Sensitivity to Azoxystrobin Among Isolates of *Uncinula necator*: Baseline Distribution and Relationship to Myclobutanil Sensitivity

Francis P. Wong and **Wayne F. Wilcox,** Department of Plant Pathology, Cornell University, New York Agricultural Experiment Station, Geneva 14456

ABSTRACT

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Two hundred fifty-six single-conidial chain isolates of *Uncinula necator* were assayed for their sensitivity to azoxystrobin and myclobutanil. These isolates were collected from two sites in New York in 1999: an "organic" vineyard where no synthetic fungicides have been used (baseline population) and a commercial vineyard having a history of compromised powdery mildew control with myclobutanil (demethylation inhibitor [DMI]-resistant population). Mean coefficients of variance for a leaf disk assay used to test fungicide sensitivities were 31% for azoxystrobin and 41% for myclobutanil. Baseline ED_{50} values ranged from 0.0037 to 0.028 μg/ml (mean 0.0097 μg/ml) for azoxystrobin and from 0.0049 to 0.69 μg/ml (mean 0.075 μg/ml) for myclobutanil. A shift in the mean ED_{50} value for azoxystrobin to 0.018 μg/ml was observed in the DMI-resistant population; with the strongest shift observed for isolates collected from vines treated exclusively with myclobutanil (0.024 μg/ml). For the 256 tested isolates, there was a moderate, but statistically significant, correlation between azoxystrobin and myclobutanil sensitivities (R^2 = 0.36, $P < 0.001$). Tests with three other strobilurin fungicides (kresoxim-methyl, pyraclostrobin, and trifloxystrobin) indicate clear differences in the intrinsic activity of these compounds against *U. necator*, and the applicability of the methods developed with azoxystrobin for assays with pyraclostrobin and trifloxystrobin. Isolates from the high and low ends of the azoxystrobin sensitivity distribution (15× difference in mean ED_{50} values) were equally controlled in planta by protectant or postinfection treatment with azoxystrobin at 250 μg a.i./ml, but postinfection application at lower rates (2.5 and 25 μg a.i./ml) resulted in a 41 and 44% decrease, respectively, in the control of the low-sensitivity isolates versus high-sensitivity isolates. The results of this study document the baseline sensitivity distribution of *U. necator* to azoxystrobin, provide evidence of partial cross-sensitivity between azoxystrobin and myclobutanil, and illustrate the potential selection for individuals with reduced sensitivity (quantitative range) to azoxystrobin by postinfection application and reduced rates of this fungicide.

Grapevine powdery mildew, caused by the obligate biotrophic ascomycete *Uncinula necator* (Schwein.) Burrill, is a major fungal disease of grapevine worldwide (31). Without the implementation of control measures, primarily the regular application of fungicides, this disease can cause severe losses in yield and fruit quality. The strobilurin fungicides are part of the Q_oI class of fungicides, which inhibit mitochondrial respiration by blocking electron flow through the electron transport chain by binding to the Q_o site of the cytochrome bc_1 complex (17). Several strobilurin fungicides have been registered recently or are currently under development for the control of *U. necator*. These materials are unique in the sense that they are the first site-specific crop protection compounds that are effective against *Ascomycetes, Basidiomycetes,* and *Oomycetes* (1–3,15,18,27,28,39).

Corresponding author: F. P. Wong
E-mail: frank.wong@ucr.edu

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A number of species of plant-pathogenic fungi and oomycetes developed practical resistance to the strobilurin fungicides soon (one to three seasons) after their use became widespread, including *Blumeria graminis*, *Mycosphaerella fijiensis*, *Plasmopara viticola*, *Pseudoperonospora cubensis*, *Sphaerotheca fuliginea*, and *Venturia inaequalis* (11,12,17). At least two potential mechanisms of resistance to strobilurins have been demonstrated for fungi in vitro, target site mutations in cytochrome bc_1 (5,8,25,41) and the induction of alternative respiration (41,42). However, the exact mechanism has not been confirmed for all of the aforementioned cases of practical resistance.

One of the first commercial strobilurin fungicides, azoxystrobin, was labeled for use on grapevine in the United States in 1997 and has been shown to be highly effective for the control of *U. necator* and other fungal pathogens of this crop, including the oomycete *P. viticola* (37,38). In order to minimize the risk of resistance development, product labels for azoxystrobin and other strobilurin fungicides specify that these materials be used a limited number of times during a growing season, mandating their rotation with fungicides that have different modes of action. For *U. necator*, the sterol demethylation inhibitors (DMIs) represent one such rotational partner. The DMIs have been a cornerstone of grapevine powdery mildew management since the early 1980s, and although shifts toward insensitivity and practical resistance have occurred in some vineyards (10,16,40), they still provide adequate control of the disease in many others. In New York, azoxystrobin and DMI fungicides are often used together in rotational programs to manage both grapevine powdery mildew and the development of resistance to each group of compounds.

Recent developments in fungicide resistance research have suggested the need for a closer examination of the relationship between DMI and strobilurin sensitivities. First, studies with *V. inaequalis* in New York (W. Köller and W. F. Wilcox, *unpublished*) have indicated that strobilurin and DMI sensitivities are not completely independent traits for this pathogen. Second, related work on the interaction of fenarimol and dodine also indicated that there is an unexpectedly positive relationship between sensitivities to these two unrelated fungicides in *V. inaequalis* (21,24). There is also recent evidence in *V. inaequalis* that there is a potential predisposition of fungicide resistance to develop more rapidly to multiple classes of chemistries (22). Third, recent investigations of multidrug resistance in fungi have indicated that cross-resistance can develop to unrelated compounds (6,7). Finally, there have been anecdotal observations that strobilurin resistance developed first in pathogens that had been heavily exposed to DMI fungicides. If there is a sufficient lack of independence for DMI and strobilurin sensitivities, then resistance-management practices could be compromised, and current fungicide-use strategies would have to be reconsidered.

Therefore, the broad objective of this study was to examine the relationship between sensitivities to strobilurin and DMI fungicides within *U. necator* populations, using azoxystrobin and myclobutanil, respectively, as representative compounds, and to relate these findings to practical resistance-management programs. Specific sub-objectives were to (i) develop a method for assaying the sensitivity of *U. necator* isolates to azoxystrobin, (ii) use the method to determine the baseline sensi-

tivity distribution for this pathogen-fungicide combination, and (iii) explore the utility of this method with respect to examining the sensitivity of *U. necator* to other strobilurin fungicides.

MATERIALS AND METHODS

Fungicides. Technical grade azoxystrobin (98% active, Syngenta Crop Protection, Greensboro, NC), kresoxim-methyl (93% active, BASF Corporation, Research Triangle Park, NC), myclobutanil (93% active, Dow AgroSciences, Indianapolis, IN), pyraclostrobin (93% active, BASF Corporation) and trifloxystrobin (96% active, Bayer Corporation, Kansas City, KS) were used for this study unless otherwise noted. The fungicides were dissolved in acetone to provide stock solutions containing 10 mg/ml of each fungicide. All stock solutions were stored at –20°C in 2-ml cryovials (Fisher, Pittsburgh, PA) sealed with Parafilm.

For field and greenhouse studies, the fungicides were applied as formulated product: Nova 40W (40% active, Dow AgroSciences) and Abound 2.08F (23% active, Syngenta Crop Protection).

***U. necator* populations.** Two sites were chosen for this study, and *U. necator* populations from both had been characterized in 1995 for myclobutanil sensitivity (10). The first site was an "organic" vineyard in an isolated location in Burdett, NY, where neither DMI nor strobilurin fungicides have been used. *U. necator* isolates from this site were considered representative for baseline populations with respect to both fungicides, and for the purpose of this study, this vineyard was considered the "baseline site." The second site was a commercial vineyard with a long history of DMI use and high frequency of DMI-resistant *U. necator* isolates (10). For the purpose of this study, this was referred to as a "DMI-resistant site." Since 1996, a number of experimental fungicide spray programs had been imposed there to assess their effects on further shifts toward fungicide insensitivity within the *U. necator* population (35). For this study, samples were collected from three such experimentally imposed treatments: (i) no fungicides throughout the growing season, (ii) six applications of myclobutanil (112 g a.i./ha); and (iii) four applications of azoxystrobin (200 g a.i./ha) alternated with two of myclobutanil (112 g a.i./ha) (Table 1). Fungicide applications were initiated at approximately 2 weeks prebloom and continued at 2-week intervals until veraison. Commercial fungicide formulations were applied with a hooded-boom sprayer at 207 kPa in a volume of 467 liters/ha for the two prebloom sprays and 936 liters/ha thereafter. Each treatment was replicated three times in randomized complete blocks, with each individual plot consisting of five rows of 15 vines. Disease was assessed 1 to 2 weeks before harvest (September) based upon disease severity as measured by the percentage area infected of 25 grape clusters from the center of each replicated plot. Severity ratings were made using the Barrett-Horsfall scale before conversion to percentage cluster area infected.

Collection and maintenance of isolates. In late-August of 1999, approximately 150 infected leaves were sampled arbitrarily from a block of approximately 1,000 *Vitis* × *labruscana* cv. Concord vines at the baseline site. Sixty-eight single-chain isolates were obtained from this site, as described below.

In September of 1999, approximately 50 infected leaves were sampled from each replicate plot of the three experimentally imposed fungicide treatments located at the DMI-resistant site, 2 weeks after the last fungicide application. To minimize inter-plot interference, samples were taken from the young, terminal leaves of the nine center vines within the three middle rows of each plot, then approximately 60 single-chain isolates were obtained for each of the treatments (Table 1) and maintained subsequently, as described below.

During the sampling procedure, young, infected terminal leaves were placed individually into small plastic bags, transported to the lab, and incubated for 1 day in humid chambers to promote additional sporulation. Sporulating colonies were excised from the leaves and placed into 60-mm petri dishes containing approximately 15 ml of 1.5% water agar (Difco, Detroit, MI). Approximately 3 days later, single-conidial chain isolates were obtained by transferring a single chain of conidia to 12-mm-diameter, surface-disinfested *Vitis vinifera* cv. Chardonnay leaf disks with the adaxial surface exposed, using a single camel hair attached to a 15-mm disposable glass Pasteur pipette with paraffin wax. Transfer tools were disinfested by placing them in 70% ethanol for 10 min, then 95% ethanol for 30 s, and were thoroughly air-dried before use. Transfers were made with the aid of a dissecting microscope. Leaf disks (12 mm diameter) were prepared as described previously (10,37), with the exception of surface disinfesting leaves for 30 s in a solution of calcium hypochlorite (0.86 g/liter) instead of ethanol (10), or sodium hypochlorite and Tween 20 (37). Vines were grown in a greenhouse that was maintained at 37°C to reduce contamination by *U. necator* (9,40). Only the highly susceptible, expanding, translucent leaves from leaf positions 2 and 3 distal to the shoot tip were used, as *U. necator* infection and growth on these are more uniform than on older leaves (9,10). Inoculated leaf disks were incubated at ambient room temperature (20 to 24°C) with alternating 12-h periods of darkness and fluorescent light.

Approximately 7 to 10 days later, a single-conidial chain from a newly sporulating colony was transferred to a new set of leaf disks (prepared as described above) to obtain a single-chain isolate. For each isolate, the procedure was conducted three times in succession over three generations of the fungus to ensure the purity of the single-chain isolates. A total of 256 single-chain isolates was obtained in this way (Table 1), and the isolates were maintained by biweekly transfers of several chains of conidia to freshly prepared leaf disks, which were maintained as described previously.

Fungicide sensitivity assays. Assays were based upon the technique described in detail by Erickson and Wilcox (10). Briefly, Chardonnay leaf disks were prepared as described above, taking care to excise them from areas of the leaves without major veins (≥1 mm wide), to minimize any radial distortion of the expanding *U. necator* colonies. Excised disks were randomized and placed into glass beakers containing azoxystrobin or myclobutanil solutions ranging from 0.00049 to 8.0 µg/ml at fourfold dilutions, in type I H_2O (HPLC-grade nanofiltered sterile water), or type I H_2O only (check treatment). Solutions were derived from technical grade fungicides dissolved in acetone, and acetone concentrations (1×10^{-5} to 0.8 mg/ml) were not normalized, as acetone concentrations of ≤5 mg/ml were shown previously (10) not to affect the growth of *U. necator* under these conditions.

Leaf disks were submerged in the solutions for 1 h, with regular agitation, then they were blotted dry between clean paper towels. Four disks per fungicide treatment were placed, with the adaxial surface exposed, into 60-mm petri dishes containing approximately 15 ml of 1.5% water agar. After incubation overnight at ambient room temperature, each disk in a petri dish was inoculated at a single point with two to three conidial chains (approximately 15 to 30 conidia) from an individual *U. necator* isolate. Inoculated leaf disks were incu-

Table 1. Origin and size of samples of *Uncinula necator* isolates

Population	Group	Treatment	Timing[z]	No. of isolates
Baseline	Bl	None	…	68
DMI-resistant	Em	Myclobutanil (112 g a.i./ha)	1–6	69
	Ea	Myclobutanil (112 g a.i./ha)	1, 4	58
		Azoxystrobin (200 g a.i./ha)	2, 3, 5, 6	
	Ec	None	…	61

[z] Fungicide applications were initiated approximately 2 weeks before bloom in mid-June, and applied thereafter at 2-week intervals.

bated at ambient room temperature (20 to 25°C) with alternating 12-h periods of fluorescent light and darkness.

Seven days after inoculation, mycelial expansion from the point of inoculation on each leaf disk was determined using a 32× ocular micrometer and a stereomicroscope. Three measurements of radial expansion were made for each of the roughly circular colonies, and the mean values for the four replicate leaf disks per treatment were used to calculate the relative growth (RG values, average radial expansion of the check treatment) for each isolate × concentration combination. Dose response curves for individual isolates were generated by plotting the RG values against the $\log_{10}$ of the fungicide concentration used (Microsoft Excel 7.0, Seattle, WA), and the $\log_{10}$ effective doses for 50% growth inhibition of mycelial growth ($\log_{10}$ ED_{50} values) were calculated from the regression equation generated through the linear portion of the sigmoidal curve.

Assay reproducibility. To determine the reproducibility of the test, azoxystrobin and myclobutanil sensitivities were assayed five times for each of six isolates. Three isolates were chosen from both the baseline and DMI-resistant sites, to best represent the range of fungicide sensitivities among the sample population. For each repeat of the test, in which all six isolates were assayed, a new stock solution of either azoxystrobin or myclobutanil was prepared. The mean of the ED_{50} values, variance, coefficient of variance, and 95% confidence intervals were calculated for each isolate based upon the five replicated tests. Since ED_{50} values are typically log-normally distributed, the appropriate formulas (4,13) were used to calculate these values using the natural log of the ED_{50} values (Table 2). A mean coefficient of variance was calculated for both the azoxystrobin and myclobutanil assays, based upon the results from these six isolates. The least significant difference ($\alpha = 0.05$) was calculated based upon the mean $\log_{10}$ ED_{50} values for the six isolates using single-factor analysis of variance and Fisher's LSD (Minitab, v. 12.10, State College, PA).

Distributions of U. necator *sensitivities to azoxystrobin and myclobutanil.* After the assay reproducibility was known, the test was conducted once for each of the 256 isolates of *U. necator.* For each run of the experiment, approximately 10 isolates were assayed, employing solutions ranging from 0.0020 to 8.0 µg/ml for myclobutanil, and from 0.00049 to 2.0 µg/ml for azoxystrobin. New fungicide stock solutions were prepared after two runs of the test. Histograms were constructed for $\log_{10}$ ED_{50} values based upon the least significant difference among the fungicide sensitivities of the six isolates tested for assay reproducibility (approximately 0.20 $\log_{10}$ units), as described above. Fungicide sensitivities between the baseline population and the unsprayed checks from the DMI-resistant site (Bl versus Ec) were compared using Student's *t* test (Minitab v. 12.10), using the unsprayed check population as a second baseline population for the comparison of fungicide sensitivities of isolates recovered from vines where no selection pressure for shifts in sensitivity was applied. Differences among the three populations from the DMI-resistant site were analyzed by ANOVA and Fisher's LSD (Minitab v. 12.10). Statistics were performed on the normally distributed $\log_{10}$-transformed ED_{50} values. Log_{10} ED_{50} values for azoxystrobin and myclobutanil of each individual isolate were correlated to determine if a relationship existed between azoxystrobin and myclobutanil sensitivities, examining all populations collectively and each individual population separately (Minitab v. 12.10). To determine if a single discriminatory dose could be used to accurately evaluate fungicide sensitivities, as has been suggested for evaluating *V. inaequalis* sensitivities to DMI fungicides (32), $\log_{10}$ ED_{50} values were regressed against the RG values obtained for each isolate at 0.0078, 0.031, and 0.13 µg of azoxystrobin per ml (MS Excel 7.0). Likewise, for myclobutanil, $\log_{10}$ ED_{50} values were regressed against the RG values obtained at 0.50 µg/ml, a discriminatory dose used but not validated by a previous study (35).

Sensitivity of U. necator *isolates to pyraclostrobin, kresoxim-methyl, and trifloxystrobin.* To determine if our *U. necator* assay was applicable to strobilurin fungicides other than azoxystrobin, and to compare the relative intrinsic activities of these against *U. necator*, the technique was used to test 26 isolates spanning the range of sensitivities to azoxystrobin for their sensitivities to kresoxim-methyl, pyraclostrobin, and trifloxystrobin. Fungicide solutions ranged in concentration from 0.00012 to 8.0 µg/ml for kresoxim-methyl and from 0.00012 to 0.50 µg/ml for pyraclostrobin and trifloxystrobin. Histograms based upon the $\log_{10}$ ED_{50} values were constructed, and differences in the sensitivities of these isolates to azoxystrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin were analyzed by ANOVA and Fisher's LSD (Minitab v. 12.10). Regression analysis was used to compare the $\log_{10}$ ED_{50} values of individual isolates for each of the fungicides (Minitab v. 12.10).

Influence of azoxystrobin sensitivity differences on disease control. To determine if the differences in sensitivity to azoxystrobin detected among *U. necator* isolates have practical implications with respect to disease control, a seedling assay was utilized to test the effect of different azoxystrobin dosages against a composite of five isolates of high sensitivity (Bl-5, 38, 65, 84, 85; mean $ED_{50} = 0.0040$ µg/ml) versus a composite of five isolates of low sensitivity (Em-17, 45, 47, 48, 63; mean $ED_{50} = 0.061$ µg/ml). The seedling bioassay was based upon previous work investigating the physical mode of action of azoxystrobin against *P. viticola* (38). Bioassays with *U. necator* were performed using azoxystrobin in both protectant and postinfection modes. For each run of the protectant assays, sets of six replicate 6-week-old *V. vinifera* cv. Riesling seedlings, each having four to five true leaves, were sprayed until runoff 1 day before inoculation with commercially formulated azoxystrobin (Abound 2.08F) at concentrations of 0, 0.25, 2.5, 25, and 250 µg a.i./ml, using a modified paint sprayer at a pressure of 207 kPa. To facilitate their subsequent identification, the first three leaves larger than 2.5 cm in diameter were marked with small strips of tape around the petioles; then fungicide solutions were applied until runoff.

To prepare the inoculum, five surface-disinfested leaf sections (approximately 30 × 60 mm) kept on 1.5% water agar in 60-mm petri dishes were inoculated with several chains of conidia from individual *U. necator* isolates, one set of five leaves for each individual isolate. Leaf sections were incubated at room temperature as described above. Two weeks later, three infected leaf sections (those with the most abundant

Table 2. Formulas used for calculations of assay reproducibility

Term	Formula[z]	Reference
Mean	$e^{\mu+\frac{\sigma^2}{2}}$	Casella and Berger (4)
Variance	$e^{2(\mu+\sigma^2)} - e^{2\mu+\sigma^2}$	Casella and Berger (4)
Coefficient of variance	$\frac{e^{2(\mu+\sigma^2)} - e^{2\mu+\sigma^2}}{e^{\mu+\frac{\sigma^2}{2}}}$	Casella and Berger (4)
Confidence interval upper limit	$e^{\mu+\frac{\sigma^2}{2}+\frac{\sigma H_{1-\alpha}}{\sqrt{n-1}}}$	Gilbert (13)
Confidence interval lower limit	$e^{\mu+\frac{\sigma^2}{2}+\frac{\sigma H_{\alpha}}{\sqrt{n-1}}}$	Gilbert (13)

[z] μ and σ^2 are the mean and variance, respectively, of the natural log of the ED_{50} values of individual isolates determined from the leaf disk assays. $H_{1-\alpha}$ and H_α are the Land's coefficients used for calculating the upper and lower confidence intervals for a specified level of α.

sporulation) for each isolate from either the high or the low sensitivity group were placed into a 50-ml screw-top centrifuge tube containing 25 ml of 0.0005% Tween 20 in type I H_2O, and vortexed for 30 s (total of 15 leaf sections per treatment). The conidial suspension was adjusted to a final concentration of 5×10^5 conidia per ml with additional 0.0005% Tween 20 in type I H_2O. Seedlings were inoculated immediately thereafter by spraying the conidial suspension from a height of 1 m in a fine mist using a modified paint gun (at 207 kPa). Tests using the two different sensitivity groups were performed on different dates to minimize the effect of cross-interference between groups. Each seedling received a 5-s misting, in which approximately 2.5×10^5 conidia were sprayed onto the plant. The mist droplets dried quickly, usually within 5 to 10 min. Thirty minutes after inoculation, seedlings were moved to a greenhouse and transferred into customized 1 × 1 × 1.5 m (W × L × H) Plexiglas containment chambers, each with its own source of HEPA-filtered air to minimize the probability of contamination from other *U. necator* sources. Seedlings were incubated at 25°C for 14 days with ambient lighting; then disease severity on the three marked leaves was determined by overlaying a 100-mm plastic petri plate, marked with a 1 × 1 cm grid above (but not contacting) the leaves, and determining both the diseased and total surface area of the leaf.

To test the postinfection activity of azoxystrobin against the two sensitivity groups, the same procedures were used, except that untreated seedlings were inoculated and placed into the containment chambers after a 30-min drying period. Five days after inoculation, when colonies on the leaves could be visually detected, seedlings were removed from the chambers, sprayed with the same concentration range of azoxystrobin, then allowed to air-dry for 2 h before being returned to the containment chambers. Disease was assessed 14 days after inoculation, as described above. Each experiment was conducted three times, using six replicate seedlings per treatment. Disease severity data were normalized using the arcsine $\sqrt{y}$ transformation, and the means of each replicate set of six seedlings were analyzed by multivariate ANOVA using the general linear model procedure of SAS (SAS 6.0, SAS Institute, Cary, NC). The sum of squares was partitioned using orthogonal contrasts to determine the levels of significance and main sources of variation for the treatment interactions. Additional Student's *t* tests were performed to compare disease severity data between the low- and high-sensitivity groups for each concentration of azoxystrobin used (Minitab v. 12.10) for both protectant and postinfection assays.

RESULTS

Fungicide sensitivity assays. *Assay reproducibility.* For the six isolates examined in five repeated runs of the assay, the coefficients of variance for each isolate ranged from 10 to 29% with respect to azoxystrobin assays and from 11 to 126% with respect to myclobutanil assays. The mean coefficients of variance for these assays were 31 and 41%, respectively (Table 3). The 95% confidence intervals ranged from 62 to 268% and from 47 to 1099% of the mean ED_{50} values for individual isolates subjected to the azoxystrobin and myclobutanil assays, respectively (Table 3). On average, the 95% confidence intervals ranged from 76 to 293% and 79 to 154% of the mean values, respectively, for azoxystrobin and myclobutanil assays. The least significant difference ($\alpha = 0.05$) separating mean ED_{50} values beyond that attributable to experimental error was 0.17 and 0.23 $\log_{10}$ units, respectively, for the azoxystrobin and myclobutanil assays.

Baseline distribution of U. necator *sensitivities to azoxystrobin and myclobutanil.* Among the isolates from the baseline population (Bl), individual ED_{50} values for azoxystrobin ranged from 0.0037 to 0.028 µg/ml (separated by a factor of 7.5), with a mean value of 0.0097 µg/ml (Fig. 1A). In comparison, there was an approximate twofold difference in the mean azoxystrobin ED_{50} value of isolates recovered from the unsprayed checks from the DMI-resistant population (Ec), with individual values for the 61 isolates ranging from 0.0034 to 0.048 µg/ml (separated by a factor of 14), with a mean of 0.018 µg/ml (Fig. 1A). The mean $\log_{10}$ ED_{50} values of these two sensitivity distributions were significantly different according to Student's *t* test ($P < 0.001$). Furthermore, the difference in the mean $\log_{10}$ ED_{50} values for these populations (0.27) exceeded the least significant difference value of 0.17 $\log_{10}$ units of the assay, indicating that the observed differences were not merely due to any imprecision in repeated assays.

For myclobutanil sensitivities, individual ED_{50} values for isolates from the baseline population ranged from 0.0049 to 0.69 µg/ml (mean = 0.075 µg/ml), being separated by a factor of approximately 140 (Fig. 1B). These results are similar to those previously reported from the same location in 1995 (10). Individual ED_{50} values for isolates recovered from the unsprayed vines from the DMI-resistant site ranged from 0.021 to 3.7 µg/ml (separated by a factor of approximately 180), with a mean value of 0.92 µg/ml. This 12-fold difference in mean ED_{50} values was significant ($P < 0.001$) according to Student's *t* test. Even more pronounced was the difference in sensitivities between the median categories for the two populations, whose midpoint values were separated by a factor of nearly 30 (Fig. 1B).

Field performance of azoxystrobin and myclobutanil and sensitivity shifts over a single season. For the 1999 season, the myclobutanil and azoxystrobin–myclobutanil spray programs at the Dresden site, where the DMI-resistant population was collected, provided 86 and 91% control of disease caused by *U. necator*, respectively, based upon the severity of cluster infections during a year of low disease pressure. In contrast, data from 1998 (when disease pressure was higher) showed 63 and 93% control under the same experimental treatments. Differences between these two treatments were only significant for the 1998 growing season (Duncan-Waller *k*-test, $\alpha = 0.05$, W. F. Wilcox, *unpublished*). In 1996 and 1997, where azoxystrobin was absent from the spray programs, only 84 and 76% control of disease was obtained, respectively, when myclobutanil was used exclusively during the growing season (W. F. Wilcox, *unpublished*).

Of the individual isolates from the DMI-resistant population, those from subpopulation Em (six myclobutanil sprays) had the lowest sensitivity to azoxystrobin, with a mean ED_{50} value of 0.024 µg/ml and a

Table 3. Reproducibility of assay for determining ED_{50} values for azoxystrobin and myclobutanil against six isolates of *Uncinula necator*

Isolate	ED_{50} (µg/ml) azoxystrobin			ED_{50} (µg/ml) myclobutanil		
	Mean[x]	95% CI[y]	CV[z]	Mean[x]	95% CI[y]	CV[z]
Bl-1	0.008	(0.007-0.011)	0.22	0.015	(0.012-0.024)	0.33
Bl-3	0.016	(0.013-0.023)	0.29	0.058	(0.027-0.632)	1.26
Bl-6	0.011	(0.009-0.014)	0.22	0.012	(0.009-0.018)	0.35
Em-3	0.013	(0.010-0.020)	0.35	1.304	(1.168-1.477)	0.12
Em-4	0.024	(0.015-0.065)	0.66	1.499	(1.359-1.675)	0.11
Em-5	0.013	(0.012-0.014)	0.10	0.619	(0.497-0.848)	0.27
Mean			0.31			0.41

[x] Mean ED_{50} value based upon $\log_e$-transformed ED_{50} values obtained from five repeated assays.
[y] 95% confidence interval based upon $\log_e$-transformed ED_{50} values.
[z] The coefficient of variance based upon $\log_e$-transformed ED_{50} values.

range of 0.0047 to 0.075 μg/ml (Fig. 2A). In contrast, isolates from the azoxystrobin–myclobutanil spray program (Ea) had a mean ED_{50} of 0.015 μg/ml (range = 0.0046 to 0.033 μg/ml). Analysis of variance for the three subpopulations indicated that differences among subpopulations were significant ($P < 0.001$), and multiple comparisons indicated that sensitivities of isolates from the Ea and Ec populations were similar to each other but significantly different from those from the Em population (Fisher's LSD, $\alpha = 0.05$).

Within the DMI-exposed subpopulations, isolates recovered from vines subjected to six myclobutanil sprays (Em) were even less sensitive to myclobutanil than were isolates from unsprayed vines (Ec) or those treated with the azoxystrobin–myclobutanil rotation (Ea), as evidenced by an increased value for the midpoint of the median sensitivity category and differences in mean ED_{50} values (1.2, 0.91, and 0.92 μg/ml, respectively) (Fig. 2B). Analysis of variance indicated that the differences among subpopulations were significant ($P < 0.001$), and multiple comparisons indicated that the mean sensitivities of isolates from subpopulations Ea and Ec were similar but significantly different from those of subpopulation Em (Fisher's LSD, $\alpha = 0.05$).

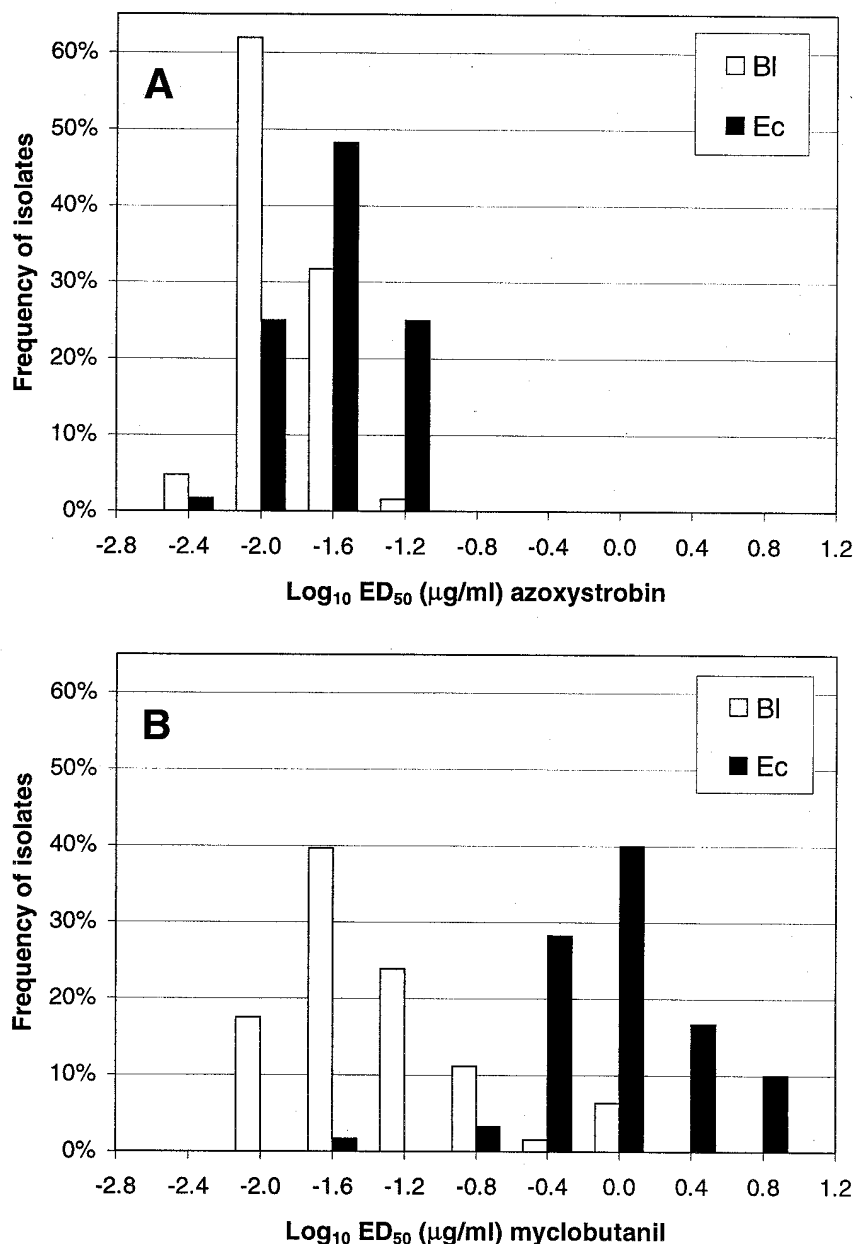

Fig. 1. Distribution of sensitivities for isolates of *Uncinula necator* collected from a site previously unexposed to either demethylation inhibitor (DMI) or strobilurin fungicides (Bl, n = 68) and the unsprayed check vines from a DMI-resistant site (Ec, n = 61) **A,** azoxystrobin and **B,** myclobutanil. Discreet categories of ED_{50} values for individual isolates are organized based upon the least significant difference of 0.20 $\log_{10}$ units ($\alpha = 0.05$). Indicated values represent the midpoint for each sensitivity category.

Cross-sensitivity to azoxystrobin and myclobutanil among isolates of U. necator. Regression analysis of $\log_{10}$-transformed ED_{50} values for azoxystrobin and myclobutanil for all 256 isolates indicated a moderate but highly significant linear relationship between sensitivities to these two fungicides (Fig. 3A). However, when the baseline population and the individual subpopulations from the DMI-resistant site were analyzed separately, it was apparent that this relationship was most pronounced among isolates that had been subjected to intensive selection pressure for tolerance to myclobutanil (Fig. 3C), and not evident in other treatments.

Relative aggressiveness of U. necator *isolates with respect to azoxystrobin and myclobutanil sensitivities.* To determine if sensitivities to either azoxystrobin or myclobutanil were related to the aggressiveness of individual isolates, $\log_{10}$-transformed ED_{50} values for each fungicide were regressed against the mean radial expansion on the untreated (check) leaf disks for all 256 isolates. Regression analysis indicated no linear relationship between colony expansion and azoxystrobin sensitivity ($R^2 = 0.001$, $P = 0.690$) and only a very weak negative linear relationship between colony expansion and myclobutanil sensitivity ($R^2 = 0.018$, $P = 0.028$).

Validity of single discriminatory doses for determining U. necator *sensitivities to azoxystrobin and myclobutanil.* There was a strong linear relationship between RG values obtained at the tested discriminatory dose concentrations of 0.031 μg/ml for azoxystrobin and 0.50 μg/ml for myclobutanil and the $\log_{10} ED_{50}$ values with respect to individual *U. necator* isolates (Fig. 4). For the three discriminatory doses of azoxystrobin analyzed, the RG values obtained at 0.031 μg/ml appeared to be more appropriate for estimating useful $\log_{10} ED_{50}$ values. For RG values determined for azoxystrobin at 0.031 μg/ml, the regression line was described by the equation: $\log_{10} ED_{50(\text{azoxystrobin})} = 1.23(\text{RG}) - 2.19$ ($R^2 = 0.64$, $P < 0.001$). The range of ED_{50} values that could be calculated based upon this equation would be 0.0064 to 0.11 μg/ml. Likewise, for calculating ED_{50} values based upon the RG values for myclobutanil at 0.50 μg/ml, the relationship was described by: $\log_{10} ED_{50(\text{myclobutanil})} = 2.17(\text{RG}) - 1.62$ ($R^2 = 0.81$, $P < 0.001$). The range of ED_{50} values that could be calculated based upon the RG values at this dose of myclobutanil would be 0.024 to 3.5 μg/ml.

Sensitivity of selected U. necator *isolates to pyraclostrobin, kresoxim-methyl, and trifloxystrobin.* Similar leaf disk assays of *P. viticola* sensitivities to strobilurin fungicides indicated that this technique was better suited for tests with azoxystrobin than for tests with kresoxim-methyl and trifloxystrobin (37). Within its limitations,

the technique indicated substantial differences among the intrinsic activities of the three strobilurins against this pathogen. The leaf disk assay was applicable to all strobilurin fungicides tested except kresoxim-methyl. ED_{50} values for kresoxim-methyl could not be calculated for all of the isolates tested due to a combination of the phytotoxicity of the compound to leaf disks at concentrations ≥4.0 µg/ml and its relatively low intrinsic activity against *U. necator* (Fig. 5). As seen in previous work (37), trifloxystrobin caused similar phytotoxicity on leaf disks at concentrations ≥0.5 µg/ml, but its intrinsic activity against *U. necator* was sufficient to allow the determination of ED_{50} values when used in the assays at lower, nonphytotoxic concentrations.

Pyraclostrobin had the highest intrinsic activity of the four strobilurin fungicides tested, with individual ED_{50} values for the 26 isolates tested ranging from 0.0016 to 0.010 µg/ml, with a mean value of 0.0044 µg/ml (Fig. 5). For trifloxystrobin, individual ED_{50} values ranged from 0.0058 to 0.052 µg/ml, with a mean value of 0.015 µg/ml (Fig. 5). Azoxystrobin sensitivities ranged from 0.0042 to 0.055 µg/ml, with a mean value of 0.013 µg/ml (Fig. 5). Analysis of variance and multiple comparisons indicated that mean ED_{50} values for azoxystrobin and trifloxystrobin were not statistically different from each other, but both were statistically different compared with pyraclostrobin (Fisher's LSD, $\alpha = 0.05$).

There was a strong correlation between azoxystrobin and trifloxystrobin $\log_{10} ED_{50}$ values ($R^2 = 0.75$, $P < 0.001$) (Fig. 6A). However, $\log_{10} ED_{50}$ values for pyraclostrobin did not correlate as well with those of azoxystrobin ($R^2 = 0.21$, $P = 0.018$) or trifloxystrobin ($R^2 = 0.24$, $P = 0.010$) (Fig. 6B and C).

Influence of azoxystrobin sensitivity on disease control. Within the relatively narrow range of azoxystrobin sensitivities that we detected in our field samples of *U. necator*, differences affected disease control only when the fungicide was used at fractions of the recommended rate, and effects on disease control were more pronounced when it was used in a postinfection rather than a protective mode. Protectant applications provided 100% control of the composite populations of both high and low sensitivity at the recommended rate of 250 µg a.i./ml and also at 10% of this rate (Fig. 7A). When seedlings were treated

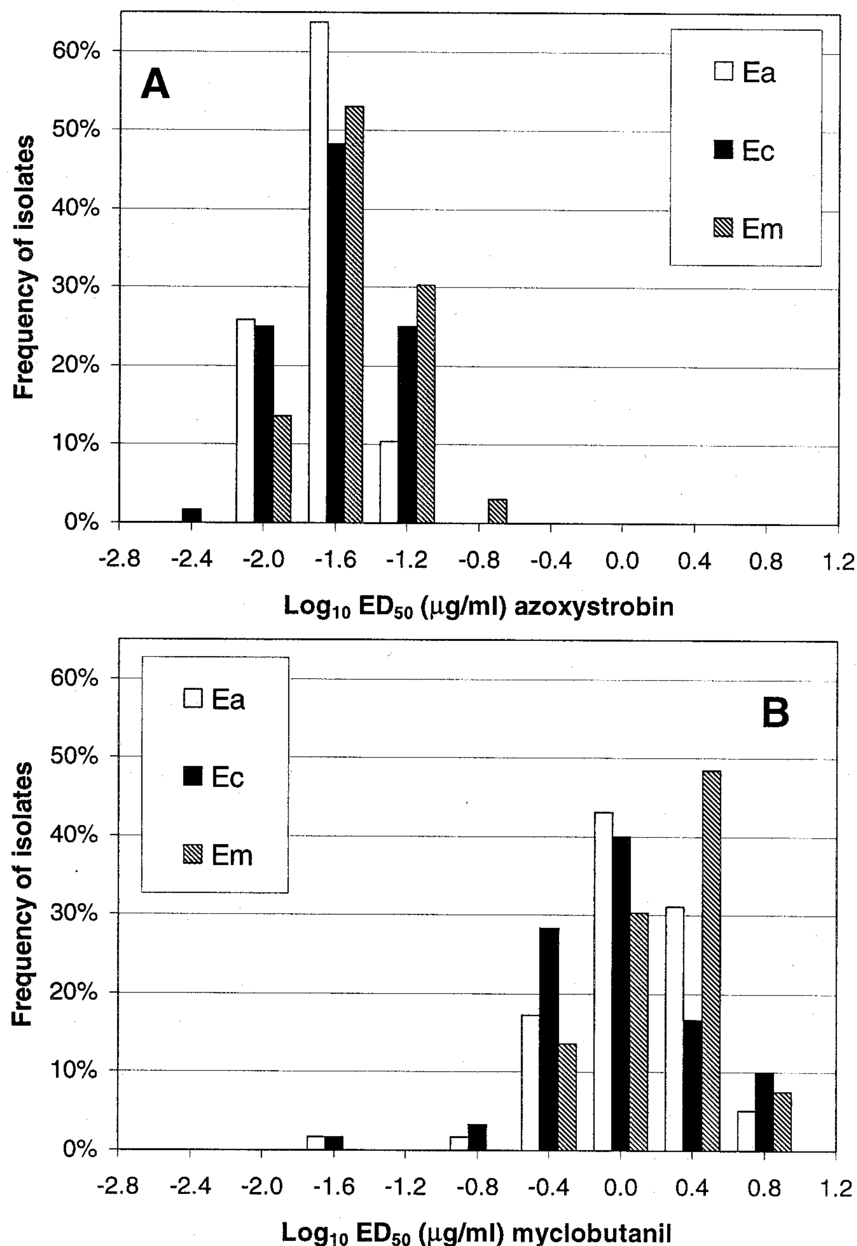

Fig. 2. Distribution of sensitivities to azoxystrobin and myclobutanil among isolates of *Uncinula necator* exposed to different experimental treatments from a demethylation inhibitor (DMI)-resistant site. Distribution of sensitivities for the DMI-resistant subpopulations, Ea (azoxystrobin and myclobutanil in rotation, n = 58), Ec (unsprayed, n = 61), and Em (six myclobutanil applications, n = 69) for **A,** azoxystrobin and **B,** myclobutanil. Discreet categories of ED_{50} values for individual isolates are organized based upon the least significant difference of 0.20 $\log_{10}$ units ($\alpha = 0.05$). Indicated values represent the midpoint for each sensitivity category.

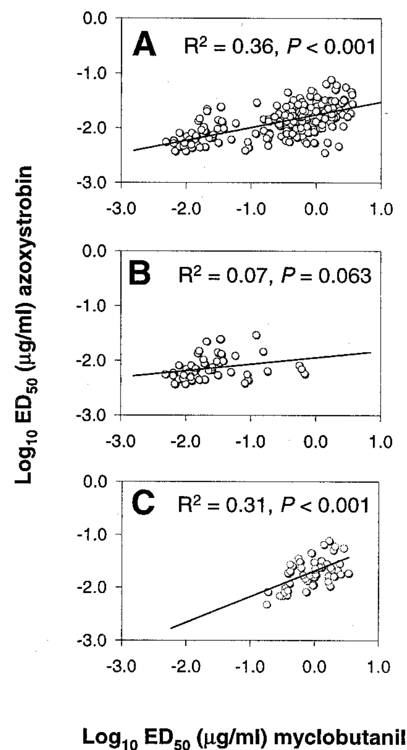

Fig. 3. Relationship between sensitivities to azoxystrobin and myclobutanil among individual isolates of *Uncinula necator*. The coefficients of determination and significance levels are shown for the equations derived from regressing the $\log_{10} ED_{50}$ values for azoxystrobin on $\log_{10} ED_{50}$ values for myclobutanil for **A,** all tested isolates (n = 256), **B,** all isolates from the baseline population (n = 68), and **C,** all isolates from subpopulation Em (six myclobutanil applications, n = 69).

with 2.5 μg a.i./ml azoxystrobin, control of the low-sensitivity isolates was 73%, as opposed to 91% for the high-sensitivity group. At a rate of 0.25 μg a.i./ml, control of the low- and high-sensitivity groups was 26 and 39%, respectively. Analysis of variance indicated a significant difference between the response of the low- and high-sensitivity isolates against the four rates of azoxystrobin (Fig. 7A, Table 4). However, pairwise comparisons of disease development between groups indicated no significant differences for the control of either group at any given rate (Student's *t* test, α = 0.05) (Fig. 7A). Analysis of variance indicated no significant difference between the runs of the experiments ($P = 0.90$), indicating that the analysis of the means of the treatments in three replicated experiments was statistically appropriate.

In contrast, although postinfection applications of azoxystrobin provided virtually complete control of both *U. necator* populations at the 250 μg a.i./ml rate, such applications at lower rates were pronouncedly less effective against isolates of low versus high sensitivity to the fungicide. For example, whereas a rate of 25 μg a.i./ml provided 98% control of the high-sensitivity group, it provided only 54% control for the low-sensitivity groups. Similarly, there was a 41% difference between these groups with respect to disease severity following treatment with 2.5 μg a.i./ml (Fig. 7B). Such differences are also reflected in the comparative analyses of variance for the protectant versus postinfection (Table 4) assays, e.g., the main effect of pathogen sensitivity and the interactive effect of sensitivity × fungicide concentration were both far greater under postinfection conditions. Individual comparisons between high- and low-sensitivity groups at each concentration of azoxystrobin used, using Student's *t* tests, indicated no significant differences between the two sensitivity groups except for the 0.25 and 25 μg a.i./ml treatments in the postinfection assay ($P = 0.015$ and 0.012, respectively) (Fig. 7B). Similar to the results for the protectant assays, differences between repeated runs of the experiment were not significant ($P = 0.25$), and analysis of the means of the treatments across the three experiments was statistically appropriate.

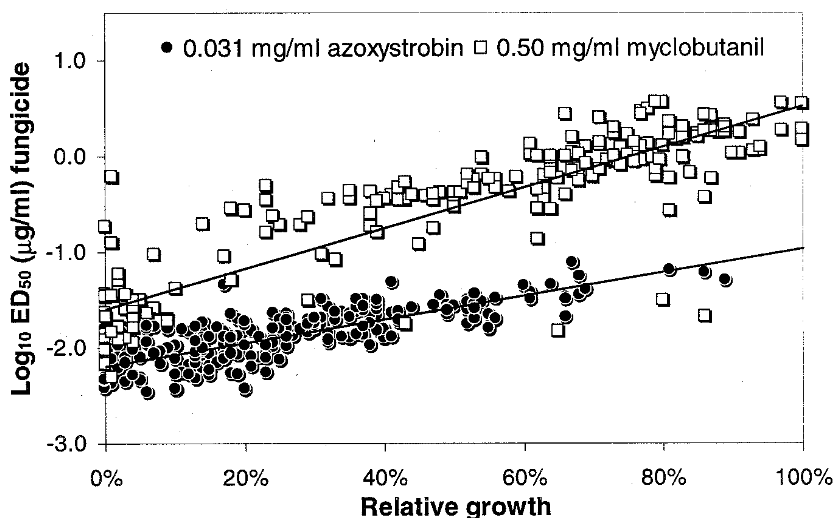

Fig. 4. Relationship between the relative growth (compared with the check treatment) of individual isolates of *Uncinula necator* on grape leaf disks treated with a single discriminatory dose of azoxystrobin at 0.0078 μg/ml or myclobutanil at 0.50 μg/ml and the $\log_{10}$ ED_{50} values determined for the same isolates for each respective fungicide.

DISCUSSION

In this study, the baseline sensitivity of *U. necator* to azoxystrobin has been described, and according to the sample size of 68, 95% of all isolates will fall into the described baseline sensitivity range with 95% confidence (26). Based upon the composite population of baseline and DMI-resistant isolates, where limited applications of azoxystrobin have been used, the sample size of 256 isolates (with azoxystrobin ED_{50} values ranging from 0.0034 to 0.075 μg/ml and a mean of 0.017 μg/ml) would represent between 98 and 99% of all isolates with 95% confidence (26). The narrow distribution of azoxystrobin sensitivities is similar to those previously de-

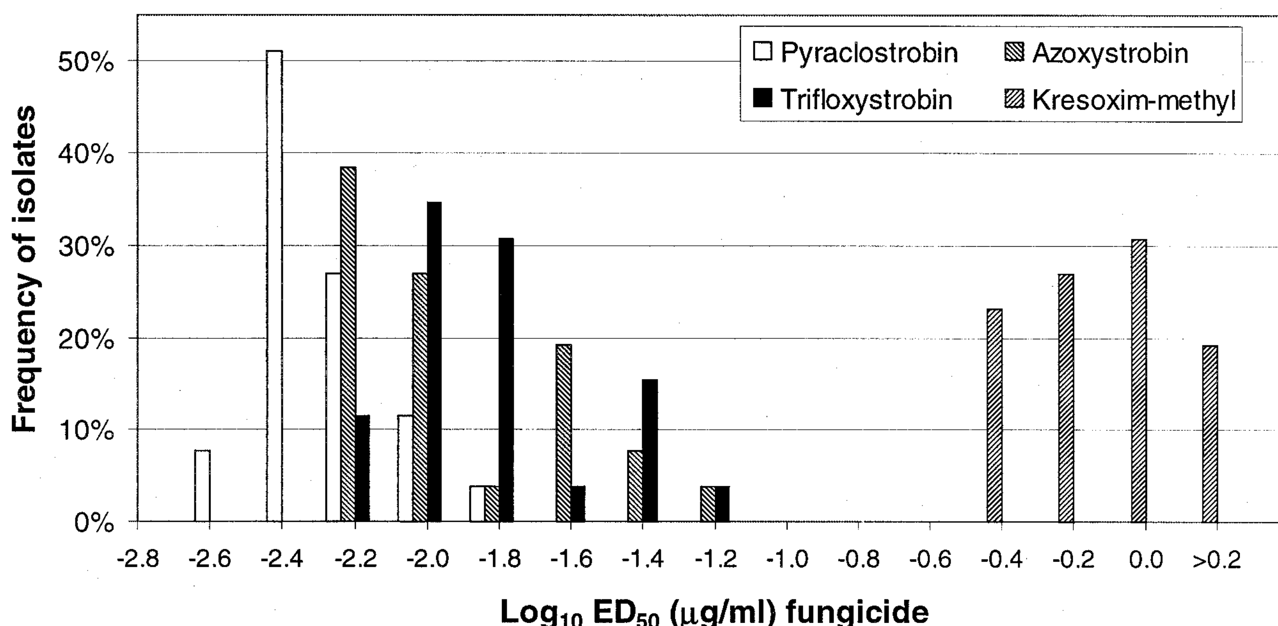

Fig. 5. Distribution of sensitivities to azoxystrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin among 26 selected isolates of *Uncinula necator*. Discreet categories of ED_{50} values for individual isolates are organized based upon the least significant difference of 0.20 $\log_{10}$ units (α = 0.05). Indicated values represent the midpoint for each sensitivity category.

scribed for *Mycosphaerella graminicola* (14) and *P. viticola* (37) to azoxystrobin and *V. inaequalis* to kresoxim-methyl (29,30), and are in clear contrast with the much broader distribution of sensitivities to DMI fungicides documented in this and other (10,16,40) studies on *U. necator*.

For future monitoring of *U. necator* sensitivities to azoxystrobin, the use of a single discriminatory dose of 0.031 μg/ml would be useful for characterizing isolates within the range of sensitivities detected for the 256 isolates of *U. necator* used in this study. However, isolate sensitivities outside of this range, which may include those that may develop qualitative resistance to azoxystrobin, would not be adequately discriminated, mandating that an additional higher discriminatory dose be used. The discriminatory dose of 2.0 μg/ml could suffice; only 18 of the 256 isolates tested grew at this concentration, with growth being 2% or less relative to the untreated check. Isolates that were not inhibited by this higher dose of azoxystrobin would be likely to have qualitative resistance to this fungicide. Since no isolates with this phenotype were detected in this study, additional work would have to be performed to confirm the presence of resistance, but this higher dose would appear to be a logical choice for a discriminatory dose that would allow for the detection of highly resistant isolates.

In contrast, the single discriminatory dose of 0.50 μg/ml appears to be applicable for future monitoring of *U. necator* sensitivities to myclobutanil. Relative growth values at this dose could be used to classify isolates representing a wide range of sensitivities as either myclobutanil sensitive or resistant (10).

The results of the comparative study of *U. necator* sensitivities to azoxystrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin illustrate a number of points. Kresoxim-methyl appeared to be several magnitudes lower in intrinsic activity compared with azoxystrobin, pyraclostrobin, and trifloxystrobin. However, kresoxim-methyl has been shown to provide very good control of grapevine powdery mildew in the field (33,34,36), indicating that the intrinsic activity of these strobilurin fungicides as determined by the excised leaf disk assay does not fully reflect the field performance of the fungicides. These results, in combination with a previous study on *P. viticola* and strobilurin fungicides (38), indicate that other physical properties of fungicides (vapor activity, redistribution, absorption, breakdown, etc.), in addition to intrinsic activity, contribute to the efficacy of a fungicide under field condi-

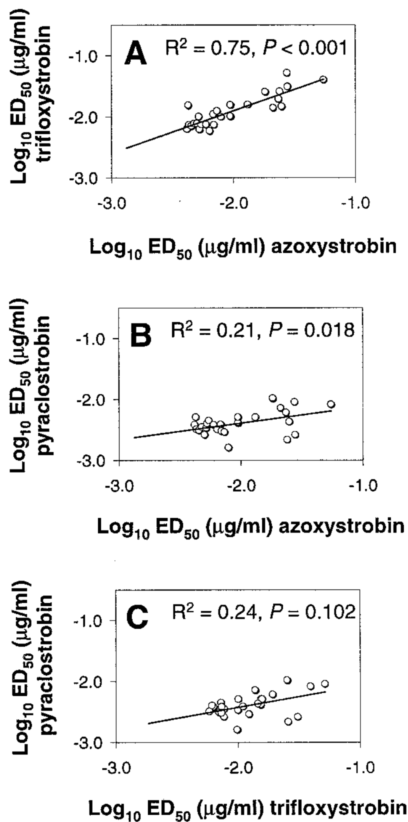

Fig. 6. Relationships among sensitivities to azoxystrobin, pyraclostrobin, and trifloxystrobin among 26 selected isolates of *Uncinula necator*. The coefficients of determination and significance levels are shown for the equations derived from regressing **A,** $\log_{10}$ ED_{50} values for trifloxystrobin on $\log_{10}$ ED_{50} values for azoxystrobin, **B,** $\log_{10}$ ED_{50} values for pyraclostrobin on $\log_{10}$ ED_{50} values for azoxystrobin, and **C,** $\log_{10}$ ED_{50} values for pyraclostrobin on $\log_{10}$ ED_{50} values for trifloxystrobin.

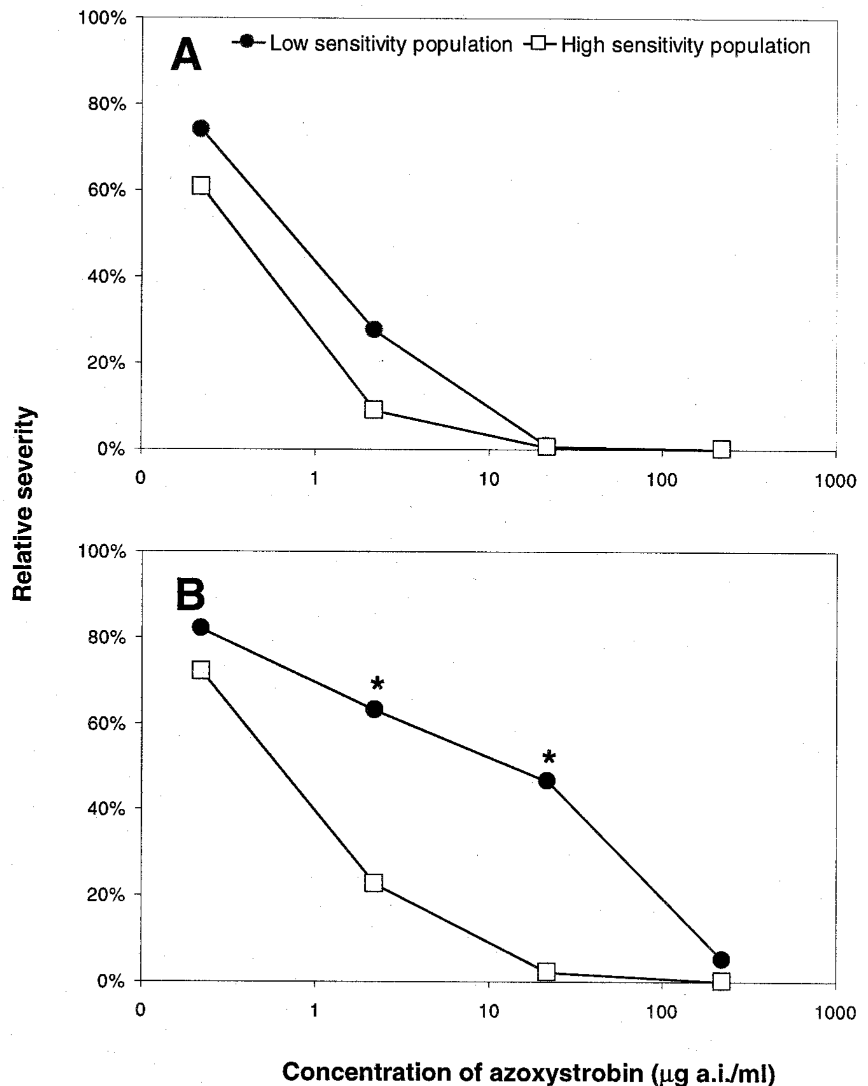

Fig. 7. Disease control provided by azoxystrobin on *Vitis vinifera* cv. Riesling seedlings inoculated with two composite populations of *Uncinula necator* with differing sensitivities to the fungicide (mean ED_{50} values = 0.0040 and 0.061 μg/ml, respectively). Disease severity is expressed as the percent surface area infected on the leaves of fungicide-treated relative to that on the dH_2O-treated checks, 14 days after inoculation. Each data point represents the mean value for 54 rated leaves over three replicated runs of the experiment. **A,** Seedlings were treated with fungicide 1 day before inoculation. **B,** Seedlings were treated with fungicide 5 days after inoculation. Significant differences (Student's *t* test, $\alpha < 0.05$) between high- and low-sensitivity populations within individual treatments are designated by (*).

tions, although the contributions of each of these components are not fully understood.

Interestingly, *U. necator* sensitivities to azoxystrobin, pyraclostrobin, and trifloxystrobin did not correlate as strongly or as uniformly as expected, based upon the existence of cross-resistance between fungicides of this group for a number of plant pathogens (18). However, the results were comparable to those found for cross-sensitivities published for *U. necator* to the DMI fungicides fenarimol, myclobutanil, and triadimenol (10). Perhaps our results were due to the narrow range of sensitivities of the isolates tested to the strobilurins and the limited sample size used. Nevertheless, it would have been interesting to have had tested isolates with a wider range of sensitivities, including those with high levels of strobilurin insensitivity, to make these comparisons and document cross-resistance between strobilurins for *U. necator*. However, the present study appears to be the first published record to document the sensitivity distribution of this pathogen to strobilurin fungicides.

As seen with previous studies using grape leaf disks and strobilurin fungicides (37), there was considerable phytotoxicity caused by kresoxim-methyl at concentrations higher than 4.0 μg/ml, a concentration that did not inhibit fully the radial expansion of the isolates of *U. necator* that were tested. Alternate methodologies were not pursued for the purposes of this study, but further studies indicated that treating the leaf disks 24 h after excision with a spray suspension of formulated kresoxim-methyl (Sovran 50WP) allowed for the application of fungicide as high as 64 μg/ml without phytotoxicity. Although not applicable for this study, this method would allow for the application of this type of leaf disk assay for future sensitivity monitoring for *U. necator* to kresoxim-methyl.

The development of practical resistance to strobilurin fungicides by several plant-pathogenic fungi and oomycetes appears to be caused by the rapid buildup of highly resistant phenotypes, resulting in qualitative, disruptive shifts in their sensitivity distributions with respect to these materials (11,12,17). However, the risk of quantitative, directional shifts that have conferred practical resistance to DMI fungicides for many plant pathogens, including *U. necator* (10,40), is unclear for the strobilurins. Within this context, it is important to note the pronounced difference in the range of sensitivities that were detected in the baseline population with respect to azoxystrobin versus myclobutanil (i.e., 7.5- versus 140-fold, respectively). Typically, practical resistance to DMI fungicides has developed when fungicide rates that controlled the vast majority of the pathogen population fail to provide adequate control of the least sensitive phenotypes at the edge of such broad ranges, allowing them to build up to damaging levels (10,20,23).

In contrast, our results suggest that the risk and impact of quantitative shifts in sensitivity to azoxystrobin, although measurable, may be relatively minor with respect to the narrow range of sensitivities that were detected among isolates of *U. necator*. Even for the least-sensitive *U. necator* isolates that we found, azoxystrobin provided complete disease control in a 1-day protectant assay when used at a rate as low as 10% of that recommended for field use, although longer intervals between fungicide treatment and inoculation were not tested to determine the duration of this activity. Nevertheless, such reduced-rate applications made 5 days after inoculation clearly provided a selective pressure favoring the least-sensitive individuals within the pathogen population and an attendant reduction in disease control. Thus, maintenance of full recommended fungicide rates, uniform spray coverage to protect all susceptible host tissues, and the avoidance of extended application intervals (particularly if used in a postinfection mode, either intentionally or by default, e.g., on leaf tissues that have emerged since the last fungicide application) should minimize this risk. However, these techniques are unlikely to affect the selection for a qualitative, disruptive shift toward resistance, since it is primarily dependent on the number of selective events (fungicide applications) to which the pathogen is exposed (30).

It is within the context of potential quantitative shifts in sensitivities to strobilurins among populations of *U. necator* that the relationship between sensitivities to azoxystrobin and myclobutanil must be considered. Our results show a lack of independence between sensitivities to these two unrelated fungicides. In this study, we have referred to this as "cross-sensitivity" versus "cross-resistance," since this relationship is not equivalent to the case when a genetic change in an individual confers resistance to a whole class of related chemistries (18). This relationship between azoxystrobin and myclobutanil sensitivities is similar to that which has been documented for the relationship between sensitivities to dodine and DMI fungicides for *V. inaequalis* (21). The mechanism underlying this relationship is unknown. An active efflux mechanism has been suggested as a potential contributor to the expression of pleiotropic resistance to fungicides (6,7,19), but such activity in plant-pathogenic fungi is still poorly understood. Alternatively, it is possible that the development of fungicide resistance in individual isolates of a plant-pathogenic fungus may predispose those individuals to more rapid development of resistance to additional classes of unrelated fungicides (double resistance) (22). Nevertheless, relative to the baseline site with no exposure to either DMI or strobilurin fungicides, sensitivities to azoxystrobin had shifted at the DMI-resistant site, where prolonged usage of DMI fungicides has led

Table 4. Analysis of variance for disease severity for *Vitis vinifera* cv. Riesling seedlings treated with protectant or postinfection applications of azoxystrobin at 0 to 250 μg a.i./ml and inoculated with isolates of *Uncinula necator* of either high or low azoxystrobin sensitivity

		Disease severity[w,x]			
		Protectant[y]		Postinfection[z]	
Source of variation	**df**	**MS**	***F***	**MS**	***F***
Sensitivity	1	0.0341	7.79 *	0.8603	205.81 ***
Concentration	4	0.9116	208.12 ***	0.4067	97.30 ***
Linear	1	3.4313	783.39 ***	1.5535	371.65 ***
Quadratic	1	0.1195	27.28 ***	0.0005	0.13
Cubic	1	0.0944	21.55 ***	0.0088	2.10
Quartic	1	0.0011	0.26	0.0640	15.32 ***
Sensitivity × concentration	4	0.0114	2.60	0.0664	15.88 ***
Linear	1	0.0040	0.92	0.0352	8.43 **
Quadratic	1	0.0218	4.97 *	0.1899	45.42 ***
Cubic	1	0.0095	2.17	0.0403	9.65 **
Quartic	1	0.0103	2.34	0.0000	0.00
Error	20	0.0044		0.0042	

[w] *F* values are significant at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***) as noted.
[x] Disease severity was analyzed following arcsine square root transformation of the data.
[y] Protectant applications were made 1 day before inoculation.
[z] Postinfection applications were made 5 days after inoculation.

to the development of practical resistance to myclobutanil.

Although a shift in azoxystrobin sensitivity was apparent in the population treated exclusively with myclobutanil, the extent of this shift was not severe enough to compromise control by azoxystrobin, as evidenced by the seedling assays, when 10% to full rates of the fungicide were used in a protectant manner or when full rates were applied postinfection. Furthermore, rotational programs incorporating both azoxystrobin and myclobutanil provided good control of powdery mildew in both years where it had been tested, in addition to halting further shifts toward myclobutanil insensitivity in 1999, compared with isolates recovered from the unsprayed and myclobutanil-only treated vines. The historical performance of myclobutanil at the DMI-resistant site clearly indicated that the performance of this fungicide was compromised, and the control provided by myclobutanil-only spray programs would not be acceptable commercially. The spray programs in 1998 and 1999 that included azoxystrobin provided superior disease control, indicating that azoxystrobin contributed to the control of DMI-resistant individuals that would otherwise not have been as well controlled in a myclobutanil-only spray program. Thus, based on our results, we suggest that such programs rotating strobilurin and DMI fungicides are viable options for limiting the use of both materials while providing commercially acceptable disease control.

For practical resistance to the strobilurins by *U. necator*, the risk and timing of resistance development is still unclear. The results presented by this study provide important information to assist in monitoring shifts in sensitivity over the long term. Additionally, we provide information on an unexpected relationship in *U. necator* between azoxystrobin and myclobutanil sensitivities, that can serve as a model for other DMI-strobilurin interactions as well as provide information for additional studies on cross sensitivities between otherwise unrelated fungicides.

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