Sensitivity of *Uncinula necator* to Benomyl, Triadimefon, Myclobutanil, and Fenarimol in California

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ABSTRACT

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Sensitivity of *Uncinula necator* subcultures to benomyl and the demethylation-inhibiting (DMI) fungicides triadimefon, myclobutanil, and fenarimol was assessed in 1993, 1994, and 1995 with leaf disk bioassays. In 1993, 1994, and 1995, 81.8, 96, and 96.7% of the subcultures, respectively, did not grow on leaf disks treated with 30 mg of benomyl per liter, whereas growth of the remaining subcultures was inhibited by more than 90%. Median EC₅₀ values of triadimefon, myclobutanil, and fenarimol decreased from 1993 to 1994, and those of triadimefon decreased again from 1994 to 1995. In the same period, median EC₅₀ values of all three DMI fungicides increased in a vineyard never exposed to DMI fungicides. The highest means and ranges of EC₅₀ values found were those of triadimefon. Means and ranges were lower for myclobutanil and lowest for fenarimol, reflecting differences in inherent activities of the fungicides and potential for development of resistance. Pairwise correlations between EC₅₀ values of each DMI fungicide were positive and confirmed earlier indications of cross resistance.

Control of powdery mildew in grape, caused by *Uncinula necator* (Schwein.) Burrill, is of high priority in disease management in California vineyards. Sulfur has been used for mildew control for nearly 150 years (7), but other fungicides have become available more recently. The fungicide benomyl (Benlate, E. I. Du Pont de Nemours & Co., Agricultural Products Dept., Barley Mill Plaza, Wilmington, DE 19898) was registered for powdery mildew control by the Environmental Protection Agency (EPA) in 1994. Benomyl has been used in California vineyards since 1972 to control *Botrytis cinerea*, causal agent of gray mold on grape. Currently, no information is available about the frequency of benomyl-resistant strains of *U. necator* populations in California. Sterol biosynthesis-inhibiting fungicides that affect demethylation (demethylation-inhibiting [DMI] fungicides) are of crucial importance in grape powdery mildew control programs. Triadimefon (Bayleton, Bayer Corp., Agricultural Chemical Division, P.O. Box 4913, Kansas City, MO 64120) was introduced in 1982 and was the most important tool for disease control for a number of years (7). In 1989, two additional DMI fungicides, myclobutanil (Rally, Rohm and Haas Co., 727 Norris-

town Road, Spring House, PA 19477) and fenarimol (Rubigan, Dow Elanco, 9330 Zionsville Rd., Indianapolis, IN 46248) were introduced for grape powdery mildew control. Reduced sensitivity to triadimefon was suspected in 1985 and confirmed in 1986 and 1990 (8,18). Sensitivity of *U. necator* subcultures was lowest to triadimefon, intermediate to myclobutanil, and highest to fenarimol (18). The cause of the differing sensitivity to triadimefon, myclobutanil, and fenarimol is not known.

Cross resistance to DMI fungicides was reported in a number of fungal plant pathogens, including *Erysiphe graminis* f. sp. *hordei* (2), *Cladosporium cucumerinum*, *Pyrenophora teres* (12), *Sphaerotheca fuliginea* (12,25), *Rhynchosporium secalis* (13), and *Pseudocercospora herpotrichoides* (16). A recent study on *P. teres* indicated that resistance to DMI fungicides is generally, but not always, genetically correlated (21). Indications of cross resistance were also reported in *U. necator* (27), but the results of this study were based on a limited number of isolates, and correlations among sensitivities to different DMI fungicides were not determined.

Our first objective was to monitor sensitivity to the three DMI fungicides and benomyl over time. Our second objective was to compare EC₅₀ values of the three DMI fungicides between monoconidial isolates and subcultures of *U. necator*, which were also used in a previous study (8). Our third objective was to determine whether EC₅₀ values of triadimefon, myclobutanil, and fenarimol were correlated in monoconidial isolates. Significant correlations would confirm indications of

cross resistance among the three DMI fungicides.

MATERIALS AND METHODS

A leaf disk bioassay was performed as described previously (8) with some modifications. The leaves were collected from *Vitis vinifera* L. 'Carignane' cuttings instead of seedlings. Fully expanded, 6-day-old leaves were used to produce leaf disks. Leaf disks were immersed for 30 min in an aqueous fungicide solution prepared as described (8) and drained of excess liquid before being placed in the petri dishes lined with moistened paper towel and Miracloth (Calbiochem-Novabiochem, La Jolla, CA). The remaining film of solution was allowed to dry overnight. Leaf disks were inoculated with conidia from one inoculum source of *U. necator* by means of a vacuum-operated settling tower (23). This inoculation method separated individual conidia and provided a uniform inoculum density among the leaf disks. For each inoculum source of *U. necator* and each fungicide concentration, three petri dishes, each containing eight leaf disks, were used. Inoculum densities were determined from the number of conidia observed on microscope slides located in the settling tower during inoculation. Inoculum densities were assessed with a hemacytometer. The average inoculum density ranged from 1,500 to 4,000 conidia/cm². Ten days after inoculation, leaf disks were scored for percent surface area colonized by *U. necator* to the nearest 10%. Estimates of coverage and EC₅₀ values calculated with this method were similar to those obtained with the Horsfall-Barratt rating system used previously (8). The percentage of inhibition at each fungicide concentration was determined by comparison to water-treated, inoculated leaf disks. The EC₅₀ value for each *U. necator* subculture was determined by logit transformation of the percentage of inhibition and regression against the natural logarithm of the fungicide concentrations.

Sensitivity to DMI fungicides and benomyl in California in 1993, 1994, and 1995. In 1993, 1994, and 1995, sampling for *U. necator* was performed in 25 vineyards throughout California. In total, 49, 44, and 50 samples consisting of mildew-infected leaves or berries were collected from the same vineyards in each successive year. Subcultures of *U. necator* were obtained from infected berries or leaves from

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separate individual vines and used to inoculate 4- to 6-week-old Carignane grapevine seedlings. The seedlings were grown in 5-cm pots and maintained at 35 to 37°C before inoculation. Prolonged exposure to these temperatures prevented contamination with *U. necator* prior to inoculation (5). After inoculation with *U. necator*, seedlings were encased in clear plastic tubes as described previously (8). Each mildew sample was transferred to grape seedlings and its EC₅₀ values of triadimefon, myclobutanil, and fenarimol were determined with the leaf disk bioassay described in the previous section. In the same years, five, five, and nine samples, respectively, were collected from individual vines in the Yuba County vineyard described previously as containing wild-type isolates (8). These samples were processed and analyzed for EC₅₀ values as described for the other samples. Because the data obtained were not normally distributed and a closer approximation to normality could not be accomplished by transformation, the data were analyzed for each fungicide separately for each combination of years by means of Wilcoxon-Mann-Whitney's two-sample tests in the NPAR1WAY procedure of SAS (SAS Institute Inc., Cary, NC). The probability threshold was set at 0.0167 to control the error rate for multiple comparisons.

U. necator subcultures were also tested for sensitivity to benomyl. For this purpose, leaf disks were treated with a solution of 30 mg of benomyl per liter, dried overnight, and inoculated with conidia of a *U. necator* subculture as described previously. The percent inhibition was calculated from the difference between the colonized surface area coverage estimated on water-treated, inoculated leaf disks, and colonization on leaf disks treated with 30 mg of benomyl per liter. Additionally, in 1993 and 1994, two and five *U. necator* subcultures, respectively, were collected in New York vineyards and incorporated into this study. In 1994 and 1995, California *U. necator* subcultures not completely inhibited on the benomyl-treated leaf disks were reisolated in order to generate new inoculum. The new inoculum was used to perform an additional bioassay on water- and benomyl-treated leaf disks. The purpose was to determine whether the surviving *U. necator* colonies actually were more resistant or had escaped exposure to benomyl. When inhibition by benomyl was less than 90%, the leaf disk surface areas colonized on benomyl- and water-treated leaf disks were assessed for significant differences by means of two-sample *t* tests.

Comparison of EC₅₀ values of DMI fungicides for heterogeneous and homogeneous *U. necator* inoculum sources.

One monoconidial isolate was produced from each of the *U. necator* subcultures collected in 1995 with a previously described method (6). Each monoconidial isolate subsequently was analyzed for EC₅₀ values of triadimefon, myclobutanil, and fenarimol as described in the leaf disk bioassay section. The differences between EC₅₀ values of triadimefon, myclobutanil, and fenarimol were calculated for 59, 58, and 54 pairs, respectively, each consisting of a monoconidial isolate and its parental subculture. The differences were not normally distributed and normality could not be accomplished by transformation. Therefore they were analyzed separately for each fungicide for a median significantly different from zero by means of the signed rank test in the UNIVARIATE procedure of SAS. The probability threshold was 0.05.

EC₅₀ correlations. The monoconidial isolates described in the previous section, and seven additional monoconidial isolates collected in 1991, maintained as described in the resistance monitoring section, were used to determine EC₅₀ correlations. Sixty-five isolates were used to calculate the correlation between EC₅₀ values of triadimefon and myclobutanil, 61 for the correlation between EC₅₀ values of triadimefon and fenarimol, and 61 for the correlation between EC₅₀ values of myclobutanil and fenarimol. In 1996, additional *U. necator* samples were collected as described in the leaf disk bioassay section and cultured from single conidia. Twenty-two isolates were used to calculate the correlation between EC₅₀ values of triadimefon and myclobutanil, 25 for the correlation between EC₅₀ values of triadimefon and fenarimol and 25 for the correlation between EC₅₀ values of myclobutanil and fenarimol. The EC₅₀ values were not normally distributed and normality of the distributions could not be accomplished for all fungicides by transformation. Pairwise correlation coefficients were determined by means of Spearman's rank correlation test in the CORR procedure of SAS.

Table 1. Means and ranges of EC₅₀ values of triadimefon, myclobutanil, and fenarimol for Californian *Ucinula necator* subcultures in 1993, 1994, and 1995, and significance of median differences between each pairwise combination of years

Year	Subcultures (no.) ^y	Fungicide	EC ₅₀ values (mg/liter)	
			Mean	Range
1993	48	Triadimefon	38.1 a ^z	2.24 to 78.8
1994	44	Triadimefon	28.1 b	0.92 to 75.3
1995	50	Triadimefon	18.1 c	0.21 to 42.2
1993	49	Myclobutanil	6.07 a	0.11 to 20.7
1994	44	Myclobutanil	3.17 b	0.31 to 19.9
1995	50	Myclobutanil	3.35 b	0.01 to 19.7
1993	49	Fenarimol	0.86 a	0.09 to 1.68
1994	44	Fenarimol	0.58 b	0.02 to 1.49
1995	50	Fenarimol	0.58 b	0.01 to 1.71

^y Number of subcultures obtained from samples of mildew infected leaf or fruit grape tissue collected from California vineyards.

^z Different letters indicate medians significantly different from those in other years (Wilcoxon-Mann-Whitney two-sample test, $\alpha = 0.0167$). The test was performed separately for each fungicide.

Table 2. Numbers of *Ucinula necator* subcultures collected in 1993, 1994, and 1995, and their origin and percentage of inhibition on leaf disks treated with 30 mg of benomyl per liter^z

Year	Subcultures		Inhibition at 30 mg of benomyl per liter (%)					
	Origin	(no.)	0-50	50-90	90-95	95-99	99-100	100
1993	California	55	0	0	0	6	4	45
1994	California	50	0	0	1	1	0	48
1995	California	60	0	0	0	0	2	58
1993	New York	2	1	1	0	0	0	0
1994	New York	5	5	0	0	0	0	0

^z Percentage of inhibition calculated as difference between average percentage of leaf disk area colonized by *U. necator* 10 days after inoculation on benomyl-treated and untreated leaf disks. Latter difference in colonization was divided by average percentage of colonization of untreated leaf disks and multiplied by 100%. Twenty-four leaf disks served as replications for each treatment and subculture.

RESULTS

Sensitivity to DMI fungicides and benomyl in California in 1993, 1994, and 1995. Mean EC₅₀ values of triadimefon, myclobutanil, and fenarimol decreased from 1993 to 1994 (Table 1). Additionally, the mean EC₅₀ value of triadimefon decreased further from 1994 to 1995. Analysis of medians indicated that decreases were significant. Means of EC₅₀ values of myclobutanil and fenarimol were similar and medians were not significantly different between 1994 and 1995. The range of EC₅₀ values of myclobutanil and fenarimol did not change considerably from 1993 to 1995.

The mean EC₅₀ value of triadimefon for the five subcultures originating from the wild-type Yuba County vineyard in 1993 was 3.37 mg of triadimefon per liter, and

EC₅₀ values ranged from 1.93 to 5.30 mg/liter. In 1994, EC₅₀ values averaged 24.3 mg/liter and ranged from 1.48 to 66.5 mg/liter. In 1995, the mean was 15.2 and the range 6.84 to 30.4 mg/liter. Means of EC₅₀ values of myclobutanil in 1993, 1994, and 1995 were 0.37, 1.63, and 1.49 mg/liter and ranges were 0.12 to 0.62, 0.09 to 6.0, and 0.26 to 4.37 mg/liter, respectively. Means of EC₅₀ values of fenarimol were 0.17, 0.25, and 0.27 mg/liter and ranges were 0.13 to 0.22, 0.08 to 0.72, and 0.10 to 0.47 mg/liter in 1993, 1994, and 1995, respectively. For myclobutanil and fenarimol, the wide ranges and small sample sizes did not reveal significant differences between medians in any pairwise combination of years. For triadimefon, only medians calculated for 1993 and 1995 were significantly different (Wilcoxon-Mann-Whitney two-sample test, $\alpha = 0.0167$).

In 1993, 1994, and 1995, 81.8, 96.0, and 96.7%, respectively, of all subcultures tested were completely inhibited at 30 mg of benomyl per liter (Table 2). When *U. necator* was inhibited by more than 95%, it could not be reisolated from the benomyl-treated leaf disks. In 1994, the subculture demonstrating 90 to 95% inhibition was reisolated successfully and demonstrated only 30% inhibition after the second exposure to benomyl. Colonization of benomyl-treated leaf disks by the latter isolate was not significantly different from that of water-treated leaf disks (two-sample test, $\alpha = 0.05$). Subcultures from New York examined in 1993 and 1994 were less inhibited by benomyl than the subcultures from California. The two New York subcultures examined in 1993 were inhibited by 43 and 53%. Colonization of benomyl-treated leaf disks by either subculture was significantly less than colonization of water-treated controls (two-sample test, $\alpha = 0.05$). In 1994, only two of the five New York subcultures were inhibited by benomyl. One showed 14% inhibition and the other 27%, but the surface area colonized on benomyl-treated leaf disks did not differ from that on the respective control discs (two-sample test, $\alpha = 0.05$). The other three New York subcultures were not inhibited by benomyl.

Comparison of EC₅₀ values of DMI fungicides for heterogeneous and homogeneous *U. necator* inoculum sources. Fifty-nine monoconidial isolates were subcultured successfully from the respective heterogeneous parent subcultures. The means of EC₅₀ values of myclobutanil and fenarimol for the monoconidial isolates were lower than those for the heterogeneous subcultures, but the differences were significant only for fenarimol (Table 3). For triadimefon, the difference between EC₅₀ values for these two groups was very small. For triadimefon, one monoconidial isolate had an EC₅₀ value over 35 mg/liter, whereas three parent subcultures had EC₅₀ values over 35 mg/liter. Four of 58 subcultures had EC₅₀ values of myclobutanil

between 7 and 20 mg/liter, but EC₅₀ values for all monoconidial isolates were less than 7 mg/liter. Seven out of 54 monoconidial isolates, with parent subcultures possessing

EC₅₀ values of fenarimol ranging from 0.3 to 1.8 mg/liter, had EC₅₀ values less than 0.2 mg/liter. Only one parent isolate, with an EC₅₀ value of less than 0.2 mg/liter,

Table 3. Means and ranges of EC₅₀ values of triadimefon, myclobutanil, or fenarimol (mg/liter) for heterogeneous and monoconidial *Uncinula necator* isolates, and the average of differences between EC₅₀ values for each pair of heterogeneous and monoconidial isolates

Fungicide	Number ^w	EC ₅₀ values(mg/liter)				
		Heterogeneous subcultures		Monoconidial isolates ^x		Avg. pairwise difference ^y
		Means	Range	Means	Range	
Triadimefon	59	17.64	0.21 to 42.2	17.29	0.68 to 48.9	0.35
Myclobutanil	58	3.12	0.01 to 19.7	2.28	0.07 to 6.39	0.84
Fenarimol	54	0.57	0.01 to 1.71	0.44	0.01 to 1.76	0.13***z

^w Number of subcultures and monoconidial isolates examined.

^x Each monoconidial was derived from its heterogeneous parent subculture.

^y Differences were calculated by subtraction of the EC₅₀ value for each monoconidial isolate from that of its respective parent subculture and taking the average of all differences.

^z Median difference significantly different from zero (signed rank test, $\alpha = 0.05$).

Fig 1. Pairwise correlations between EC₅₀ values for *Uncinula necator* of triadimefon and myclobutanil in (A) 1995 and (B) 1996, of triadimefon and fenarimol in (C) 1995 and (D) 1996, and of myclobutanil and fenarimol in (E) 1995 and (F) 1996. All correlations were significant ($P < 0.01$, Spearman's rank correlation).

produced a monoconidial isolate with an EC₅₀ value higher than 0.3 mg/liter. These observations suggested that the analysis of monoconidial isolates reduced the chances of detecting the least sensitive individuals with EC₅₀ values of triadimefon, myclobutanil, and fenarimol, exceeding 35, 7, and 1 mg/liter, respectively.

EC₅₀ correlations. In 1995 and 1996, all correlation coefficients were positive (Fig. 1; $P < 0.01$). For the isolates evaluated in 1996, triadimefon, myclobutanil, and fenarimol had mean, minimum, and maximum EC₅₀ values of 5.73, 0.63, and 23.7 mg/L, 0.80, 0.01, and 3.37 mg/L, and 0.21, 0.01, and 1.01 mg/L, respectively.

DISCUSSION

Resistance to benomyl is present in the California subcultures tested, but at much lower frequencies than in the New York subcultures. The average inoculum density used in the leaf disk bioassays was about 2,500 conidia/cm². Similar percentages of leaf disk surface area may be colonized as a result of inoculation with conidial densities of 120 and 2,500/cm² (H. L. Ypema, unpublished data). Therefore, colonization of leaf disks treated with 30 mg of benomyl per liter could be similar to that of control leaf disks when at least 5% (120/2,500) were benomyl-resistant. This demonstrates persistence of benomyl-resistant individuals under absence of the fungicide. In 1993, inoculations with 55 California *U. necator* subcultures resulted in 10 colonies on a total of 1,320 benomyl-treated leaf disks. In 1995, inoculations with 60 subcultures resulted in two colonies on a total of 1,440 benomyl-treated leaf disks. The small size of the observed colonies suggested that they originated from single conidia. If all the conidia inoculated onto the leaf disks are considered to be independent with respect to benomyl-resistance, the frequency of benomyl-resistant conidia was 9.5×10^{-6} in 1993 and 1.8×10^{-6} in 1995. If we assume that only the conidia of the subcultures from which the benomyl-resistant colonies originated were independent, frequency of benomyl-resistant conidia would have been 5.3×10^{-5} in both years. Benomyl has been used since 1972 to control gray mold on grapes, and *U. necator* in California therefore may have been exposed to benomyl inadvertently for several years. The results of this study do not indicate that the prior use of benomyl has resulted in an increased frequency of benomyl-resistant *U. necator* in California. In field experiments performed in 1994, benomyl provided excellent control of grape powdery mildew (W. D. Gubler, unpublished). However, resistance developed in New York and control failure resulted after only a few years of benomyl use (20).

Resistance monitoring in California in 1993, 1994, and 1995 indicated reductions in means of EC₅₀ values of triadimefon,

myclobutanil, and fenarimol from 1993 to 1994, and of triadimefon from 1994 to 1995. In the case of triadimefon, one reason for reductions in two consecutive years may have been that triadimefon has not been used frequently in the vineyards from which the subcultures were collected. This may have led to a reduction in frequency of individuals possessing the lowest sensitivity to triadimefon. The reduction in means of EC₅₀ values of myclobutanil and fenarimol occurred only from 1993 to 1994. Both products are still used frequently, but in alternation with sulfur. In 1994 and 1995, powdery mildew epidemics appeared later in the growing season than in 1993, due primarily to more unfavorable temperatures throughout California. Since the rate of asexual reproduction decreases at temperatures above the optimum of 25°C (5), the selection rate for the least sensitive individuals may have been lower in the growing seasons of 1994 and 1995 than in that of 1993.

In 1993, EC₅₀ values of the three DMI fungicides for subcultures from the Yuba County vineyard were similar to those found in 1990 (8). In 1994, they were much higher and covered a much broader range than in 1993 in the absence of DMI fungicide use. Subcultures were collected at the same site in both years, but vines adjacent to this site were grafted with plant material obtained from Oregon and from Kern and Sonoma counties in California early in 1994. In 1995, the subcultures were collected from three sites in this vineyard and higher EC₅₀ values of the three DMI fungicides were found in all three. The increase in EC₅₀ values suggested introduction and establishment of DMI-resistant *U. necator* in the vineyard. Wind dispersal of conidia over long distances has not been demonstrated for *U. necator*, but has been reported for *E. graminis* f. sp. *hordei* in Northern Europe (9). The Yuba County vineyard is separated from the closest neighboring vineyard by 20 km and establishment of wind-dispersed, DMI-resistant *U. necator* may be a rare event. The observations in the Yuba County vineyard suggest that long-distance transport of DMI-resistant inoculum is possible in the form of dormant mycelium in grape buds (24), or cleistothecia on the bark of plant material (7,19). Irrespective of their means of introduction, the introduced strains appeared to be sufficiently competitive to become established in the vineyard. Vineyard managers may emphasize control of powdery mildew in vineyard locations where vines from elsewhere were introduced either by planting or grafting. Emphasis on control of *U. necator* on imported plant material does not only concern introduction of strains with decreased DMI sensitivity, but also strains resistant to benomyl.

Leaf disk bioassays performed with heterogeneous subcultures resulted in a higher

number of EC₅₀ values exceeding 35, 7, and 1 mg/liter for triadimefon, myclobutanil, and fenarimol, respectively, than did assays performed with monoconidial isolates. The frequency of these least sensitive individuals in the subcultures may have been low, but their presence in the subcultures may have increased the magnitude of the generated EC₅₀ values because of their ability to grow on leaf disks treated with higher fungicide concentrations. The presumed low frequencies of the least sensitive individuals would have limited their chances of selection in the process of culturing from single conidia. A lower frequency of the least sensitive individuals in the monoconidial isolates also may explain why the mean EC₅₀ values of triadimefon, myclobutanil, and fenarimol were generally lower for the monoconidial isolates than for the heterogeneous subcultures. Whether heterogeneous subcultures or monoconidial isolates should be used in the leaf disk bioassay depends on the purpose for which it is used. An investigation focusing on detection of highest levels of resistance may take advantage of the larger sample size in *U. necator* subcultures. However, genetic studies, sensitivity correlation studies, and studies of population structure require monoconidial isolates in order to more accurately determine the frequency of a trait in a population.

The differing sensitivity of *U. necator* subcultures to triadimefon, myclobutanil, and fenarimol reported in a previous study (8) was confirmed in this study and was also apparent in monoconidial *U. necator* isolates. Reduced efficacy has been reported more frequently for triadimefon than for myclobutanil and fenarimol (8, 18), which can be explained by the lower inherent activity of triadimefon and its longer history of use, compared with myclobutanil and fenarimol. The primary target site of triadimefon, myclobutanil, fenarimol, and other DMI fungicides is inhibition of C-14 demethylation in fungal sterol biosynthesis (14,26). Inhibition of Δ^{22} -desaturase in ergosterol biosynthesis of *Ustilago maydis* has been reported as an additional target site of triarimol, a pyrimidine with a chemical structure similar to that of fenarimol (22). However, ergosta-5,24(24')-dien-3 β -ol has been reported as the principal sterol in *Podosphaera leucotricha*, *Sphaerotheca fuliginea*, and *E. graminis* f. sp. *hordei* (17). The functional sterol in *E. graminis* f. sp. *hordei* has also been described as $\Delta^{5,24,(28)}$ -ergostadienol and Δ^{22} -desaturation has not been reported to occur in the biosynthesis of this sterol (1). It may be that sterol biosynthesis in *U. necator* follows pathways more similar to those in *E. graminis*, *P. leucotricha*, and *S. fuliginea* than to those in other fungi. Even if action mechanisms of triadimefon, myclobutanil, and fenarimol do not differ in *U. necator*, mechanisms conferring resistance to each fungicide may be different.

Several mechanisms of resistance to DMI fungicides have been reported in fungi, such as reduced uptake in *Aspergillus nidulans* (3), *Penicillium italicum* (4), and *Septoria tritici* (11), and modifications in sterol synthesis in other fungi (14). Which of these mechanisms is involved in DMI resistance of powdery mildew fungi is unknown. Analysis of *E. graminis* f. sp. *hordei* suggested that neither altered sterol metabolism, reduced uptake, nor triadimenol metabolism played a major role in triadimenol resistance (10). At present, differences in inherent activity of triadimefon, myclobutanil, or fenarimol cannot be explained by differences in additional target sites or resistance mechanisms.

All pairwise correlations were highly significant and indicated that cross resistance existed among triadimefon, myclobutanil, and fenarimol. This result confirmed the previously reported indications of cross resistance to DMI fungicides on a smaller number of *U. necator* isolates (27). Cross resistance also provides an explanation for a previously reported decrease in sensitivity to triadimefon, myclobutanil, and fenarimol in *U. necator* in a vineyard site during a period in which only triadimefon was applied (8). The use of triadimefon on grapes in California since 1982 may have selected not only for *U. necator* with decreased triadimefon sensitivity, but also for *U. necator* with decreased sensitivity to myclobutanil and fenarimol. Because most California vineyards have been subjected to applications of all three DMI fungicides, reduced DMI sensitivity of *U. necator* could have been acquired through both independent selection and cross resistance. At concentrations used commercially, myclobutanil and fenarimol may suppress the least sensitive isolates sufficiently to prevent an epidemic, but they may select for *U. necator* less sensitive to all three DMI fungicides if control is not complete. To control *U. necator* and manage resistance to triadimefon successfully, fenarimol and myclobutanil should be used at concentrations that also suppress *U. necator* populations with reduced sensitivity, a strategy that showed promise in control of *Venturia inaequalis* with decreased sensitivity to fenarimol (15). Sufficient coverage and alternation of DMI fungicides with broad spectrum fungicides should further delay development of DMI resistance.

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