# Genetic Correlations in Resistance to Sterol Biosynthesis-Inhibiting Fungicides in *Pyrenophora teres*

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Supported in part by Hatch Project NYC-153416, USDA Competitive Grant 91-37303-6429, to M. G. Milgroom and by an NSERC Postgraduate Scholarship (Canada) to T. L. Peever.
We would like to thank D. Dewing, G. M. Hoffmann, H. Meier, S. Slopek, B. Steffenson, and A. Tekauz for sampling *P. teres* populations. We also appreciate the excellent technical assistance of Aigars Brants, Karen Lee, and Yang Zhou.
Accepted for publication 25 June 1993.

## ABSTRACT

Peever, T. L., and Milgroom, M. G. 1993. Genetic correlations in resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres*. Phytopathology 83:1076-1082.

We investigated cross-resistance in *Pyrenophora teres* to five sterol biosynthesis-inhibiting fungicides (SBIs) by calculating genetic correlations in resistance. Two approaches were used in separate experiments to estimate the correlations: In one method, progeny sampled from crosses of *P. teres* were used to determine "among-family" genetic correlations; in the other method, replicated isolates sampled from different *P. teres* populations were used to calculate "among-isolate" genetic correlations. Resistances to some members of the demethylation inhibitor (DMI) group of SBIs (triadimenol, propiconazole, imazalil, and fenarimol) were highly genetically correlated in both experiments, consistent with the hypothesis that many of the same genes, or genes in gametic disequilibrium, control resistance to these DMIs. However, resistances to several other pairs of DMIs had correlation coefficients that were not significantly different from zero, indicating that independent genes may control resistance to these pairs. Genetic correlations in resistance to DMIs and the morpholine SBI fenpropimorph appeared to differ from the DMI-DMI correlations, and many of the coefficients were not significantly different from zero, consistent with the hypothesis that independent genes control resistance to DMIs and fenpropimorph. Some of the genetic correlation coefficients in resistance to DMIs and fenpropimorph, however, were not significantly different from one, indicating that in certain *P. teres* populations, the same genes or genes in gametic disequilibrium controlled resistance to these pairs of SBIs. Many of the correlation coefficients in resistance to any given pair of SBIs differed among populations of *P. teres*, indicating significant genetic differentiation among these populations. The variability in correlation coefficients observed between DMI-DMI and DMI-fenpropimorph combinations and among populations indicates that no single model of cross-resistance among SBIs and among all *P. teres* populations is appropriate. Among-isolate genetic correlations provided more accurate estimates of cross-resistance among SBIs than the among-family estimates and represent the most evolutionarily relevant approach to studying genetic correlations in resistance in *P. teres* and other predominantly asexually reproducing fungi.

*Additional keywords:* evolution, fungicide resistance, quantitative genetics.

Cross-resistance to fungicides has been defined as "resistance to two or more toxicants that is mediated by the same genetic factor" (10). Cross-resistance has important implications for the evolution and management of fungicide resistance but has received relatively little research attention from plant pathologists. Mixtures or alternations of fungicides have been suggested as practical ways to slow the evolution of resistance (2,5,12,17). The effectiveness of these strategies may depend in part on how resistances to the selected fungicides are correlated genetically in the target pathogen. Therefore, understanding the genetics of cross-resistance to various fungicides is an important component of resistance management. The evolution of resistance to fungicides involves changes in the frequencies of genes controlling resistance in populations of the pathogen. If the genes that control resistance to one fungicide also confer resistance to another fungicide, then this other fungicide cannot be used in management schemes to lower the frequency of resistance genes in the population.

Sterol biosynthesis-inhibiting fungicides (SBIs) have become very important in agriculture in recent years, and there is much interest in preventing the development of resistance to them, as has occurred with other systemic fungicides (17). The success of strategies designed to delay the evolution of resistance to SBIs may depend largely on knowledge of the genetics of resistance and cross-resistance to SBIs in the target fungal population.

Cross-resistance to SBIs has traditionally been studied by determining the resistance phenotypes of a relatively small number of isolates of a given fungal species to different SBIs (3,6,11, 13–15,24,27). These studies have revealed some general patterns of cross-resistance to SBIs. Cross-resistance has generally been found among the demethylation-inhibiting (DMI) group of SBIs (3,13,27) but has not been found between DMIs and the morpholine group of SBIs (3,11,27). All members of the DMI group of SBIs have been shown to have the same mode of action (1,16), and this observation has led some researchers to conclude that there must be a common mechanism of resistance to DMIs (2,11,12). This assumption makes sense if the resistance mechanism involves alteration of the DMI target site; however, currently no evidence exists to suggest that resistance to any DMI is due to target site modifications (16).

Despite the general patterns of cross-resistance to SBIs that have been observed, the results obtained in many of these studies have been quite variable (6,14,15,24). The variability among studies may be the result of the different fungal species and/or SBIs used in each study or of the very small sample sizes in many of these studies. In addition, cross-resistance studies completed to date have compared only resistance phenotypes and not resistance genotypes and therefore do not necessarily provide any information about genetic factors controlling resistance. These problems limit the inferences that can be drawn from these studies regarding the evolution of SBI resistance in pathogen populations.

Cross-resistance can be thought of formally in terms of a correlation in resistance to two or more fungicides. A high positive correlation between resistances to two fungicides is indicative of cross-resistance. Correlations can be phenotypic—that is, caused by the combined effects of genetic and environmental factors—or genetic—that is, due to genetic factors alone. If resistances to two fungicides are correlated genetically, then the same genetic factors (genes) or genes in gametic disequilibrium control resis-

tance to both fungicides (9).

Genetic correlations have been used extensively in plant and animal breeding (9) and ecological genetic studies (28) to determine whether the same genetic factors control two or more phenotypic characters. Estimation of genetic correlations requires an experimental design in which genetic and environmental sources of variation in phenotypes can be partitioned. Such methods have been used rarely in plant pathology (7,8) and have only recently been used to study cross-resistance to fungicides (20,21,23). Genetic correlations have been proposed as a method for studying the evolution of pesticide resistance (29) and seemed to us to be the most appropriate approach for investigating genetic relationships among resistances to SBIs.

In the present study, we used two experimental approaches to estimate genetic correlations in resistance to five SBIs in *Pyrenophora teres* Drechs., the causal agent of net blotch of barley (*Hordeum vulgare* L.). *P. teres* was chosen for this research because it can be crossed in the laboratory, it is easily grown in vitro, and techniques for determining SBI resistance phenotypes have been developed (22). In the first experiment, progeny from *P. teres* crosses were sampled and used to estimate "among-family" genetic correlations in resistance. In the second experiment, replicated *P. teres* isolates sampled from five different populations were used to estimate "among-isolate" genetic correlations in resistance. The calculation of genetic correlations allowed us to quantify cross-resistance relationships among the five SBIs and to compare these relationships in different populations of *P. teres*.

## MATERIALS AND METHODS

**SBI fungicides.** Five SBIs were chosen to represent different chemical classes within the SBI group of fungicides and were obtained as technical-grade materials from their manufacturers: the DMI fungicides triadimenol (triazole class, Mobay Chemical Corp., Kansas City, MO), propiconazole (triazole class, Ciba-Geigy Corp., Greensboro, NC), fenarimol (pyrimidine class, DowElanco Corp., Greenfield, IN), and imazalil (imidazole class, Janssen Pharmaceutica, Piscataway, NJ) and the morpholine fungicide fenpropimorph (morpholine class, BASF Corp., Research Triangle Park, NC). All DMIs tested to date have the same mode of action, whereas morpholines appear to have a different mode of action (1,16).

**SBI resistance assays.** A radial growth assay on fungicide-amended medium (22) was used to determine the resistance phenotype of each *P. teres* isolate. Isolates were stored on sterile filter papers, retrieved from storage, and cultured on malt extract agar (MEA) containing 1.5% malt extract (William's Brewing, San Leandro, CA) and 2% agar (Difco), as described previously (22). Isolates were grown on MEA for 5–7 days at 25 C in the dark, and 4-mm-diameter mycelial plugs were cut from the margins of the colonies.

Mycelial plugs were placed mycelium-down on 9-cm plastic petri plates containing MEA amended with 1% ethanol (controls) or with a single discriminatory concentration of one of the five SBIs (solubilized in ethanol to a final concentration of 1%). Single discriminatory concentrations of each SBI were chosen by screening a sample of the parental isolates on three to five concentrations of each SBI. The concentration that suppressed radial growth to approximately 50% of that of the controls was chosen for the resistance assays. The inhibition of radial mycelial growth on a single concentration of an SBI close to the $EC_{50}$ value has been shown to be the most appropriate response to measure with SBI fungicides (26) and has also been shown in *P. teres* to be highly correlated with $EC_{50}$ values determined with a series of concentrations (22).

Plates were incubated for 6–7 days at 25 C in the dark, and the radial growth of each isolate was measured (22). Two replicates of each isolate were used in all assays to estimate the environmental or assay variance.

**Experiment 1.** In the first experiment, among-family genetic correlations in resistance to the five SBIs were estimated from progeny sampled from seven crosses of *P. teres*. This method involved a conventional full-sib breeding design commonly used to partition variance in quantitative genetics and breeding studies (9). The experiment was performed in two blocks consisting of independent progeny samples and two resistance assays performed at separate times. Each block was analyzed separately.

*Crosses of* P. teres. Crosses were made in all combinations for 30 isolates sampled from a single *P. teres* population in North Dakota and other isolates in our culture collection. The method of R. D. Cartwright and R. K. Webster (*personal communication*) was used for performing crosses, and the details have been published previously (22). Seven fertile full-sib crosses of *P. teres* were produced in the laboratory (out of several hundred possible crosses), and 10 ascospore progeny were randomly sampled from each cross. Five ascospore progeny from each cross were used in each block. Ascospore progeny obtained from each cross were stored on sterile filter papers as previously described (22).

The parents of the crosses and their geographic origins are shown in Table 1. B. Steffenson (North Dakota State University) provided the North Dakota isolates and isolate ONT102-1A-B from Ontario, Canada. Isolates ND89-14-B and ND89-18-B were sampled from a single barley field in 1989, and the other North Dakota isolates were sampled at one time from a different field in 1991. E. Sheridan (Wellington University) provided the New Zealand isolates. Each isolate was sampled from a different field and on a different date.

*Data analysis.* Radial growth on each SBI was measured and expressed as a proportion of growth of the control to correct for differences among isolates in in vitro growth rates (8), and each replicate had its own control. Component correlations (9) were used to estimate genetic correlations in resistance to the 10 pairs of SBIs. Genetic variance and covariance components needed to calculate these correlation coefficients were estimated from analysis of variance (ANOVA) and analysis of covariance (ANCOVA) tables in which the variance in the resistance to each SBI or the covariance in the resistance to pairs of SBIs was partitioned into genetic and environmental components (8). The ANOVA and ANCOVA models used to estimate the appropriate variance and covariance components are shown in Table 2.

TABLE 1. Isolates of *Pyrenophora teres* used as parents in crosses for experiment 1

| Cross number | Parents | Geographic origin of parents |
|---|---|---|
| 1 | ND89-14-B × ND89-18-B | North Dakota |
| 2 | S1-B × R3-B | New Zealand |
| 3 | GP1-B × ONT102-1A-B | GP1-B, New Zealand; ONT102-1A-B, Ontario, Canada |
| 4 | 13Y-B × 7d-B | New Zealand |
| 5 | ND91-33-4 × ND91-33-6 | North Dakota |
| 6 | ND91-33-26 × ND91-33-12 | North Dakota |
| 7 | ND91-33-20 × ND91-33-29 | North Dakota |

TABLE 2. Analysis of variance (ANOVA) or analysis of covariance (ANCOVA) models for partitioning the variance or covariance in sterol biosynthesis inhibitor resistance phenotypes into genetic and environmental components in experiments 1 and 2

| Source | df | EMS[a] |
|---|---|---|
| Experiment 1 | | |
| Families | 6 | $\sigma^2 + 2\sigma^2_P + 10\sigma^2_F$ |
| Progeny (families) | 28 | $\sigma^2 + 2\sigma^2_P$ |
| Replicates (families) (progeny) | 35 | $\sigma^2$ |
| Experiment 2 | | |
| Isolates | 24[b] | $\sigma^2 + 2\sigma^2_I$ |
| Replicates (isolates) | 25 | $\sigma^2$ |

[a] Expected mean squares (variance or covariance components: $\sigma^2_F$ = families, $\sigma^2_P$ = progeny, $\sigma^2$ = replicates, $\sigma^2_I$ = isolates.
[b] Degrees of freedom for a population sample of 25 isolates.

Among-family genetic correlation coefficients were calculated for experiment 1 from the following equation:

$$r_G = \sigma_F(x,y) / \sqrt{\sigma_F^2(x) \cdot \sigma_F^2(y)}, \quad (1)$$

where $r_G$ is the among-family genetic correlation coefficient, $\sigma_F(x,y)$ is the among-family genetic covariance in resistance to a given pair of SBIs ($x$ and $y$) obtained from the appropriate ANCOVA table for that pair of SBIs, and $\sigma_F^2(x)$ and $\sigma_F^2(y)$ are the among-family genetic variances in resistance to the SBIs $x$ and $y$, respectively, obtained from separate ANOVA tables for resistance to each SBI.

The ANOVA and ANCOVA models were identical (Table 2), and three sources of variation were identified: families, progeny (within families), and replicates (within progeny and families). The expected mean squares of the model (Table 2) allowed estimation of the among-family genetic component of variance—$\sigma_F^2(x)$ or $\sigma_F^2(y)$—and the covariance—$\sigma_F(x,y)$—for each SBI or pair of SBIs. These estimated components of variance were then used in equation 1 to calculate the genetic correlation coefficients in resistance.

MINITAB 7.1 statistical software (Minitab Inc., State College, PA) was used for the analysis of the variance components. Covariance sums of squares were calculated by a program we developed for EXCEL 4.0 spreadsheet software (Microsoft Corp., Seattle, WA, and the appropriate ANCOVA tables were generated by hand. Error estimates for the correlation coefficients were determined from the formulas given by Ennos and Swales (8). Ninety-five percent confidence intervals for the correlation coefficients were constructed by multiplying the standard error of the estimates of the correlation coefficients by $Z_{0.05}(1.96)$.

**Experiment 2.** In the second experiment, we used replicated isolates sampled from five different *P. teres* populations to estimate among-isolate genetic correlations in resistance to the five SBIs. This method takes advantage of the fact that *P. teres* is haploid, reproduces predominantly asexually in the field, and can be clonally replicated in the laboratory. Isolates sampled from each population were analyzed in separate resistance assays.

*Sampling* P. teres *populations.* We obtained 107 single-conidial isolates of *P. teres* from five barley fields in different parts of the world (Table 3). For the purposes of this study, isolates sampled from the same field were considered members of the same *P. teres* population.

*Data analysis.* Resistance phenotype for each isolate on each SBI and among-isolate genetic correlations were determined by methods similar to those described for experiment 1. The resistance phenotypes of each population sample were determined in single resistance assays performed at different times.

The variance and covariance in resistance phenotype for each SBI or pair of SBIs were partitioned into environmental and genetic components by a one-way ANOVA or ANCOVA with a slightly different model (4,8) from that described for experiment 1 (Table 2). Replicates of isolates provided an estimate of the environmental or assay variance. Among-isolate genetic correlation coefficients in resistance to each pair of SBIs were estimated from equation 1 with the appropriate variance components derived from the ANOVA or ANCOVA model. For experiment 2, $r_G$ is the among-isolate genetic correlation coefficient, and $\sigma_F(x,y)$ in equation 1 is replaced by $\sigma_I(x,y)$, the among-isolate genetic covariance in resistance to a given pair of SBIs ($x$ and $y$) obtained from the ANCOVA table for this pair of SBIs. Similarly, $\sigma_I^2(x)$ and $\sigma_I^2(y)$, the among-isolate genetic variances in resistance to the SBIs $x$ and $y$, respectively, obtained from separate ANOVA tables for resistance to each SBI, replace $\sigma_F^2(x)$ and $\sigma_F^2(y)$, respectively, in equation 1.

The ANOVA and ANCOVA models were identical (Table 2), and two sources of variation could be identified: isolates and replicates within isolates. The expected mean squares of the model allowed estimation of the among-isolate component of variance—$\sigma_I^2(x)$ or $\sigma_I^2(y)$—and covariance—$\sigma_I(x,y)$—for each SBI or pair of SBIs. These estimated components of variance were then used in equation 1 to calculate the among-isolate genetic correlation coefficients in resistance. Variance and covariance sums of squares in resistance to each SBI or pair of SBIs and confidence intervals were obtained as described for experiment 1.

We also used a nested ANOVA model with three sources of variation identified (population, isolates, and replicates) to compare the variance in resistance genotype for each SBI among the five populations of *P. teres.* Variance in resistance among populations and within populations (among isolates) was tested with an $F$ test (19). $F$ values for variation among populations were obtained by dividing mean square for population by mean square for isolates. $F$ values for variation among isolates were obtained by dividing mean square for isolates by mean square for replicates.

## RESULTS

**Experiment 1.** Separate analyses of the two blocks of experiment 1 produced similar results for both blocks. Results of only one block are presented.

*Resistance phenotypes.* Mean radial growth of the parents and the progeny on each SBI varied between 24 and 67% of that of the controls (Table 4). Radial growth proportions in this range have been shown to be appropriate for discriminating SBI-resistance phenotypes (22). Highly significant genetic variation in resistance was detected among the parental isolates and among the progeny isolates for each SBI (data not shown). This result indicates significant among-family genetic variation in resistance to each of the SBIs tested in this study.

The distributions of the progeny resistance phenotypes appeared to be bimodal for triadimenol and fenarimol and continuous for propiconazole, imazalil, and fenpropimorph (data not shown). The bimodal distribution of resistance phenotypes to triadimenol has been observed previously, and a single, major genetic locus has been shown to confer a large phenotypic difference between the sensitive and the resistant distributions, while several additional genes appear to confer smaller phenotypic effects (22). The present results suggest that a single, major genetic locus may also control resistance to fenarimol in *P. teres,* but more crosses and progeny must be analyzed to demonstrate this conclusively.

*DMI-DMI correlation coefficients.* Two of six correlation coefficients in resistance to the DMIs were significantly different from 0 and not significantly different from 1 (Table 5). This suggests that the same genes may control resistance to triadimenol and fenarimol and to imazalil and propiconazole. This result is interesting because the DMIs in both of these pairs are from different chemical classes.

Correlation coefficients in resistance to the two DMIs from the triazole class (triadimenol and propiconazole) as well as two other DMI-DMI combinations were not significantly different from 0 or −1, suggesting that different genes may control resistance to these pairs of DMIs or that resistances may be negatively genetically correlated.

TABLE 3. Sources of *Pyrenophora teres* isolates used in experiment 2

| Population | *n* | Sampling date | Location | Barley cultivar | Collector |
|---|---|---|---|---|---|
| New York | 25 | July 1990 | Cattaraugus Co. | Unknown | D. Dewing |
| Manitoba | 11 | July 1985 | Winnipeg | Unknown | A. Tekauz |
| North Dakota | 25 | July 1991 | Stutsman Co. | Robust | B. Steffenson |
| Germany | 25 | July 1991 | Deggendorf | Trixi | G. M. Hoffmann |
| Alberta | 21 | August 1991 | Innisfail | Harrington | S. Slopek |

The large errors associated with most of these estimates (except triadimenol-fenarimol and imazalil-propiconazole) resulted in extremely large confidence intervals for the coefficients (Table 5). This level of error makes it difficult to draw any definite conclusions regarding genetic correlations in resistance among DMIs in this experiment.

*DMI-fenpropimorph correlation coefficients.* None of the correlation coefficients in resistance to DMIs and fenpropimorph were significantly different from 0 (Table 5), which suggests that different genes may control resistance to DMIs and fenpropimorph. Three of the DMI-fenpropimorph correlations were not significantly different from either 0 or 1, again indicating the high level of error associated with the estimates. The large confidence intervals obtained for all of these estimates made it difficult to reach any definite conclusions regarding genetic correlations in resistance to DMIs and fenpropimorph in this experiment.

**Experiment 2.** *Variation in SBI resistance phenotypes.* Variation in resistance to each SBI was partitioned into among- and within-population genetic components with a nested ANOVA (Table 6). The variation among the five populations was highly significant ($P < 0.001$) for every SBI, which indicates that these *P. teres* populations are genetically differentiated for resistance to each of the SBIs examined. Highly significant genetic variation ($P < 0.001$) was also found among the isolates within each population for all SBIs (Table 6).

TABLE 4. Mean sterol biosynthesis inhibitor resistance phenotypes (as a percentage of the control) of the parents of the crosses and the progeny in experiment 1[a]

| Isolates | Number of isolates | Fungicide | | | | |
|---|---|---|---|---|---|---|
| | | Triadimenol | Fenarimol | Propiconazole | Imazalil | Fenpropimorph |
| Parents | 14 | 36 (4) | 37 (4) | 36 (2) | 67 (1) | 29 (1) |
| Progeny | 35 | 33 (3) | 31 (2) | 32 (1) | 56 (1) | 33 (2) |

[a]Standard errors of the means are shown in parentheses.

TABLE 5. Among-family genetic correlation coefficients for resistance to four demethylation inhibitors (DMIs) and the morpholine fenpropimorph calculated from five progeny samples from each of seven crosses of *Pyrenophora teres* in experiment 1[a]

| Fungicide | DMI | | | Fenpropimorph |
|---|---|---|---|---|
| | Fenarimol | Propiconazole | Imazalil | |
| Triadimenol | 0.73* (0.37,1.08) | −0.25 (−1.14,0.64) | −0.21 (−1.19,0.77) | 0.05 (−0.91,1.00) |
| Fenarimol | | −0.06 (−0.51,0.39) | −0.09 (−1.06,0.88) | 0.03 (−0.92,0.98) |
| Propiconazole | | | 0.84* (0.59,1.08) | 0.64 (−0.02,1.29) |
| Imazalil | | | | 0.59 (−0.23,1.42) |

[a]An asterisk signifies that the coefficient is significantly different from 0 and not significantly different from 1 at $P = 0.05$. The correlation coefficients were calculated from equation 1 in the text. Ninety-five percent confidence intervals calculated as described in the text are given in parentheses.

Mean resistance phenotypes for each population and each SBI are presented in Table 7. Resistance phenotypes varied between 20 and 79% of the controls, similar to the range found for the parental isolates in experiment 1. The mean propiconazole and fenpropimorph resistance phenotypes of isolates from the Alberta population were significantly higher than the combined means of the other populations.

*DMI-DMI correlation coefficients.* Genetic correlations in resistance to triadimenol-propiconazole and imazalil-propiconazole in the North Dakota population are illustrated in Figure 1A and B, respectively. The distribution of resistance phenotypes is bimodal for triadimenol and continuous for propiconazole and imazalil.

Among-isolate genetic correlation coefficients for resistance to the DMIs varied both among DMI-DMI combinations and among populations (Table 8). Correlation coefficients were generally high in the Manitoba, North Dakota, Germany, and Alberta populations. Among these four populations, only two of 24 correlation coefficients were not significantly different from 0, and four were not significantly different from 1 at the 95% probability level. The New York population appeared to have slightly lower correlation coefficients than the other populations, with two of the six coefficients not significantly different from 0.

Mean correlation coefficients were calculated for each DMI-DMI combination (Table 8). The mean correlation coefficients for the fenarimol-triadimenol and propiconazole-imazalil combinations appeared to be higher than those for the other combinations, as observed in experiment 1, but the differences were not statistically significant ($P > 0.10$) according to an a posteriori test with a single degree of freedom (19).

*DMI-fenpropimorph correlation coefficients.* Correlation coefficients varied both among DMI-fenpropimorph combinations and among populations (Table 9). Coefficients for three of the DMI-fenpropimorph pairs were significantly different from 0 in some populations and not in others. Correlation coefficients in resistance to propiconazole-fenpropimorph were significantly different from 0 in every population sampled. Eight of 20 (40%) of the among-isolate genetic correlations in resistance obtained were not significantly different from 0, while three of 20 (15%) were not significantly different from 1. None of the DMI-fenpropimorph correlation coefficients obtained in this experiment were significantly less than 0, which suggests that there is no negative cross-resistance between DMIs and fenpropimorph in these *P. teres* populations.

## DISCUSSION

We used two methods to evaluate cross-resistance relationships to five SBI fungicides in *P. teres*. The calculation of genetic correlations in resistance has allowed us to determine the genetic control of resistance to a given pair of SBIs by separating the genetic and environmental causes of the correlations. This approach to the study of cross-resistance among SBIs is more quantitative than previous approaches.

In general, we found positive correlations in resistance among the DMI group of SBIs, while correlations in resistance to DMIs and fenpropimorph were usually lower, with many not significantly different from 0. These results suggest that many of the same genes confer resistance to the DMIs, whereas resistances to DMIs and fenpropimorph may be under independent

TABLE 6. Analysis of variance for resistance to sterol biosynthesis inhibitors among and within *Pyrenophora teres* populations in experiment 2[a]

| Source | df | Triadimenol | | Fenarimol | | Propiconazole | | Imazalil | | Fenpropimorph | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | MS | *F* | MS | *F* | MS | *F* | MS | *F* |
| Population | 4 | 0.718 | 8.7*** | 0.679 | 9.3*** | 0.684 | 23.3*** | 0.624 | 13.5*** | 0.872 | 22.8*** |
| Isolates | 102 | 0.083 | 59.0*** | 0.073 | 95.3*** | 0.029 | 18.9*** | 0.046 | 25.0*** | 0.038 | 18.4*** |
| Replicates | 107 | 0.001 | | 0.001 | | 0.002 | | 0.002 | | 0.002 | |

[a]MS = mean square. *F* is the *F* value for population (MS population/MS isolates) and for isolates (MS isolates/MS replicates). Three asterisks indicate significance at $P < 0.001$.

genetic control. Despite these general cross-resistance patterns, some correlation coefficients among the DMIs were not significantly different from 0, and some of the DMI-fenpropimorph correlation coefficients were not significantly different from 1. These exceptions suggest that cross-resistance relationships among SBIs are quite variable and that no single model of cross-resistance among SBIs is appropriate for all *P. teres* populations.

Correlation coefficients that were not significantly different from 1 were obtained for resistance to fenarimol-triadimenol and imazalil-propiconazole in experiment 1 and in several populations in experiment 2, suggesting that the same genes or sets of genes control resistance to these pairs of DMIs. A high degree of correlation in resistance phenotype to prochloraz (in the same chemical class as imazalil) and propiconazole was also observed in a previous study with another plant pathogen (15).

Genetic correlation coefficients in resistance to the remaining four pairs of DMIs were not significantly different from 0 in experiment 1, but most were significantly greater than 0 in experiment 2. The differences between the two experiments in correlation coefficients probably stem from differences between the "population" of parental isolates used in experiment 1 and the population samples used in experiment 2 in DMI resistance gene frequencies and/or degrees of gametic disequilibrium. The only DMI-DMI correlation coefficients that were not significantly different from 0 were from the New York population in experiment 2. These low correlation coefficients were due to the fact that a resistance allele at a single locus, which confers a major phenotypic effect on resistance to triadimenol (22) and fenarimol, was not present in the New York population.

The high genetic correlation coefficients in resistance to most pairs of DMIs observed in this study agree with the results of previous studies that have also demonstrated cross-resistance among DMIs (3,13,27). Other studies failed to detect consistent patterns of cross-resistance to all the DMIs tested (6,14). This variability may be due to the small number of isolates used in

TABLE 7. Mean resistance phenotypes (as a percentage of the control) for each sterol biosynthesis inhibitor and each *Pyrenophora teres* population in experiment 2

| Population | *n* | Triadimenol | Fenarimol | Propiconazole | Imazalil | Fenpropimorph |
|---|---|---|---|---|---|---|
| New York | 25 | 20 | 23 | 50 | 52 | 52 |
| Manitoba | 11 | 53 | 58 | 53 | 74 | 56 |
| North Dakota | 25 | 42 | 41 | 55 | 75 | 57 |
| Germany | 25 | 49 | 49 | 46 | 50 | 41 |
| Alberta | 21 | 41 | 47 | 76 | 69 | 79 |
| LSD[a] | | 13 | 12 | 8 | 10 | 8 |

[a] Least significant difference ($P = 0.05$) calculated from the mean square errors in Table 6.

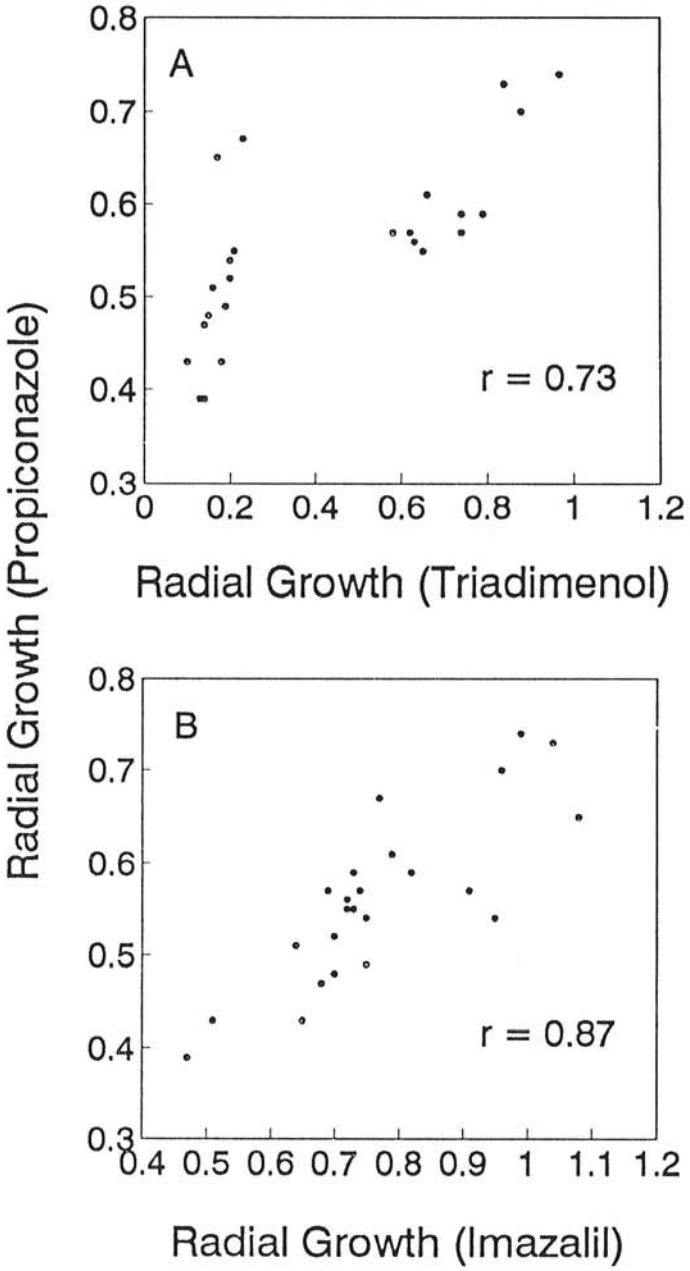


**Fig. 1.** Radial growth (as a proportion of the control) of North Dakota isolates of *Pyrenophora teres* on **A,** propiconazole and triadimenol and **B,** propiconazole and imazalil. Genetic correlation coefficients (*r*) are shown. Each data point represents the mean of two replicates.

TABLE 8. Among-isolate genetic correlation coefficients for resistances to pairs of demethylation inhibitors (DMIs) estimated for isolates sampled from different *Pyrenophora teres* populations in experiment 2[a]

| Population | *n* | Fenarimol-triadimenol | Fenarimol-propiconazole | Fenarimol-imazalil | Imazalil-triadimenol | Imazalil-propiconazole | Propiconazole-triadimenol |
|---|---|---|---|---|---|---|---|
| New York | 25 | 0.49 (0.15,0.83) | 0.54 (0.26,0.82) | 0.61 (0.35,0.87) | −0.23 (−0.65,0.19) | 1.00 (0.97,1.03) | −0.32 (−0.73,0.10) |
| Manitoba | 11 | 0.87 (0.71,1.02) | 0.43 (−0.05,0.92) | 0.28 (−0.27,0.83) | 0.60 (0.22,0.98) | 0.71 (0.40,1.02) | 0.71 (0.41,1.00) |
| North Dakota | 25 | 0.99 (0.98,1.00) | 0.70 (0.50,0.90) | 0.54 (0.26,0.82) | 0.53 (0.24,0.82) | 0.87 (0.77,0.97) | 0.73 (0.55,0.91) |
| Germany | 25 | 0.93 (0.87,0.99) | 0.76 (0.57,0.95) | 0.57 (0.29,0.85) | 0.59 (0.32,0.86) | 0.83 (0.67,0.99) | 0.82 (0.65,0.99) |
| Alberta | 21 | 0.97 (0.95,0.99) | 0.52 (0.20,0.84) | 0.67 (0.43,0.91) | 0.60 (0.33,0.87) | 0.85 (0.72,0.95) | 0.48 (0.13,0.83) |
| Mean[b] | | 0.84 | 0.61 | 0.56 | 0.39 | 0.87 | 0.45 |

[a] Confidence intervals calculated as described in the text are given in parentheses.
[b] Mean correlation coefficient for each DMI-DMI combination (weighted by the population sample size).

these studies, the different geographic origins of isolates, or the fact that these studies did not attempt to separate genetic and environmental causes of correlation.

Our observation that resistances to triadimenol-fenarimol and propiconazole-imazalil were more highly correlated genetically than those to other DMI-DMI combinations is interesting because triadimenol and fenarimol are from different chemical classes of SBIs (triazole vs. pyrimidine), as are propiconazole and imazalil (triazole vs. imidazole). Despite the fact that triadimenol and propiconazole are both triazoles, correlation coefficients in resistance to these two SBIs were not significantly different from 0 in experiment 1, indicating that different genes may control resistance to these two SBIs. This lack of a high positive correlation between resistance to triadimenol and resistance to propiconazole has been observed in other studies (15,22). These results suggest that resistances to DMIs are generally, but not always, genetically correlated and that cross-resistance "subgroups" that do not correspond to chemical structure may exist among the DMIs.

All of the genetic correlation coefficients estimated for resistances to DMIs and fenpropimorph in experiment 1 and seven of 20 (35%) coefficients estimated in experiment 2 were not significantly different from 0. This result is consistent with the hypothesis that different genes control resistance to DMIs and fenpropimorph. Other cross-resistance studies have also demonstrated that resistances to DMIs and morpholines are uncorrelated (3,11,13,27). Seven of eight (88%) correlation coefficients that were not significantly different from 0 in experiment 2 were obtained for resistances to triadimenol-fenpropimorph and fenarimol-fenpropimorph. These results indicate that resistances to DMIs and fenpropimorph are genetically correlated in some populations of *P. teres* and not in others and suggest that resistances to propiconazole-fenpropimorph and imazalil-fenpropimorph may be more highly correlated genetically than resistances to triadimenol-fenpropimorph and fenarimol-fenpropimorph.

Highly significant genetic differentiation was detected among populations and among isolates (within populations) for each SBI pair in experiment 2. This differentiation reflects differences in gene frequencies controlling resistance in these populations and was likely responsible for the variability observed among the correlation coefficients in experiment 2 (9). Variability in correlation coefficients within and between classes of SBIs (DMIs and morpholines) and among populations is important because it means that no single model of cross-resistance is appropriate for a given pair of SBIs in all populations of the pathogen. In terms of SBI resistance management, such variability implies that no single management strategy is likely to be adequate for all combinations of SBIs and all populations of the pathogen. The observation that DMI-fenpropimorph combinations may be genetically correlated in certain populations raises questions about the potential effectiveness of employing DMI-morpholine mixtures to delay the evolution of resistance (2,12,17).

The genetic correlation coefficients calculated in experiment 1 were associated with a very high degree of error, which severely limited the inferences that could be made regarding the genetic control of resistance to these SBIs from this part of the study. The high degree of error was likely due to the limited number of crosses used in this experiment compared to other genetic correlation studies (9,28). Unfortunately, we were able to generate only seven successful full-sib crosses among isolates in our collection. More complex analyses of the data in experiment 1 employing both blocks yielded similar correlation coefficients and did not reduce the confidence intervals appreciably. The fact that the parents of the crosses used in experiment 1 were sampled from diverse geographic locations, combined with the great variability in correlation coefficients observed among populations in experiment 2, limits the inferences that can be drawn from experiment 1. Attempts were made to produce 10–20 full-sib families from a single population of *P. teres* in North Dakota. Hundreds of crosses were made between isolates sampled from this population, but only three produced fertile pseudothecia. The low success rate in crossing these *P. teres* isolates was interesting because our success rate with other isolates was relatively high, even with isolates sampled from different geographic locations (22). Although the reasons for the low number of successful crosses from the North Dakota population are unknown, the result may be another indication that this population does not undergo much sexual reproduction under field conditions. The problems encountered in creating a sufficient number of families for this analysis, the high degree of error associated with the estimates, and more careful consideration of the reproductive biology of *P. teres* have led us to question the evolutionary significance of the approach taken in experiment 1 and to focus instead on investigating cross-resistance among SBIs via replicated isolates sampled from single populations, as in experiment 2.

*P. teres* can reproduce both asexually via conidia and sexually via ascospores. A single generation of sexual reproduction occurs between growing seasons of barley (25), in contrast to the several generations of asexual reproduction that occur during the barley growing season. At present, we do not know whether sexual reproduction occurs in all *P. teres* populations, nor do we know the relative contribution of sexual versus asexual reproduction to the pool of initial inoculum in populations where recombination occurs. Among-family genetic correlations in resistance to SBIs may be important in populations where regular sexual reproduction occurs and where the SBI is applied early in the growing season of the crop. For example, sexual reproduction may break up resistances that were correlated because of gametic disequilibrium due to asexual reproduction in the previous season and that could then be selected in the first asexual generation by SBIs applied to the seed. However, if sexual reproduction does not occur or if it makes only a small contribution to the pool of initial inoculum relative to asexual reproduction, then among-isolate genetic correlations may be much more important

TABLE 9. Among-isolate genetic correlation coefficients for resistances to demethylation inhibitor (DMI)-fenpropimorph pairs estimated for isolates sampled from different *Pyrenophora teres* populations in experiment 2[a]

| Population | *n* | Triadimenol-fenpropimorph | Propiconazole-fenpropimorph | Imazalil-fenpropimorph | Fenarimol-fenpropimorph |
|---|---|---|---|---|---|
| New York | 25 | −0.25 (−0.66,0.16) | 0.95 (0.90,1.00) | 0.96 (0.92,1.00) | 0.65 (0.42,0.88) |
| Manitoba | 11 | 0.27 (−0.27,0.80) | 0.56 (0.15,0.96) | 0.72 (0.42,1.01) | 0.12 (−0.46,0.70) |
| North Dakota | 25 | 0.46 (0.14,0.78) | 0.71 (0.51,0.91) | 0.75 (0.57,0.93) | 0.48 (0.17,0.79) |
| Germany | 25 | 0.22 (−0.21,0.65) | 0.45 (0.08,0.82) | 0.25 (−0.18,0.68) | 0.00 (−0.44,0.44) |
| Alberta | 21 | 0.21 (−0.20,0.62) | 0.62 (0.34,0.90) | 0.69 (0.46,0.92) | 0.36 (−0.01,0.73) |
| Mean[b] | | 0.17 | 0.67 | 0.67 | 0.35 |

[a] Confidence intervals calculated as described in the text are given in parentheses.
[b] Mean correlation coefficient for each DMI-fenpropimorph combination (weighted by the population sample size).

than among-family genetic correlations in resistance. Among-family genetic correlations tell us if the same (or closely linked) genes control resistance to a given pair of SBIs. However, if selection occurs during the asexual or clonal phase of pathogen reproduction, then whether the same genes or different genes control resistance does not matter as long as they are found in the same isolate or clone of the pathogen (18).

Determining cross-resistance in terms of among-isolate genetic correlations allows us to separate genetic and environmental causes of correlation. It does not, however, allow us to distinguish between correlations caused by the same genes and correlations caused by independent genes in gametic disequilibrium. Distinguishing between these two causes may not be very important from an evolutionary perspective if *P. teres* reproduces predominantly or exclusively asexually, as all genes are effectively linked in each clonal lineage. In such cases it may be more appropriate to think of cross-resistance in terms of among-isolate genetic correlations rather than in terms of among-family genetic correlations estimated from crosses. We propose that the best approach to studying cross-resistance among fungicides in any predominantly asexually reproducing pathogen is to estimate among-isolate genetic correlations in resistance from large numbers of replicated isolates sampled from single populations of the pathogen.

Cross-resistance has important implications for the evolution of SBI resistance in the field as well as for the practical management of resistance. Genetically correlated traits do not evolve independently (8,29), and therefore, high positive genetic correlations in resistance to a pair of SBIs mean that isolates that are resistant to one member of the pair will also be resistant to the other member. This precludes replacing one SBI by the other or using them in mixtures and/or alternations in order to manage resistance. Because of the high positive genetic correlations in resistance to most DMI-DMI combinations and some DMI-fenpropimorph combinations observed in this study, combinations of these SBIs must be used with caution in resistance management schemes with *P. teres* and possibly other fungi as well.

Genetic correlation coefficients of 0 suggest that different genetic factors control resistance to each SBI. Replacing one SBI to which resistance has developed with another to which resistance is genetically uncorrelated may restore disease control temporarily but will select for an additional set of resistance genes. The result of mixing or alternating SBIs with uncorrelated resistance mechanisms, such as some of the DMI-morpholine combinations examined in this study, may be the eventual selection of individuals resistant to both classes of SBIs through independent genetic factors. This consideration, combined with the fact that resistances to many pairs of SBIs may be highly correlated genetically, raises questions about the potential effectiveness of mixtures for managing SBI resistance.

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