Inheritance of Triadimenol Resistance in *Pyrenophora teres*

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ABSTRACT

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Progeny from crosses between isolates of *Pyrenophora teres* sensitive and resistant to the sterol demethylation-inhibiting fungicide (DMI) triadimenol revealed that resistance segregates at a single, major genetic locus. Crosses between isolates from different geographic regions (Canada, New Zealand, and the United States) indicated a lack of fertility barriers between isolates from these regions and indicated that resistance to triadimenol is conferred by alleles at the same genetic locus. When the ascospore progeny from the same crosses were grown on media containing propiconazole, a closely related DMI, resistance appeared to segregate in a quantitative manner. These different segregation patterns for resistance to two very closely related DMIs (both in the triazole class of DMIs) may be attributable to how the parents were selected for the crosses or to different phenotypic effects associated with DMI resistance genes. The results presented here indicate that the inheritance of DMI resistance may not be the same for different pathogens and DMIs; therefore, it is unlikely that any one model regarding the inheritance of resistance to DMIs would be appropriate.

Additional keywords: fungicide resistance.

Resistance to systemic fungicides has evolved rapidly in some populations of plant pathogenic fungi. Although the theoretical bases of resistance evolution are well established (13,15), we lack empirical knowledge of the factors controlling the evolution of resistance in the field, such as the reproductive biology of the pathogen, frequencies of resistance in the field, rates of selection, and fitness costs associated with resistance. One of the relevant factors controlling resistance evolution may also be the way in which resistance is inherited. Resistance to several systemic fungicides has been shown to be determined by a single major genetic locus (2,24). Resistance to other fungicides appears to be determined quantitatively (6,7).

The mode of inheritance of resistance has also been thought to correlate to the fungicide rather than the pathogen (18,20). The mode of inheritance of fungicide resistance may play a role in how rapidly resistance evolves in a pathogen population, and anecdotal field observations have supported this generality (4). One way this may be explained is that resistance conferred by single genetic loci, such as resistance to benomyl or metalaxyl, confer very high levels of resistance, which result in greater additive genetic variance relative to quantitatively determined resistances (24,25,27). This is because single-gene resistances are distributed into a limited number of discrete phenotypic classes, whereas quantitatively inherited resistances are distributed continuously. The rate at which resistance evolves in a population is directly proportional to the amount of additive genetic variance in resistance and thus, all other factors being equal, single gene resistances will have greater additive genetic variance and will evolve faster (13). Another reason quantitatively inherited resistances evolve more slowly is hypothesized fitness costs. It has been speculated (4) that quantitatively determined resistances may have greater overall fitness costs than single gene resistances because several resistance genes, each with an associated fitness cost, would confer a higher overall fitness cost than resistance determined by a single genetic factor. It must be emphasized, however, that although there has been speculation about fitness costs associated with sterol demethylation-inhibiting fungicide (DMI) resistance, there is no experimental data that has demonstrated such costs.

Resistances to the systemic fungicides benomyl and metalaxyl, which are generally thought to be qualitatively inherited (2,24), evolved to high frequencies in pathogen populations only a few

years after their introduction into agriculture (3,21). In contrast, resistance to DMIs has generally evolved more slowly, and differences between sensitive and resistant phenotypes (resistance factors) are much smaller than for benomyl or metalaxyl (10,25). Genetic studies of DMI resistance in *Erysiphe graminis* DC. f. sp. *hordei* Ém. Marchal revealed a continuous distribution of resistance phenotypes in the progeny, suggesting quantitative inheritance of resistance (6,7). Another indication that resistance may be quantitatively inherited is that continuous distribution of DMI resistance phenotypes is usually found when pathogen populations are sampled (19,22). Mean levels of resistance to DMIs have increased slowly in most plant/pathogen systems (19), and this has led to a belief that the apparently slower evolution of resistance to DMIs compared to other systemic fungicides is because resistance is inherited in a quantitative manner and that decreased fitness is associated with high levels of resistance (10). However, quantitative inheritance of resistance to DMIs in *E. g. hordei* contrasts markedly with a study by Stanis and Jones (25), who found that resistance to the DMI fenarimol in *Venturia inaequalis* was inherited at a single genetic locus.

In contrast to research with field-collected isolates, the genetics of resistance to DMIs has been investigated more thoroughly using laboratory-generated resistant mutants. Thirty mutants of *Nectria haematococca* var. *cucurbitae* that were resistant to triadimenol had mutations that all mapped to the same genetic locus when the resistant mutants were crossed (8). These mutations produced a large effect on the triadimenol resistance phenotype. Several mutants resistant to two other DMIs, imazalil and fenarimol, have been generated, and resistance has been mapped to eight genetic loci for each fungicide (8,27). Each of these loci provided a small increase in the resistance phenotype.

The contradictory results obtained in genetic studies of DMI resistance in field-collected pathogens led us to reexamine the question of inheritance of resistance to DMI fungicides. We chose *Pyrenophora teres* Drechs., which causes net blotch of barley, as our study organism, because resistance to the triazole DMI triadimenol evolved in field populations of *P. teres*, and because the fungus can be grown in vitro and induced to reproduce sexually in the laboratory. In contrast to the relatively slow evolution of resistance to DMIs observed in most plant pathogenic fungi (19), resistance to triadimenol in *P. teres* in New Zealand evolved rapidly, making triadimenol ineffective for disease control after only 3 yr of use (16). This indicated that some factors controlling the rate of evolution of resistance may be different in this fungus than in others that have been studied. One of these factors may be the way in which resistance is inherited. Therefore, our main objective was to determine the mode of inheritance of triadimenol resistance in *P. teres*. Our secondary objective was to compare this inheritance pattern to the inheritance of resistance to propiconazole, another triazole DMI, using progeny from the same crosses.

MATERIALS AND METHODS

Isolates of *P. teres*. Isolates of *P. teres* from three different geographic areas were used for making crosses. Isolates R3, GP_1, 7d, S_1, and 13Y of *P. teres* were obtained from Dr. E. Sheridan, Victoria University, Wellington, New Zealand. These were hyphal tip isolates collected from New Zealand barley fields that had been given triadimenol as a seed treatment. Isolates ND89-14, ND89-18, ONT102-1A, and ONT858-1B were single conidial isolates provided by Dr. B. Steffenson, North Dakota State University, Fargo, ND, United States; isolate PT-MN-85-41E1 was a single conidial isolate provided by Dr. A. Tekauz, Agriculture Canada, Winnipeg, Manitoba. Upon receipt of all the above isolates in our laboratory, they were induced to sporulate under near UV irradiation, and a single conidium was collected from each isolate. These single conidial isolates were denoted by the letter B following the isolate code. The isolates from North Dakota, United States (ND prefix), Ontario, Canada (ONT prefix), and Manitoba, Canada (MN prefix) were obtained from barley fields that had never been treated with triadimenol or any other fungicide.

The isolates were cultured routinely on malt extract agar (MEA), which consisted of 1.5% malt extract (William's Brewing Supplies, San Leandro, CA, United States) and 2% agar (Difco). For storage of the isolates, MEA was overlain with 1-cm^2 pieces of sterile filter paper. After mycelium had grown over the filter papers, they were peeled from the agar, placed in sterile coin envelopes, and dried in a closed plastic container with anhydrous $CaSO_4$; they were then stored at −20 C.

Triadimenol resistance phenotypes. The DMI triadimenol (Baytan) was provided as technical grade material by Mobay Chemical Corporation (Kansas City, MO, United States). The triadimenol resistance phenotypes of the above isolates were determined using a radial growth assay on MEA amended with several concentrations of triadimenol as described below. Isolates were retrieved from storage by placing small portions (approximately 4 mm^2) of dried filter paper on MEA plates. They were allowed to grow for 5–7 days at 25 C in the dark before mycelial plugs were obtained from the colony margin using a corkborer 4 mm in diameter and were inverted on MEA plates amended with six concentrations of triadimenol (0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 μg/ml) in 1% ethanol or with 1% ethanol only (control). Approximately 20 ml of MEA per plate was poured using a peristaltic pump; the MEA was stirred vigorously with a stir bar to keep the triadimenol evenly distributed. Each isolate was replicated two or three times on fungicide-amended and ethanol-only plates in each experiment. Plates were incubated at 25 C in the dark for 6 or 7 days, and the diameter of growth of the colonies on each plate was measured to the nearest millimeter by averaging two diameters taken at 90 degrees to each other and subtracting the diameter of the agar plug. The resistance phenotype of each isolate on each concentration of triadimenol was then expressed as the proportion of radial growth on the triadimenol-amended plates compared to growth on the control plates. We used this measure of resistance because it corrects for differences in growth rates between isolates. EC_{50} values (effective concentration to inhibit 50% of radial growth) were calculated for each isolate by regressing radial growth (as a proportion of the control) against log-transformed triadimenol concentrations and using the fitted regression line to estimate EC_{50} values. All six concentrations were used to determine EC_{50} values for the triadimenol-sensitive isolates, and the highest three concentrations were used to determine EC_{50}s of the triadimenol-resistant isolates. Confidence intervals for these EC_{50} values were determined by regressing log concentration against radial growth and calculating a prediction interval for each EC_{50} value (23).

The calculation of EC_{50}s is an extremely laborious process for large numbers of isolates; therefore, we wanted to determine whether a single concentration of triadimenol could be used to estimate EC_{50}s. The single concentration that was chosen was 10 μg/ml, which was approximately equal to the mean EC_{50} of all of the parental isolates (9.7 μg/ml) and provided maximum discrimination between sensitive and resistant isolates. A single concentration assay has been used previously to determine sensitivity to DMI fungicides and has been shown to correlate well to EC_{50} values; the optimum discriminatory dose was found to be approximately equal to the mean EC_{50} of the population (22). Progeny from each cross were analyzed together in a single experiment; replicated measurement of the resistance phenotype of some of the parental isolates in different experiments indicated that experiment-to-experiment variability in triadimenol resistance phenotype was negligible.

The correlation of radial growth at 10 μg/ml and EC_{50}s for the parental isolates was calculated using the nonparametric Spearman rank correlation method, because the data were bimodally distributed and not bivariate normal (23). The data for radial growth at 10 μg/ml were obtained in an experiment separate from that used to calculate the EC_{50}s and, therefore, represent independent observations for each variable. The correlation between triadimenol and propiconazole EC_{50}s of the parental isolates was calculated using the standard product moment correlation method and tested using Fisher's

z-transformation (23).

Propiconazole resistance phenotypes. Technical grade propiconazole (Tilt) was provided by Ciba-Geigy Corporation (Greensboro, NC, United States). Propiconazole resistance phenotypes of the parental isolates were determined using the same method as that described above for triadimenol. Five concentrations of propiconazole (0.01, 0.1, 0.3, 1.0, and 3.0 μ/ml) were used in the assay. EC_{50} values and corresponding prediction intervals were calculated in the same manner as described for triadimenol using the highest four concentrations of propiconazole. Isolates from storage were prepared as described above, except that they were plated on MEA containing 0.3 μg/ml propiconazole (solubilized in 0.3% ethanol) and on plates containing 0.3% ethanol only. This concentration was slightly lower than the mean EC_{50} value of propiconazole resistance for all of the isolates used as parents. Radial growth was measured as described above, and the resistance phenotypes of the isolates was expressed directly as percent radial growth of the control. Progeny from each cross were analyzed together in a single experiment.

Crosses of *P. teres*. Crosses between triadimenol-resistant (R) and triadimenol-sensitive (S) isolates were made using sterile barley straw on Sach's agar (R. D. Cartwright and R. K. Webster, *personal communication*). Conidia were produced by growing the isolates on 25% V8 juice agar under near UV irradiation (GE black light #F40BL) with an alternating photoperiod (10 h of light, 14 h of dark) at 20 C for 14 days. The colonies were flooded with sterile water and scraped to remove conidia and mycelium, and the resulting suspension was poured into 15-ml sterile plastic tubes. Conidia and mycelium were sedimented in a Beckman GP centrifuge (Beckman Instruments, Palo Alto, CA, United States) at 1,500 *g* for 3 min, and the supernatant was decanted. Conidia and mycelial fragments were resuspended in sterile water and adjusted to a concentration of approximately 20,000 conidia per milliliter using a haemocytometer. One hundred microliters of conidial suspension of each parental isolate was placed on pieces of sterile barley straw overlaying Sach's agar (26) in 9-mm plastic petri plates. Crosses were made between triadimenol-resistant and sensitive isolates in all combinations. Each isolate was also selfed to determine if the isolates we used were heterothallic as previously reported for this fungus (12). Crosses were incubated at 15 C in the dark for 6–16 wk, during which time fertile pseudothecia developed. Random ascospore samples were obtained by crushing from two to five fertile pseudothecia per cross to liberate the ascospores. The ascospores were then separated on 4% water agar and allowed to germinate. Single ascospores were placed on MEA plates with filter papers for storage as described above.

Before each triadimenol or propiconazole assay, ascospore isolates were retrieved from storage, plated on MEA, and grown for 5–7 days in the dark at 25 C. The resistance phenotypes of the progeny were determined using the single-concentration assay described above. Each ascospore isolate was replicated once, and parental isolates were replicated 10 times for the analysis of each cross and assayed at the same time as their progeny. Each cross was analyzed in a separate experiment.

Estimated number of genetic loci controlling resistance. The number of minor genetic loci segregating for triadimenol resistance and the number of genetic loci segregating for propiconazole resistance in the crosses was estimated using the method of Caten (1) from Mather and Jinks (10). This method requires estimation of the genetic variance in resistance phenotype between the progeny from each cross. To accomplish this, each progeny isolate was replicated once to obtain an estimate of the variance within the progeny (environmental or assay variance). Genetic and environmental sources of variance in resistance phenotype were partitioned using a nested ANOVA and the genetic variance in resistance phenotype for each cross determined from the appropriate variance components. Expected mean squares were obtained from a model proposed by Caten (1) for the analysis of F_1 progeny from a biparental cross of haploid fungi. The significance of genetic variance in each cross was tested by calculating a standard *F*-statistic using MINITAB 7.1 statistical software (Minitab Inc., State College, PA, United States). Heritabilities of triadimenol and propiconazole resistance for each cross were calculated by dividing the genetic variance by the total phenotypic variance (genetic plus environmental variance). The number of effective genetic factors (k) was estimated as:

$$k = \frac{(P_H - P_L)^2}{4(V)_G} \quad (1)$$

where P_H and P_L are the highest and lowest progeny resistance phenotypes, respectively, and V_G is the genetic variance in resistance. To use this estimate of the number of effective factors, we assumed no linkage between loci controlling the resistance phenotype and that the resistance loci all have equal and additive effects. This estimate is progeny sample size-dependent, because more extreme progeny genotypes are represented as the sample size increases.

RESULTS

Triadimenol and propiconazole resistance phenotypes of the parents. R and S isolates were readily distinguished when grown on a series of triadimenol concentrations (Fig. 1). EC_{50} values estimated from these curves using interpolation from fitted values in the linear regression are presented in Table 1. The mean EC_{50} values for the sensitive and resistant isolates were 1.4 and 17.9 μg/ml, respectively. Radial growth on 10 μg/ml triadimenol was highly correlated ($r_s = 0.82$, $P < 0.001$) to EC_{50} value determined using six concentrations of triadimenol (Fig. 2). Thus, we are confident that radial growth on a single concentration of triadimenol can be used in place of EC_{50} as a measure of resistance phenotype in this fungus.

In contrast to the results with triadimenol, the propiconazole resistance phenotypes of the parental isolates were not bimodally

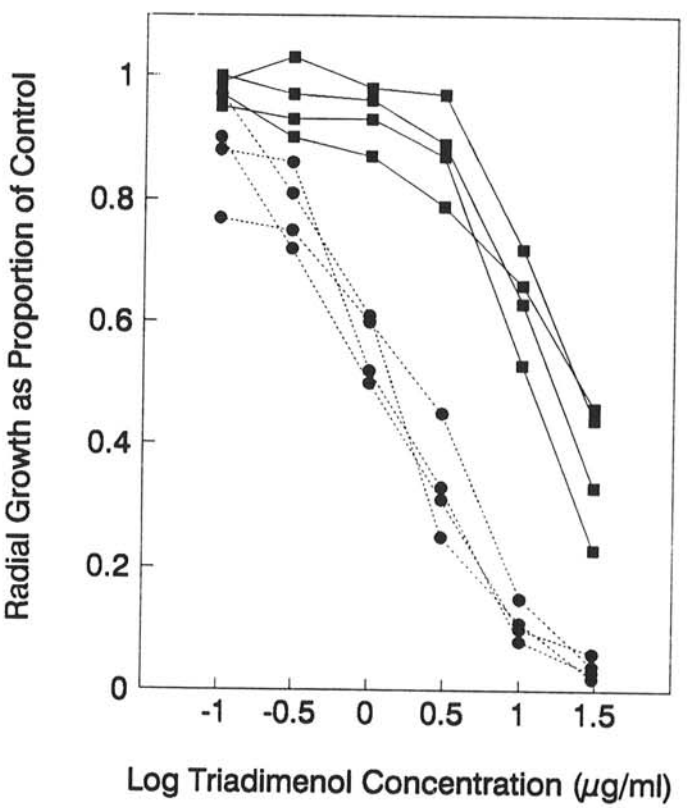

Fig. 1. Radial growth of triadimenol-sensitive (dashed lines) and triadimenol-resistant (solid lines) parental isolates of *Pyrenophora teres* on six concentrations of triadimenol. Growth measurements were made after 6 days growth on triadimenol-amended malt extract agar. Each point represents the mean of three replicates.

distributed (Table 1). Propiconazole EC_{50}s were approximately 20 times lower than the triadimenol EC_{50}s. The mean propiconazole resistance phenotype of the triadimenol-sensitive parents was not significantly different than the mean of the triadimenol-resistant parents ($P = 0.085$, t test). The correlation of triadimenol and propiconazole EC_{50}s for the parental isolates was 0.45, which was significantly different from 1 but not from 0 ($P < 0.01$).

Triadimenol resistance phenotypes of the progeny. Ascospore progeny were obtained from crosses between *P. teres* isolates both from the same and different geographical locations. This result demonstrates a lack of fertility barriers among *P. teres* isolates obtained in Canada, New Zealand, and the United States. Many of the crosses did not produce ascospores, despite the fact that the parents were of opposite mating type and produced ascospores when paired with other isolates. None of the selfed isolates produced any fertile pseudothecia.

In five of six S × R crosses, the progeny segregated into two discrete distributions in 1:1 ratios (Table 2). Four progeny distributions from S × R crosses are shown in Figure 3. An F_1 segregation ratio of 1:1 is expected for a single major gene in a haploid organism. Progeny from one cross (ND89-18-B × GP_1-B) segregated into two discrete distributions but in a ratio closer to 2:1 than 1:1 (Fig. 3D and Table 2). However, some progeny in the upper tail of the sensitive distribution of this cross were much more resistant than for any of the sensitive progeny from other crosses. If a resistance phenotype of 0.4 (midpoint of the parents) is taken as the cutoff value between the S and R classes, progeny from this cross also segregate in a 1:1 ratio (ratio 21:33, $P = 0.10$). Progeny from two R × R crosses were all resistant (Fig. 4A and B and Table 2). One cross was made between two sensitive isolates, and all the progeny from this cross were sensitive (Fig. 4C).

Segregation of additional genetic loci that contribute to the triadimenol resistance phenotype was observed in all the crosses analyzed. Since the method for estimating the number of effective factors was developed for characters that segregate continuously in a cross, we used it only for the resistant distributions of progeny from the S × R and R × R crosses. An ANOVA table for a representative cross, in which variance in triadimenol resistance phenotype is partitioned, is shown in Table 3. All except one cross had highly significant ($P < 0.001$) variance in triadimenol resistance phenotype between the progeny, which indicates the segregation of loci influencing resistance phenotype. Heritabilities of triadimenol resistance ranged from 0.34 to 0.95 with a mean heritability of 0.73. When k was estimated using triadimenol-resistant progeny from four S × R crosses and two R × R crosses, it was determined that between three and five loci influencing resistance phenotype were segregating in all but two of the crosses (Table 4). In two S × R crosses (S_1-B × R3-B and ND89-18-B × ND89-14-B), it was estimated that 6.5 and 12 loci, respectively, influenced resistance phenotype. Although the estimates of k appear to be quite variable, they demonstrate that other loci also influence the resistance phenotype in addition to the single major locus segregating in the S × R crosses. These loci will be termed *minor genes*, because they have a smaller effect on the resistance phenotype than the major gene that was observed to be segregating in the S × R crosses.

Propiconazole resistance phenotypes of the progeny. Progeny from four of the crosses that were used to determine the inheritance of resistance to triadimenol were used to determine the inheritance of propiconazole resistance. Different numbers of progeny were analyzed for some of the crosses between experiments because of the subsequent collection of more ascospores from one cross (7d-B × R3-B) and the loss of ascospore isolates in storage from others (S_1-B × R3-B and 13Y-B × 7d-B). Note that the differences between the resistance phenotypes of the parents were smaller on propiconazole than on triadimenol (Fig. 5). The progeny phenotypes were distributed continuously for each of the crosses. In contrast to the 1:1 segregation observed for triadimenol resistance, no single major locus appeared to be segregating in these crosses (Fig. 5).

The number of effective propiconazole resistance factors segregating in these crosses was determined in the same way as for triadimenol (Table 5). Highly significant levels of genetic variance in propiconazole resistance phenotype were detected between

TABLE 1. Triadimenol and propiconazole EC_{50} values of *Pyrenophora teres* isolates used as parents in crosses

	EC_{50} (μg/ml)	
	Triadimenol	Propiconazole
ND89-18-B	1.4 (0.6, 3.5)[a]	0.5 (0.3, 0.9)
S_1-B	1.1 (0.5, 2.4)	0.1 (0.0, 0.3)
7d-B	1.3 (0.5, 3.5)	0.3 (0.1, 0.8)
13Y-B	2.0 (0.9, 4.6)	0.5 (0.2, 1.2)
ONT858-1B-B	1.3 (0.3, 5.3)	0.5 (0.3, 1.0)
ND89-14-B	15.7 (9.0, 26.9)	0.4 (0.3, 0.8)
R3-B	11.4 (6.3, 20.0)	0.6 (0.3, 1.3)
GP_1-B	25.5 (15.2, 40.8)	0.5 (0.2, 1.5)
ONT102-1A-B	24.0 (16.2, 34.5)	0.6 (0.3, 1.1)
PT-MN-85-41E1-B	12.9 (7.6, 20.9)	0.8 (0.5, 1.2)

[a]Numbers in parenthesis indicate 95% prediction intervals for a single observation determined using the regression of log concentration against radial growth (proportion of control).

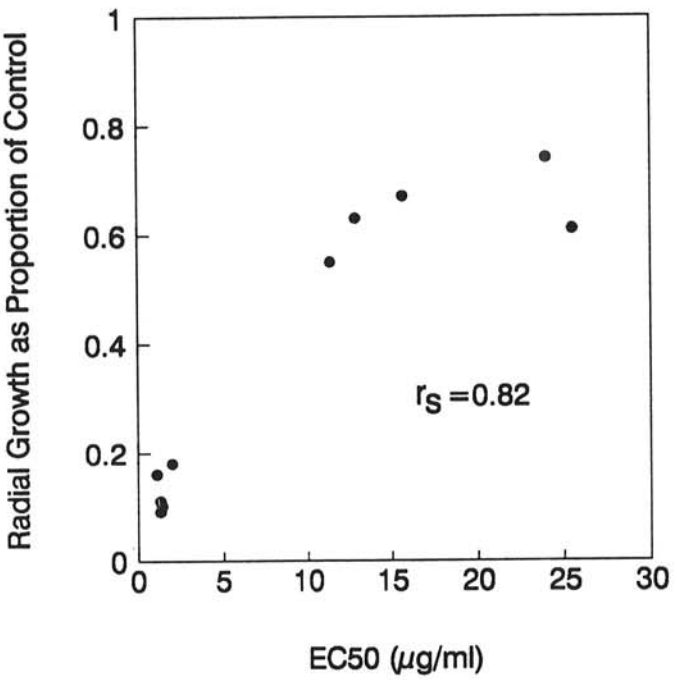

Fig. 2. Radial growth on 10 μg/ml triadimenol versus EC_{50} value for 10 isolates of *P. teres* used as parents in crosses. The correlation coefficient ($r_s = 0.82$) was obtained using Spearman's rank correlation method.

TABLE 2. Summary of segregation ratios, chi-square values, and probabilities for progeny distributions of crosses between *Pyrenophora teres* isolates on 10 μg/ml triadimenol

Cross	Parental isolates	Ratio (S:R)	χ^2	Probability[a]
S × R	ND89-18-B × ND89-14-B	44:48	0.64	0.42
	ND89-18-B × GP_1-B	36:18	6.00	0.02
	S_1-B × R3-B	22:17	0.17	0.68
	7d-B × R3-B	32:20	2.77	0.09
	ONT858-1B-B × ONT102-1A-B	24:21	0.20	0.65
	ND89-18-B × PT-MN-85-41E1	30:22	1.23	0.27
R × R	GP_1-B × ONT102-1A-B	0:55	...[b]	...
	ND89-14-B × R3-B	0:42	...	...
S × S	13Y-B × 7d-B	46:0	...	...

[a]Probability of a greater χ^2 value (1 df) under the null hypothesis of 1:1 segregation.
[b]Not calculated.

progeny from all crosses ($P < 0.001$). The heritability of propiconazole resistance was very high, with a mean value of 0.95. Between three and six loci were estimated to be controlling propiconazole resistance phenotype.

DISCUSSION

Crosses between isolates of *P. teres* have revealed that triadimenol resistance is inherited at a single major locus. Crosses between *P. teres* isolates from different geographical areas have demonstrated a lack of fertility barriers among isolates from these areas and that resistance to triadimenol is conferred by alleles at the same locus. Resistance to propiconazole, another triazole DMI, appeared to be inherited quantitatively. This difference in

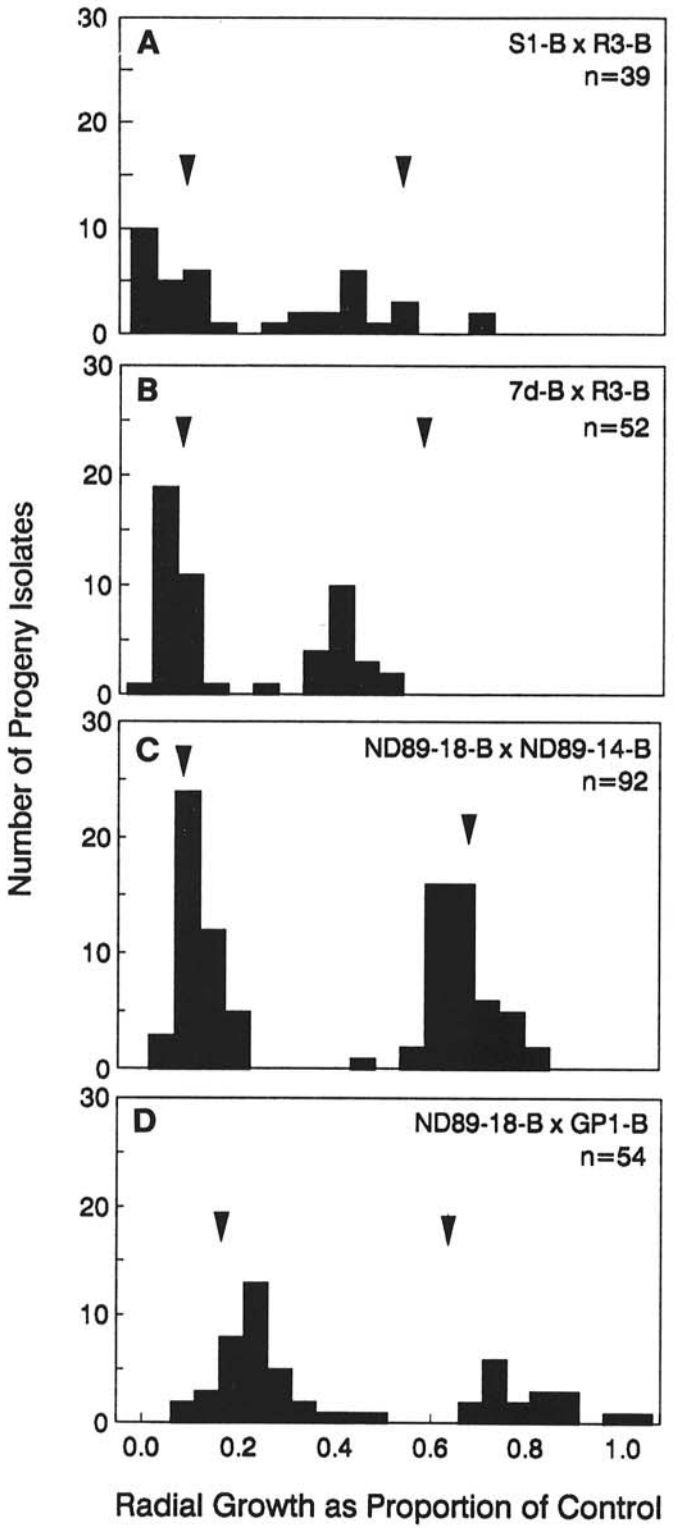

Fig. 3. Distributions of radial growth on 10 μg/ml triadimenol for progeny from four representative crosses between triadimenol-sensitive and resistant isolates of *P. teres*. Arrows indicate the mean resistance phenotypes of the parents, and *n* is the total number of progeny analyzed from each cross.

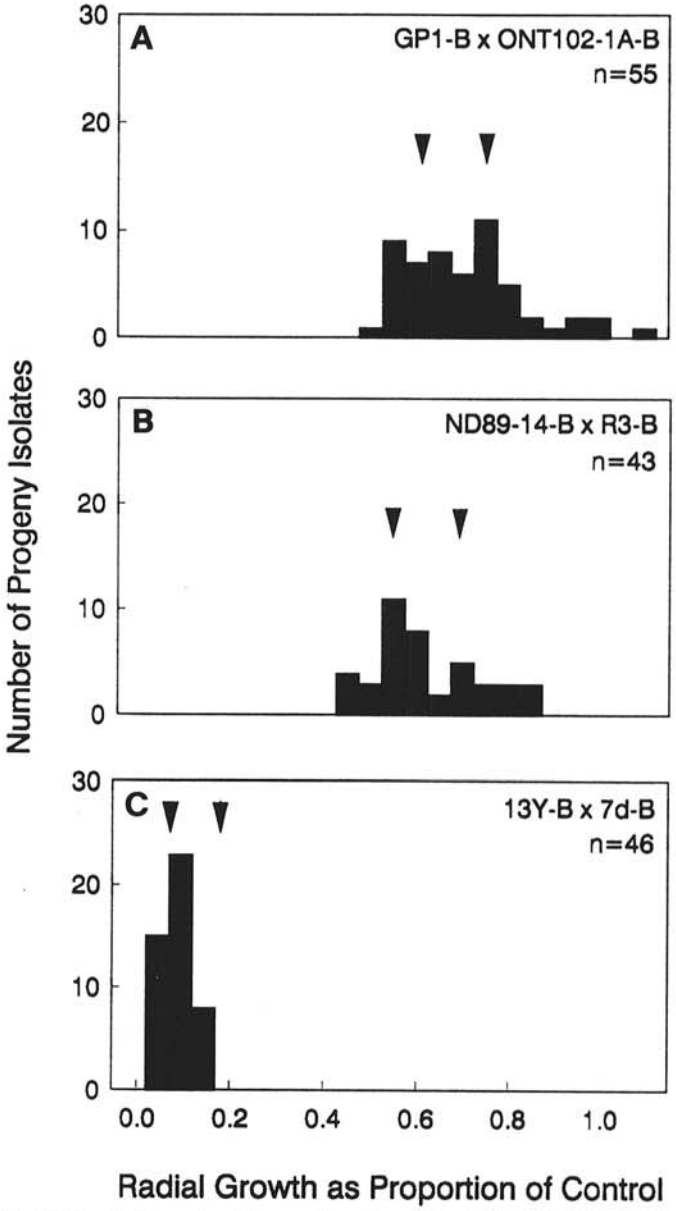

Fig. 4. Distributions of radial growth on 10 μg/ml triadimenol for progeny from **A** and **B**, two crosses between triadimenol-resistant isolates, and **C**, one cross between triadimenol-sensitive isolates of *P. teres*. Arrows indicate the mean resistance phenotypes of the parents and *n* is the total number of progeny analyzed from each cross.

TABLE 3. Representative ANOVA table showing sources of variation in triadimenol resistance phenotype of progeny from cross ND89-18-B × ND89-14-B. Expected mean squares from a nested model developed for analysis of F_1 progeny of a cross between haploid fungi[a]

Source	df	MS	*F*	*P*[b]	EMS[c]
Between progeny	47	0.0075	2.55	0.001	$\sigma^2_E + 2\sigma^2_G$
Replicates (progeny)	48	0.0029			σ^2_E
Total	95				

[a] Only triadimenol-resistant progeny were analyzed.
[b] Probability of a greater *F* value ($H_o: \sigma^2_G = 0$).
[c] Expected mean squares (σ^2_E = environmental or assay variance, σ^2_G = genetic variance).

inheritance pattern is interesting, considering that both of these fungicides belong to the triazole class of DMIs and have the same mode of action (9). Correlations between the parental EC_{50} values for triadimenol and propiconazole ($r = 0.45$) also indicate the lack of a clear cross-resistance relationship between these two DMIs in *P. teres*. This may mean that different genes control resistance to each DMI or that the locus for triadimenol resistance does not have a major phenotypic effect on propiconazole resistance. It is possible that the observed segregation pattern for triadimenol resistance is attributable to resistance loci that are closely linked. Single-progeny isolates were observed in two of the S × R crosses that had resistance phenotypes that were clearly intermediate between the sensitive and resistant progeny distributions. These intermediate types may represent rare segregants for an additional closely linked resistance locus.

The continuous segregation pattern observed for propiconazole resistance may be an artifact of the way in which the parents of the crosses were selected. The parents were selected based on their triadimenol resistance phenotypes, and the differences in parental resistance phenotypes for propiconazole were much smaller than for triadimenol. Therefore, it may be that the small sample of parental isolates used in the present study does not adequately represent the total variation for propiconazole resistance present in *P. teres* populations. Crossing isolates of *P. teres* with more extreme propiconazole resistance phenotypes will be necessary to definitively determine the inheritance of resistance to propiconazole. However, the present results indicate that a single model of the inheritance of resistance to different DMIs may not be possible, but rather that patterns of inheritance may vary depending upon the resistance phenotypes of the parents and the pathogen/DMI combination tested.

In addition to the major gene segregating for triadimenol resistance, minor genes affecting the resistance phenotype were also observed to be segregating in these crosses. These additional loci could be detected because estimates of the environmental or assay variance were available from replicates of each progeny isolate. Between three and five loci appear to be segregating for resistance to triadimenol and propiconazole in all but two of the crosses examined. The estimates of k for crosses S_1-B × R3-B and ND89-18-B × ND89-14-B were much higher than for any of the others. This may be explained by the fact that much higher levels of environmental variance were observed in these crosses than in the others, which resulted in a significant decrease in the estimate of genetic variance as a proportion of the total phenotypic variance and inflation of k. Because of the variability of the estimates of k and the restrictive assumptions necessary to estimate them, they must be considered only as rough approximations of the number of loci segregating in a cross. However, results of the ANOVA and the estimates of k indicate that, although there is a major genetic locus controlling triadimenol resistance, several other loci were segregating in these crosses, which contributed to the resistance phenotype. These additional loci must be considered when discussing the evolution of triadimenol resistance because they can modify the resistance phenotype of isolates considerably when recombined into a single isolate. They may also be significant in terms of fitness costs associated with resistance. If each resistance gene confers some costs, then fitness may be significantly affected by these minor genes. They may also control resistance to other DMI fungicides (cross-resistance) and would, therefore, prove important in resistance management schemes.

Crosses between triadimenol-resistant isolates from different parts of the world (Canada, New Zealand, and the United States) produced no sensitive progeny. This indicates that resistance in isolates from these various regions is attributable to alleles at the same genetic locus and that there was a lack of fertility barriers between populations from these regions. This lack of sexual barriers may mean that these *P. teres* populations have not been isolated geographically for very long, or that they are not isolated at all because of regular migration. *P. teres* can be seedborne (17), and global movement of the pathogen on seed has probably occurred in the recent past (16). The lack of ascospore production by any of the selfed isolates confirms previous results demonstrating heterothallism in this fungus (12) and indicates that the progeny obtained in this study were likely to be true hybrids. Unfortunately, mating type is very difficult to score in this fungus, and no other genetic markers are currently available that would have allowed us to screen for selfs directly.

Previously published data on the inheritance of resistance to DMIs in field-collected isolates of plant pathogens are contradictory. Resistance to fenarimol was qualitative in *V. inaequalis* (25), whereas resistances to ethirimol and triadimenol were quantitative in *E. g. hordei* (6,7). These three studies had methodological constraints that may have precluded accurate conclusions regarding the inheritance of resistance. Stanis and Jones (25) used a minimal inhibitory concentration assay (MIC), which was unable to detect progeny with intermediate resistance phenotypes, making it impossible to reject the hypothesis of quantitative inheritance of fenarimol resistance in *V. inaequalis*. Hollomon (6) measured germ tube length to determine the resistance phenotypes of *E. g. hordei* to ethirimol; this assay was later shown to be inappropriate for research with DMIs (14). Finally, the study of the inheritance of triadimenol resistance by Hollomon et al (7) was based on small numbers of progeny, very small numbers of crosses, and small differences in resistance phenotypes between the parents. These constraints are certainly understandable, given the difficulty of working with a pathogen such as *E. graminis*.

The importance of inheritance in controlling the rate of evolution of DMI resistance in a population of a plant pathogen will depend to a large extent upon how resistance is genetically controlled and upon the role of sexual reproduction in the biology of the pathogen. Frequencies of resistant genotypes in a population of a haploid organism will not change as a result of recombination for resistances inherited at a single locus. However, sexual recombination might change the distribution of resistant phenotypes considerably for resistances that are quantitatively inherited because of changes in epistasis and/or linkage disequilibrium (13,15). Since many plant pathogens have no known sexual stage

TABLE 4. Sources of variance in resistance phenotype and number of effective genetic factors segregating for triadimenol resistance in crosses between *Pyrenophora teres* isolates

Cross	Parental isolates	n[a]	σ^2_G[b]	σ^2_E[c]	h^2[d]	k[e]
S × R	7d-B × R3-B	20	0.00161*	0.00911	0.64	3.0
S × R	S_1-B × R3-B	17	0.00498^{NS}	0.00976	0.34	6.5
S × R	ND89-18-B × ND89-14-B	48	0.00227*	0.00293	0.44	12.0
S × R	ND89-18-B × GP_1-B	18	0.00886**	0.00189	0.82	3.7
S × R	ONT858-1B-B × ONT102-1A-B	21	0.00674**	0.00128	0.84	4.0
S × R	ND89-18-B × PT-MN-85-41E1	22	0.01016**	0.00161	0.86	3.9
R × R	GP_1-B × ONT102-1A-B	55	0.00173**	0.00093	0.95	4.9
R × R	ND89-114-B × R3-B	42	0.01249**	0.00116	0.92	3.7

[a] Number of triadimenol-resistant progeny analyzed from each cross.
[b] Genetic variance (* $P = 0.001$, ** $P < 0.001$, ^{NS} $P = 0.08$).
[c] Environmental or assay variance.
[d] Heritability = $\sigma^2_G/(\sigma^2_G + \sigma^2_E)$.
[e] Number of effective genetic factors calculated using Equation 1 in text.

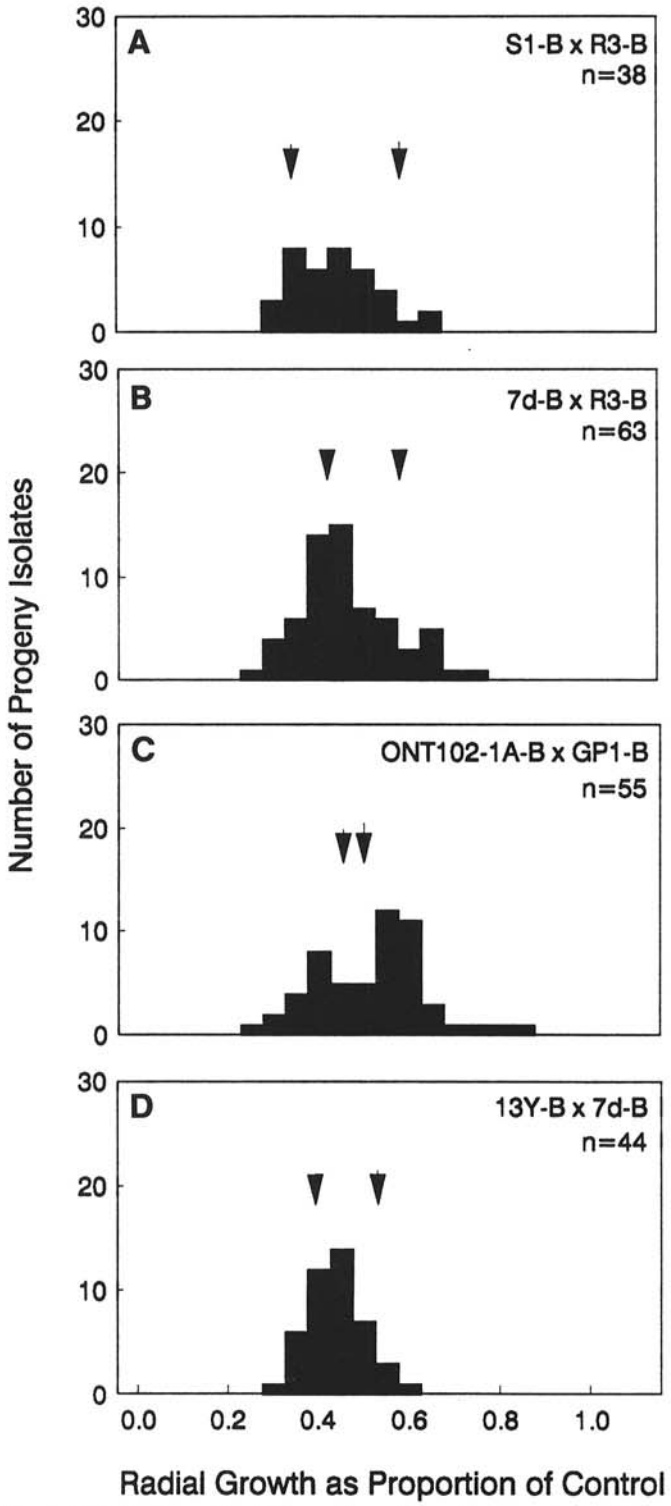

Fig. 5. Distributions of radial growth on 0.3 μg/ml propiconazole for progeny from four crosses between isolates of *P. teres*. Arrows indicate the mean resistance phenotypes of the parents, and *n* is the total number of progeny analyzed from each cross. (Crosses shown in **A** and **B** are the same as those in Fig. 3A and B.)

or have a sexual stage that occurs only once a year, it is difficult to generalize the importance of sexual versus asexual reproduction in the evolution of resistance. Even though sexual reproduction may occur only once a year, it still might be significant in increasing the genetic variance in resistance that is then selected in subsequent asexual generations. With exclusively asexual reproduction, the inheritance of resistance is irrelevant, as there is no recombination among clones, and genotypes are preserved intact from one generation to the next.

TABLE 5. Sources of variation in resistance phenotype and number of effective genetic factors segregating for propiconazole resistance in crosses between *Pyrenophora teres* isolates

Parental isolates	n^a	$\sigma^2_G{}^b$	$\sigma^2_E{}^c$	h^{2d}	k^e
7d-B × R3-B	63	0.01032*	0.00078	0.93	5.6
S_1-B × R3-B	38	0.00837*	0.00044	0.95	3.5
GP_1-B × ONT102-1A-B	55	0.01497*	0.00043	0.97	5.8
13Y-B × 7d-B	44	0.00392*	0.00021	0.95	4.3

[a]Number of progeny analyzed from each cross.
[b]Genetic variance (* $P < 0.001$).
[c]Environmental or assay variance.
[d]Heritability = $\sigma^2_G/(\sigma^2_G + \sigma^2_E)$.
[e]Number of effective genetic factors calculated using equation 1 in text.

Resistance to triadimenol is controlled by a single, major genetic locus and by several additional loci. Therefore, it is difficult to predict the effect this inheritance pattern might have on the evolution of resistance to triadimenol in populations of *P. teres*. The result may largely depend upon whether or not the minor genes controlling resistance have significant effects on fitness, and presently we do not know what phenotypic effects these loci have in vivo. The sexual stage of *P. teres* has been found in several parts of the world, but whether or not it is important epidemiologically is a matter of debate (17). In regions where sexual reproduction occurs, *P. teres* probably undergoes one sexual generation per year, followed by several asexual generations during the growing season of the crop. The sexual stage may, therefore, be important in generating variation for triadimenol resistance, which is then selected by fungicide application during the growing season. On the other hand, sexual reproduction may also disrupt highly resistant multilocus genotypes selected in the previous season. A model of the evolution of quantitatively controlled fungicide resistance has demonstrated that the effect of periodic sexual reproduction on the evolution of resistance depends to a large extent on the heritability of resistance (15). The mean heritability of triadimenol resistance calculated using the present crosses was 0.73; therefore, a seasonal sexual cycle might be expected to have little effect on the evolution of resistance. The sexual stage has not been reported from New Zealand (17), and, consequently, we do not know whether it was a factor in the rapid evolution of resistance to triadimenol observed. The rapid evolution of resistance in New Zealand may have been largely attributable to the large variance in triadimenol resistance present in *P. teres* populations before the use of triadimenol and to strong selection for resistant isolates imposed by triadimenol treatment.

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