>
<tr>
<td>Mixture</td>
<td>2</td>
<td>1.870</td>
<td>0.3753</td>
</tr>
<tr>
<td>Treatment × mixture</td>
<td>2</td>
<td>0.262</td>
<td>0.8440</td>
</tr>
<tr>
<td>Isolate × mixture</td>
<td>4</td>
<td>1.478</td>
<td>0.0019</td>
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<tr>
<td>(treatment)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Generation</td>
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<td>40.108</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment ×</td>
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<td>0.0162</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Mixture × generation</td>
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<td>0.254</td>
<td>0.4940</td>
</tr>
<tr>
<td>Treatment × mixture</td>
<td>4</td>
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<td>0.0078</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>0.295</td>
<td></td>
</tr>
</tbody>
</table>

* Degrees of freedom.

Table 2. Mean values of log(EC₅₀) and their slopes for mixtures of ratios of triadimefon-resistant and -sensitive isolates of Erysiphe graminis f. sp. tritici tested in 1991 in three generation cycles in the absence of triadimefon

<table>
<thead>
<tr>
<th>Generation</th>
<th>TM1</th>
<th>TM2</th>
<th>TM3</th>
<th>UMI</th>
<th>UM2</th>
<th>UM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.66</td>
<td>0.83</td>
<td>0.10</td>
<td>-0.34</td>
<td>-1.02</td>
<td>-0.99</td>
</tr>
<tr>
<td>3</td>
<td>-0.78</td>
<td>-0.94</td>
<td>-1.26</td>
<td>-1.31</td>
<td>-1.69</td>
<td>-1.22</td>
</tr>
<tr>
<td>5</td>
<td>-1.47</td>
<td>-2.91</td>
<td>-2.69</td>
<td>-3.02</td>
<td>-2.58</td>
<td>-3.51</td>
</tr>
<tr>
<td>Slope</td>
<td>-2.13</td>
<td>-3.74</td>
<td>-2.79</td>
<td>-2.68</td>
<td>-1.56</td>
<td>-2.52</td>
</tr>
</tbody>
</table>

* T: treated field; U: untreated field; M1: mixture 1 = 25% resistant isolate; M2: mixture 2 = 50% resistant isolate; M3: mixture 3 = 75% resistant isolate.

b Slope of mean value of log(EC₅₀) = (mean value of log(EC₅₀) at generation 5) – (mean value of log(EC₅₀) at generation 1).

c Standard error of the slopes of mean values of log(EC₅₀) = 0.38.
experiment was repeated twice, and values were averaged for the two experiments. The analysis was done using the analysis of variance procedure (ANOVA) on SAS (29). Prior to analysis, all EC$_{50}$ values were transformed to log(EC$_{50}$) values using the natural logarithm. An LSD test was applied to separate experimental means (8,27).

RESULTS

1991 experiment. There was a highly significant difference ($P < 0.05$) among generation cycles of powdery mildew of wheat in terms of log(EC$_{50}$) values of mixtures of R and S isolates (Table 1). Results of the LSD test demonstrated that the mean value of log(EC$_{50}$) of all mixtures of R and S isolates for isolate mixtures significantly decreased from $-0.13 \pm 0.11$ to $-1.20 \pm 0.11$ and $-2.70 \pm 0.11 \mu g$ triadimefon per ml when kept for one, three, and five generation cycles, respectively, in the absence of triadimefon.

Significant interaction ($P < 0.05$) among treatments and generations (Table 1) in their effects on the log(EC$_{50}$) values of R and S isolates, and results of the LSD indicated that for mixtures of R and S isolates that were collected from treated and untreated fields, the mean values of log(EC$_{50}$) decreased significantly after five generation cycles in the absence of triadimefon. In the case of those from untreated fields, the log(EC$_{50}$) value decreased from 0.54 $\pm$ 0.16 $\mu g$ triadimefon per ml in the first generation to $-0.99 \pm 0.16$ and $-2.40 \pm 0.16 \mu g$ triadimefon per ml in the third and fifth generations, respectively, in the absence of triadimefon. A similar trend was observed in mixtures of R and S isolates from mildew populations collected from untreated wheat fields where the mean value of log(EC$_{50}$) of mixtures of R and S isolates decreased from $-0.79 \pm 0.16 \mu g$ triadimefon per ml in the first generation cycle to $-1.40 \pm 0.16$ and $-3.04 \pm 0.16 \mu g$ triadimefon per ml in the third and fifth generation cycles, respectively.

The significant interaction ($P < 0.05$) between treatments, mixtures of R and S isolates, and generation cycles (Table 1) of the fungus in their effects on the mean values of log(EC$_{50}$), and the slopes (mean log(EC$_{50}$) at G5) – (mean log(EC$_{50}$) at G1)) of the mean values of log(EC$_{50}$) for mixtures of three S/R ratios. (Table 2) demonstrated that for each mixture of S/R ratios, the slope of mean values of log(EC$_{50}$) was negative, indicating that the mean values of log(EC$_{50}$) decreased after keeping these mixtures for five generation cycles in the absence of the fungicide.

1992 experiment. There was a highly significant difference ($P < 0.05$) between generation cycles in their effects on the log(EC$_{50}$) values of all isolates tested (Table 3). Results of the LSD test indicated that the mean value of the log(EC$_{50}$) of all isolate mixtures decreased from 2.39 $\pm$ 0.22 $\mu g$ triadimefon per ml in the first generation to 0.80 $\pm$ 0.22 and $-0.38 \pm 0.22 \mu g$ triadimefon per ml after keeping the mixtures of R and S isolates for three and five generation cycles, respectively, in the absence of triadimefon. The significant difference ($P < 0.05$) between isolates (blocks) indicates that blocking isolates added to the precision of the ANOVA (Table 3) with regard to treatments (generations).

DISCUSSION

The highly significant difference between generation cycles of powdery mildew in terms of the mean values of log(EC$_{50}$) in 1991 suggested that R strains of E. g. f. sp. tritici are less fit than S ones in the absence of triadimefon. Regardless of the ratio of S/R in all mixtures, there was a significant increase in sensitivity of the fungus due to competition between R and S isolates over five generation cycles in the absence of the fungicide. The significant interaction among generation cycles of powdery mildew and treatments also suggested that, for mixtures of R and S isolates from both treated and untreated wheat fields, there was a decline in resistance after keeping the mixtures for five generation cycles in the absence of triadimefon. Negative slopes of the mean values of log(EC$_{50}$) for all mixtures of R and S isolates over five generations indicated the decrease in resistance of the fungus in the absence of the fungicide. Our results agree with those reported by Dekker (10), Fuchs et al. (15), and De Waard and Van Nistelrooy (12). Dekker (10) reported that the competitive ability of mixtures of benomyl-resistant and -sensitive isolates of Sphaerotheca fuliginea (Schlechtend.:Fr.) P. Holl (90:10, 50:50, 10:90) has been tested in the absence of benomyl for five generations. He found that the percentage of resistant isolates had declined and almost disappeared even from the mixture with 90% resistant isolate. Our results are not in variance with these results. Similar results were reported for triforine in the fungus Cladosporium cucumerinum Ellis & Arth. (15) and for fenarimol in Penicillium italicum Wehmer (12). In other cases, scientists reported that resistant strains were more fit, and competed better with sensitive in the absence of fungicides (3,4,17,22,32,35).

The highly significant difference among generation cycles in terms of the mean values of log(EC$_{50}$) for mixtures of R and S isolates in 1992 indicated that there was a decline in the proportion of R isolates as a result of competition with S ones for five generations in the absence of triadimefon. R isolates of E. g. f. sp. tritici were less competitive than S ones in the absence of the fungicide. These results are similar to those reported by Buchenauer et al. (5), who found that in mixtures of triadimefon-resistant and -sensitive isolates of barley powdery mildew (50:50), the proportion of resistant isolates decreased after five passages in the absence of triadimefon. Hol- lomon (19) and Walmsley-Woodward et al. (37) also indicated reduced competitive abilities of ethirimol- and tridemorph-resistant isolates of barley powdery mildew compared with sensitive isolates in the absence of the fungicide. A similar report (18) indicated the disappearance of the resistant strain of Botrytis cinerea (Pers.:Fr.) in mixtures of R and S isolates (50:50, 10:90) cultured on vinclozolin-free medium for four generation cycles. Moorman and Lease (26) found similar results.

When fungicide resistance is accompanied by decreased fitness such as in our studies, the risk of development of resistance problems in commercial practice may be considered as moderate or low, since low fitness counteracts the build-up of resistance. This is in agreement with Dekker (11) and Beever and Byrne (2). In order to be able to extrapolate our findings to the field situation, a more extensive competition study involving a larger number of resistant and sensitive isolates, and repeated over several years, might be needed. If the outcome of such a study was similar to our findings, then this might suggest that if the use of triadimefon in Nova Scotia is discontinued for a certain period of time, and reintroduced thereafter with caution, this might help decrease the proportion and frequency of resistant isolates, and, eventually, achieve better disease control. Reentry of triadimefon must be done with caution, following monitoring studies to indicate when this might be worthwhile. The risk of reentry must be minimized by the use of fungicide mixtures, careful timing, and growing resistant cultivars of wheat.

ACKNOWLEDGMENTS

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LITERATURE CITED

2. Beever, R. E., and Byrne, R. J. W. 1982. Re


