Comparative Physical Modes of Action of Azoxystrobin, Mancozeb, and Metalaxyl Against *Plasmopara viticola* (Grapevine Downy Mildew)

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ABSTRACT

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The physical modes of action of azoxystrobin, mancozeb, and metalaxyl were evaluated on grapevine seedlings using *Plasmopara viticola* as a model pathogen. The protectant, postinfection, postsymptom, translaminar, and vapor activities of azoxystrobin were evaluated at a rate of 250 µg/ml. Azoxystrobin provided 100% disease control when applied 1 to 5 days before inoculation. Postinfection applications of azoxystrobin had little effect on the incidence of disease, but colony area and sporulation from the resultant lesions was reduced by 47 and 96%, respectively, relative to the check treatment when applied up to 5 days after inoculation. Postsymptom applications (6 days after inoculation) of azoxystrobin resulted in an 85% mean reduction of resporulation from diseased tissue relative to the check when seedlings were evaluated 1 to 14 days after treatment. Translaminar activity was greatest when the upper surface of the leaf was treated 7 days before inoculation of the lower leaf surface (94% disease control). In contrast, control was <50% when leaves were similarly inoculated 1 and 3 days after treatment. Vapor activity was not pronounced, providing maximum reductions of 5, 11, and 37%, with regard to incidence, colony area, and sporulation, relative to the check when seedlings were treated 1 to 7 days before inoculating adjacent, untreated leaves. Comparatively, mancozeb (1,790 µg/ml) provided complete control of the disease when applied 1 to 5 days before inoculation, but showed little postinfection activity in reducing disease incidence, although it exhibited moderate to high antispore activity when applied in postinfection and postsymptom modes (mean reductions of 38 and 89%, respectively, compared with the check treatments). Metalaxyl (260 µg/ml) also provided complete control of the disease when used in protectant mode, and also when applied 1 day after inoculation. Applications at 3 to 5 days after inoculation provided substantial reductions in disease severity and sporulation (mean reductions of 46 and 94%, respectively, compared with the check treatments), and postsymptom applications resulted in a mean 84% reduction in resporulation. Collectively, the results of this study illustrate the unique physical modes of action for azoxystrobin in comparison to that of two traditional protectant and systemic fungicides, and provide information on how azoxystrobin and other strobilurin fungicides with similar physical modes of action should be best used in disease management programs.

Plasmopara viticola (Berk. & Curt.) Berl. & Toni., the causal agent of grapevine downy mildew, is a highly destructive pathogen on cultivated grapevine worldwide, especially in areas where warm, wet weather occurs during the production season (10). The primary component of virtually all commercial management programs for this disease is the use of organic and inorganic fungicides. Among the fungicides recently made available for downy mildew control are the strobilurins, a new class of crop protection chemicals currently used for the management of a wide range of fungal diseases. These are unique in the sense that they are the first modern,

synthetic, site-specific fungicides to provide commercially acceptable control of fungal pathogens from all three major divisions of fungi: Oomycota, Ascomycota, and Basidiomycota (1,2,7,9,11,14).

Although the biochemical mode of action for these fungicides is well characterized (3,5,18), there is relatively little published data on their physical modes of action. This term, as described by Szkolnik (17) and elaborated upon by Köller (12), is used in reference to the observable effects of a fungicide on the host-pathogen interaction with respect to the timing and/or placement of the fungicide application, e.g., protectant, postinfection (curative), postsymptom, and vapor activities. The strobilurin fungicides also exhibit what has been termed translaminar activity (6,7,27), defined as the ability of a fungicide to penetrate through leaf tissue from a sprayed to an unsprayed lamina at concentrations sufficient to provide disease control on the unsprayed side. Understanding the extent of physical modes of action for

individual fungicides is essential for optimizing their use in disease management programs.

The strobilurin-pathogen interactions best described in the literature include azoxystrobin against *Pseudoperonospora cubensis* (9), *Puccinia recondita* (6), and *Sphaerotheca fuliginea* (9); and kresoxim-methyl against *Erysiphe graminis* var. *tritici* (7,8), *Puccinia recondita* (7,8), *Uncinula necator* (7,13), *Uromyces appendiculatus* (7), and *Venturia inaequalis* (7,8). Many of these reports include some information concerning physical modes of action, although the details of experimental procedures and/or quantitative data relative to this topic are frequently incomplete or lacking. Furthermore, there are no detailed reports concerning the physical modes of action for any of the strobilurin fungicides against the intercellular, oomycete pathogen, *P. viticola*.

Therefore, the objective of this study was to evaluate the physical modes of action (protectant, postinfection, postsymptom, translaminar, and vapor activities) of azoxystrobin against *P. viticola*, and to compare these with the activities of standard fungicides (mancozeb and metalaxyl), whose physical modes of action are generally well understood (17,19), using a seedling bioassay system. Azoxystrobin was chosen due to its well-characterized level of intrinsic activity against *P. viticola* (25), its effectiveness for the control of grapevine downy mildew in field trials (20-23), and its importance for disease control programs in the eastern United States, where it is the first fungicide available that provides commercially acceptable control of *Gignardia bidwellii*, *P. viticola*, and *U. necator*, the three most economically important pathogens of grapes in this region.

MATERIALS AND METHODS

Fungicides. Commercial formulations of azoxystrobin (Abound, 2.08 F, Zeneca Ag Products, Richmond, CA), mancozeb (Dithane 75DF, Rohm and Haas, Philadelphia, PA), and metalaxyl (Ridomil 2E, Novartis Crop Protection, Greensboro, NC) were used in all assays unless noted otherwise. Fungicides were applied at rates equivalent to those used in dilute (936 liters/ha) applications under field conditions. For each experiment, formulated product was mixed in 50 ml of dH₂O to obtain final fungicide concentrations of 250 µg/ml for azoxystrobin, 1,790 µg/ml for mancozeb, and 260 µg/ml for metalaxyl.

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For seedling bioassays, unless otherwise noted, leaves were sprayed until runoff on both the upper and lower surfaces, using a modified paint sprayer attached to a 50-ml centrifuge tube containing the fungicide solutions or dH₂O (check treatment), operating at a pressure of 206 kPa. The apparatus was cleaned thoroughly between the different fungicide treatments by rinsing the exterior under running water and then running approximately 50 ml of a dilute solution of liquid soap and dH₂O through the sprayer, followed by 50 ml of 50% acetone and then 50 ml of dH₂O.

Collection and maintenance of *P. viticola*. A single isolate of *P. viticola* (DM-15) was used for all assays, unless noted otherwise. This isolate was in the top quartile of a *P. viticola* population previously characterized (25) for sensitivity to azoxystrobin (DM-15, ED₅₀ = 0.56 µg/ml, mean baseline ED₅₀ = 0.24 µg/ml). It was maintained in petri dishes on greenhouse-grown *Vitis vinifera* cv. Gewürztraminer leaf sections (approximately 30 × 60 mm), with weekly transfers to fresh tissue, as described previously (25). An additional isolate (AR-1, ED₅₀ = 0.35 µg/ml) was maintained in the same manner for use in oospore-formation experiments. To obtain adequate amounts of inoculum for the experiments, isolates were amplified by inoculating several Gewürztraminer leaf sections 1 week beforehand (25).

Seedlings. Stratified, open-pollinated seeds of *V. vinifera* cv. Riesling were germinated under continual mist in a greenhouse at 30°C and transplanted subsequently into 6.4-cm square pots filled with a mixture of three volumes Cornell Mix and one volume sand. Seedlings were grown at 20°C and 80% relative humidity (RH) in a pathogen-free growth chamber (Percival, Perry, LA) with alternating 12-h periods of fluorescent light and darkness, unless otherwise noted. Seedlings that were approximately 6 weeks old, having three fully expanded leaves (greater than 2.5 cm in width), were used in the bioassays. Subsequent to experimental fungicide treatments, seedlings were maintained in a separate growth chamber at 20°C and 80% RH.

Seedling bioassays. Inoculation of seedlings. Unless otherwise stated, seedlings were suspended upside down in a walk-in mist chamber at 20°C immediately before inoculation. The chamber was allowed to mist for a minimum of 1 h before placing the seedlings inside, to allow it to equilibrate. A single 10-µl drop containing a suspension of 2×10^4 sporangia of isolate DM-15 per milliliter was applied to each of the four quadrats on the underside of each of the three fully expanded leaves per seedling. The inoculum was obtained by placing two to three sporulating leaf sections, cut into approximately 10-mm-wide pieces, in a 15-ml screw-top centrifuge tube containing 5 ml of sterile dH₂O, and briefly vortexing for 15 s. This initial con-

centration of sporangia was determined with the aid of a hemacytometer and diluted with sterile dH₂O to obtain the appropriate inoculum concentration. Inoculated seedlings were maintained in the mist chamber overnight in darkness, then removed and allowed to dry for approximately 2 h at room temperature before they were returned to the appropriate growth chamber.

Five days after inoculation, seedlings were placed into the mist chamber and incubated overnight in darkness at 20°C, to allow for the completion of disease development and promote the formation of sporangia. Disease was evaluated 6 days after inoculation based upon disease incidence, colony area, and sporulation intensity. Disease incidence was measured as the number of lesions that developed per seedling. Colony area was calculated as the surface area of the individual lesions formed, based upon the average of three measurements of diameters across each generally circular lesion, and applying the formula $SA = \pi(\text{diameter}/2)^2$. Sporulation intensity was determined by removing the sporulating areas of each leaf with a scalpel and forceps, and vortexing these excisions in 2 ml of a 0.05% solution of Tween 20 for 30 s in a 15-ml screw-top centrifuge tube. Sporangia concentrations were measured with the aid of a hemacytometer, using the average of three repeated measurements per sample.

Statistical analyses (ANOVA, multiple comparisons, and linear regression) were performed using MINITAB (v. 12.10, State College, PA) and SAS (v. 6.0, SAS Institute, Cary NC). The means of each experimental run of six replicate seedlings per treatment were used for the statistical analyses. Colony area data were normalized using a square-root transformation, and sporulation data were normalized using the $\log_{10}(y + 1)$ transformation function. The general linear model was used for analysis of variance, and either Duncan-Waller groupings or Dunnett's multiple comparisons were used for multiple comparisons.

Protectant and postinfection activities. Seedlings were sprayed with either azoxystrobin, mancozeb, or metalaxyl at 1, 3, or 5 days before inoculation with *P. viticola*, in order to determine the protectant activities of these fungicides. Similarly, seedlings were sprayed 1, 3, or 5 days after inoculation to determine postinfection activities. For each experimental run, the check treatment consisted of seedlings sprayed only with dH₂O 1 day before inoculation. Seedlings were allowed to dry at room temperature, and afterward they were moved to a growth chamber. Seedlings subjected to individual treatments were spatially separated in the growth chamber by a minimum of 30 cm, to minimize cross-treatment interference. Fungicides were tested in separate experiments, and

each set of these was performed three times, using six replicate seedlings per treatment. Analysis of variance and multiple comparisons were performed as described above, analyzing the effect of each fungicide separately.

Postsymptom activity. To evaluate the ability of the fungicides to arrest resporulation from diseased tissue, and to identify the duration of effectiveness when applied in this manner, seedlings were inoculated and induced to sporulate 5 days after inoculation, as described above. Six days after inoculation, sporangia were removed from the leaf underside by flushing the tissue with a steady stream of dH₂O (approximately 10 ml/s for 10 s for each leaf). Seedlings were allowed to dry at room temperature for 2 h, then sprayed with fungicide solutions (or dH₂O) and again allowed to dry at room temperature for 2 h. Then, seedlings were moved to a growth chamber and either 1, 3, 5, 7, 10, or 14 days later, sets of six seedlings for each time point were placed into the mist chamber to induce resporulation. Twenty-four hours after placement into the mist chamber, one infected leaf per seedling, each of approximately uniform size and developmental stage, was removed, and sporulation intensity was quantified as described above. The experiment was run three times, using six replicate seedlings per treatment. Analysis of variance and regression analysis were performed as described above.

Translaminar activity. To evaluate translaminar activity, the adaxial surface of the second fully expanded leaf on each seedling was treated by floating the attached, intact leaves for 5 min on 15 ml of the appropriate fungicide solution (or dH₂O for the check treatment) contained in 60-mm-diameter petri dishes. Surface tension prevented the solutions from contacting the abaxial leaf surface, and special care was taken to prevent any incidental contact of the abaxial surface with fungicides. Leaves were allowed to dry for 10 min at room temperature before returning them to the growth chamber, then they were inoculated with *P. viticola* as described above, either 1, 3, 5, or 7 days after treatment (1 day for the dH₂O check). Five days postinoculation, seedlings were placed into the mist chamber, and 24 h later, incidence, colony expansion, and sporulation intensity were evaluated as described above. The experiment was performed a total of three times, using six replicate seedlings per treatment. Analysis of variance and regression analysis were performed as described above.

Vapor activity of azoxystrobin. To test the vapor redistribution to tissues distal to the point of application, the second fully expanded leaves (distal to the shoot tip) of individual seedlings were treated with azoxystrobin (or dH₂O 1 day before inoculation), as described for the translaminar

activity assays. Treated seedlings were maintained inside the growth chamber at 20°C, 80% RH, then leaves one position above the treated leaf were inoculated either 1, 3, 5, or 7 days after treatment. Seedlings were incubated and evaluated for disease 6 days after inoculation, as described previously. To assay the vapor activity of azoxystrobin under optimal conditions, the experiment was modified by transferring fungicide-treated seedlings to translucent plastic boxes (51 cm wide × 102 cm long × 30 cm tall) with tight fitting lids (Rubbermaid, Wooster, OH), where they were maintained for the duration of the experiment (except for the overnight period in the mist chamber following inoculation). Seedlings were evaluated for disease 6 days after inoculation, as described previously. Both sets of experiments were performed a total of three times, using six replicate seedlings per treatment in each run. Analysis of variance and regression analysis were performed as described above.

Effect of postinfection and postsymptom fungicide applications on oospore formation. To test the ability of azoxystrobin, mancozeb, and metalaxyl to retard or halt the activity of *P. viticola* once it had become established within the leaf tissue, the effect of postinfection and postsymptom applications of fungicides on oospore formation was evaluated. Using standard procedures, seedlings were inoculated with a mixture containing equal ratios of two isolates of *P. viticola* (DM-15 and AR-1), which represent opposite mating types of the fungus and had produced oospores in previous experiments when co-inoculated onto detached leaf disks (24). To evaluate postinfection applications, seedlings were treated with fungicides (or dH₂O) 3 days after inoculation. Five days after inoculation, seedlings were placed in the mist chamber overnight to allow for the completion of the normal, secondary disease cycle for *P. viticola*, as described previously. Seedlings were then transferred to a growth chamber maintained at 12°C and 80% RH, with alternating 12-h periods of darkness and fluorescent light, to promote oospore formation (15). The experiment was performed three times using six replicate seedlings per treatment.

To evaluate postsymptom activities, inoculated seedlings were returned to the mist chamber 5 days after inoculation, then they were removed the following day and treated with fungicides (or dH₂O) after they had dried. Seedlings were allowed to re-dry for 2 h after treatment, then they were transferred to a growth chamber maintained at 12°C, 80% RH, with alternating 12-h periods of darkness and fluorescent light. This experiment was also performed three times using six replicate seedlings per treatment.

For both the postinfection and postsymptom experiments, seedlings were

Fig. 1. Postinfection activities of azoxystrobin, mancozeb, and metalaxyl versus *Plasmopara viticola*. For each test, one fungicide was sprayed onto six individual *Vitis vinifera* cv. Riesling seedlings per treatment either 1, 3, or 5 days after inoculation. Disease was assessed 6 days after inoculation following overnight incubation in darkness and high humidity to promote completion of the disease cycle. The experiment was performed three times for each fungicide. **A**, Disease incidence, expressed as the percentage of sporulating lesions per seedling relative to the dH₂O check treatment. **B**, Colony area, expressed as percentage of the surface area of lesions on fungicide-treated seedlings relative to those in the dH₂O check treatment. **C**, Sporulation, expressed as percentage of sporangia produced relative to the dH₂O check treatment. Within each fungicide group, columns designated with different letters are significantly different by the Duncan-Waller grouping ($P = 0.05$), and columns designated by (*) were significantly different from the check treatment (Dunnett's multiple comparison, $P = 0.05$).

removed from the growth chamber after 14 days of incubation at 12°C. Previous experiments showed that oospore formation under these conditions required approximately 10 days (24). All of the lesions that had formed on seedlings were removed, using a 10-mm-diameter cork borer. In cases where the generally circular lesions were greater than 10 mm in diameter, the center of the lesion was removed. All leaf disks from each seedling were placed into a 15-ml screw-top centrifuge tube containing 10 ml of 95% ethanol for 72 h to clear the tissue in order to aid in the detection of oospores. To quantify the number of oospores formed, four cleared leaf disks were chosen arbitrarily from each sample and rinsed in dH₂O. The total number of oospores formed in each leaf disk was counted using light microscopy at ×100 magnification. Analysis of variance and multiple comparisons were performed as described above, using the log₁₀(y + 1) transformed values of the number of oospores formed to normalize the data.

RESULTS

Protectant and postinfection activities. All three fungicides provided 100% disease control when applied in protectant mode from 1 to 5 days before inoculation. However, there were marked differences in disease control when the fungicides were applied after inoculation with *P. viticola*. For example, azoxystrobin provided modest (33%) reductions in disease incidence relative to the check treatment when applied 1 day postinoculation, whereas mancozeb provided no control and metalaxyl provided complete control (Fig. 1A). How-

ever, none of the fungicides provided significant control of disease incidence when applied 3 or 5 days after inoculation (Fig. 1A). Azoxystrobin also substantially restricted the development of those infections that did occur following postinoculation treatments, reducing the mean lesion area relative to the checks by 72, 33, and 45% when applied 1, 3, and 5 days after inoculation, respectively. As with disease incidence, these reductions were greater than those provided by mancozeb, which had no significant effect on lesion development, but less than those provided in separate tests by comparable sprays with metalaxyl (Fig. 1B). The most pronounced postinfection activity provided by azoxystrobin was with respect to sporulation from those lesions that developed subsequently, which was reduced by 86 to 96% relative to the check, with no significant difference in this effect among any of the three application timings. Metalaxyl similarly inhibited sporulation from those lesions that developed following the 3- and 5-day postinoculation sprays, whereas mancozeb reduced sporulation by only 33 to 43% across the three timings (Fig. 1C).

Postsymptom activity. All three fungicides provided pronounced and basically equivalent postsymptom activity when seedlings were evaluated from 1 to 14 days after fungicide treatment. Resporulation from fungicide-treated leaves was reduced by 77 to 98% relative to seedlings treated with dH₂O, depending on the individual treatment; in general, the greatest percent reductions occurred following the 10- and 14-day incubation periods (Fig. 2). The mean numbers of sporangia recovered

from fungicide-treated seedlings were significantly different from the check treatment ($P < 0.001$) but equivalent to each other regardless of evaluation date ($P = 0.23$).

Translaminar activity. Applications of azoxystrobin to the upper leaf surface resulted in a reduction in disease following inoculation of the lower leaf surface, the magnitude of which increased in proportion to the time elapsed between treatment and inoculation. Disease development on treated seedlings was significantly different from the dH₂O-treated checks at all time points tested ($P < 0.001$), and the response was linear over time for reductions in incidence ($P < 0.001$), colony area ($P < 0.001$), and sporulation intensity ($P = 0.02$). For example, reductions in disease incidence (relative to the check treatment) increased from 57% when the fungicide was applied 1 day before inoculation to 94% when applications were made 7 days before inoculation (Fig. 3A), with similar reductions (63 to 92%) observed for the effect on colony area (Fig. 3B). As found with the assays for postinfection activity, translaminar activity was expressed most strongly with respect to its effect on the production of sporangia from the resultant lesions, i.e., sporulation was reduced by 93 to 100% for the four azoxystrobin treatments (Fig. 3C). In comparison, mancozeb had no effect on the development of downy mildew when applied in this manner, whereas metalaxyl provided complete control (Fig. 3A to C).

Vapor activity of azoxystrobin. Apparent vapor activity of azoxystrobin on treated seedlings resulted in a time-dependent reduction in disease development with maximum reductions occurring when they were inoculated 7 days after treatment. Under optimal conditions, there was a linear response for disease development over the four time points tested with regard to disease incidence ($P = 0.03$), colony area ($P < 0.001$), and sporulation ($P < 0.001$). The effect on disease incidence was small, ranging from a 0 to 14% reduction from when seedlings were inoculated 1 day to 7 days after treatment (Fig. 4A). Colony area and sporulation were reduced by 52 and 81% relative to the check, respectively, when seedlings were inoculated 7 days after treatment (Fig. 4B and C).

For seedlings tested under open conditions (when they were not placed into closed plastic boxes), the effect of the vapor activity of azoxystrobin was less pronounced. There was no significant effect on disease incidence over the time points tested ($P = 0.83$), but linear reductions in colony area and sporulation over time were observed ($P = 0.02$ and 0.004 , respectively) (Fig. 4B and C). The strongest effects on both colony area and sporulation occurred (reductions of 11 and 37%, relative to the check, respectively) when seedlings were inoculated 7 days after treatment.

Fig. 2. Postsymptom activity of azoxystrobin, mancozeb, and metalaxyl versus *Plasmopara viticola*. For each test, six individual *Vitis vinifera* cv. Riesling seedlings were treated with fungicides 6 days after inoculation and following overnight incubation in darkness and high humidity to promote sporangium formation. These initial sporangia were flushed from the leaves immediately before fungicide applications, then re-sporulation was assessed 1, 3, 5, 7, 10, or 14 days later, following an additional overnight incubation period in darkness and high humidity. Resporulation is expressed as percentage of sporangia produced relative to the H₂O check treatment mean values for the repeated runs of the experiment.

Effects of postinfection and postsymptom fungicide applications on oospore formation. The assay used to determine the effect of fungicides on oospore formation produced variable results, with no significant differences detected among treatments ($P = 0.09$ and 0.33 for the postinoculation and postsymptom assays, respectively). Nevertheless, the mean number of oospores detected following treatment with azoxystrobin was reduced by 41% relative to the check in the postinoculation assay ($P = 0.07$, Dunnett's multiple comparisons) and by 53% in the postsymptom assays ($P = 0.82$) (Fig. 5). In comparison, oospore numbers were reduced by 53 and 29% ($P = 0.08$ and 0.73 , respectively) following treatment with metalaxyl in these respective assays, whereas reductions were 37% ($P = 0.09$) and 0% for mancozeb, respectively (Fig. 5).

DISCUSSION

It is apparent from the results of this study that the primary physical mode of action for azoxystrobin against *P. viticola* is that of a protectant fungicide with strong antispore activity. Azoxystrobin provided complete control of downy mildew when applied up to 5 days before inoculation, although the persistence of protective activity was not tested beyond this duration. In contrast, postinfection applications of azoxystrobin had only modest to negligible effects on the subsequent development of *P. viticola* lesions, providing a greater inhibition of disease severity (colony area) than of disease incidence. However, postinfection and postsymptom applications profoundly limited sporulation of the fungus from these lesions. Based on these results, we suggest that azoxystrobin controls *P. viticola* primarily on the surface of treated tissues, by (i) preventing infections via an inhibition of sporangium and/or zoospore germination (26) and (ii) restricting the production of secondary inoculum (sporangia) from those lesions that do develop, perhaps as the pathogen encounters the fungicide when it erupts through the treated zone during the sporulation process.

In addition to these surface phenomena, it appears that azoxystrobin also provides some modest but measurable activity against *P. viticola* within leaf tissues. This conclusion is supported not only by the above-mentioned results from our postinfection assays, but also by those from our previous study (25), in which we noted that relatively low concentrations of azoxystrobin similarly restricted the expansion of *P. viticola* lesions that were otherwise able to form within treated leaf disks. Although the mechanism governing this relationship is uncertain, it is possible that limited amounts of the fungicide might diffuse from the cuticle into the leaf (6) at concentrations sufficient to restrict growth

of the fungus yet too low to substantially eliminate it. Results from the oospore-formation assay, although variable and inconclusive, are nevertheless consistent with this hypothesis.

Although oospore formation can be a viable method for examining the activity of fungicides within leaf tissue against *P. viticola*, as reported in a similar study on dimethomorph (4), the test used in this

Fig. 3. Translaminal activity of azoxystrobin, mancozeb, and metalaxyl versus *Plasmopara viticola*. For each test, six individual *Vitis vinifera* cv. Riesling seedlings were treated with fungicides on the adaxial surface of the second fully expanded leaves either 1, 3, 5, or 7 days before inoculation of the abaxial leaf surface. The test was performed three times for each fungicide. **A**, Disease incidence, expressed as percentage of sporulating lesions per seedling relative to the dH₂O check treatment. **B**, Colony area, expressed as percentage of the surface area of lesions on fungicide-treated seedlings relative to those in the dH₂O check treatment. **C**, Sporulation, expressed as percentage of sporangia produced relative to the dH₂O check treatment.

study was not reliable enough to effectively detect such differences between the fungicides tested in this study. Changes in methodologies to produce oospores more

uniformly in planta would be needed to use this type of assay to produce clear results. Nevertheless, the results suggest that even a highly systemic fungicide (metalaxyl in

this case) cannot completely eradicate this fungus after it has become well established inside the leaf tissue. Practically, this underlines the concept that "eradicator" use of fungicides has its limitations, and this mode of application should be minimized under field conditions for the most effective control of fungal plant pathogens.

Additional evidence for limited movement of azoxystrobin through treated leaf tissues was provided in the tests for translaminar activity, i.e., the extent of disease control increased directly as a function of the time elapsed between treatment of the adaxial lamina and subsequent inoculation of the abaxial, culminating in virtually complete control when inoculation was delayed until 7 days after treatment. This suggests that azoxystrobin may be diffusing through the leaf from the treated surface and accumulating in the cuticle of the untreated surface at concentrations sufficient to inhibit the initiation of infection. Alternatively, the fungicide may be accumulating over time within the leaf until its concentration is high enough to inhibit the development of the fungus shortly after it penetrates. Interestingly, two characteristics of the strobilurin fungicides are their inhibition of mitochondrial respiration in both fungal and nonfungal organisms and their rapid detoxification in the cytoplasm of most nonfungal eukaryotic cells (16). Thus, although the route of azoxystrobin movement that we observed is unclear, it seems likely to be through intercellular spaces rather than directly through the living cells of the leaf. Both microscopic studies and quantitative chemical analyses might help to further elucidate the actual site of action for translaminar activity. Regardless of the mechanism, this activity has practical implications with respect to disease control under field conditions should only one leaf surface be adequately sprayed.

Although the vapor activity of azoxystrobin contributed to a modest reduction in disease development, this activity was substantially more pronounced within a small, tightly closed plastic box than within a larger and better-ventilated growth chamber. Considering that even such an "open" growth chamber provided less opportunity for the dissipation of vapors than would be expected under field conditions, it seems unlikely that vapor activity is a major component of the control that azoxystrobin can exert outdoors against *P. viticola* in typical vineyards. These results emphasize the need to develop appropriate methodologies for evaluating the vapor activities of various fungicides, in order to avoid overemphasizing their impact under common agricultural conditions.

The antispore properties of metalaxyl versus *P. viticola* were similar to those reported by Wicks and Lee (19). Furthermore, the complete and rapid control of *P. viticola* provided by the fungicide

Fig. 4. Vapor activity of azoxystrobin versus *Plasmopara viticola*. For each test, the fungicide (or dH₂O) was applied to the adaxial surface of the second fully expanded leaves of six individual *Vitis vinifera* cv. Riesling seedlings either 1, 3, 5, or 7 days before inoculation of the leaf one position above each treated leaf. In separate tests, treated seedlings were kept in either open or closed conditions after fungicide treatment. The test was performed three times under both conditions. **A**, Disease incidence, expressed as percentage of sporulating lesions per seedling relative to the dH₂O check treatment. **B**, Colony area, expressed as percentage of the surface area of lesions on fungicide-treated seedlings relative to those in the dH₂O check treatment. **C**, Sporulation, expressed as percentage of sporangia produced relative to the dH₂O check treatment.

in the translaminar assay, its excellent protective activity, and its pronounced but temporally restricted postinfection activity were consistent with expectations for a known systemic compound with a long history of efficacy against this pathogen. Nevertheless, to our knowledge, this is the first study to compare and quantify this entire range of physical modes of activity for metalaxyl (or its presently commercial enantiomer, mefenoxam) against a downy mildew. Similarly consistent with expectations for mancozeb, a traditional protectant fungicide (17) retained on the leaf surface, was its excellent protective activity coupled with a complete lack of effect on lesion development when examined in the translaminar and postinfection assays. However, its modest but significant antispore activity in the postinfection assay and its pronounced suppression of sporulation in the postsymptom assay exceeded those found in previous studies of the activity of this fungicide versus both *P. viticola* (19) and *V. inaequalis* (17).

In our tests, azoxystrobin provided protective activity against *P. viticola* that was equivalent to that provided by metalaxyl and mancozeb, but postinfection activity that was intermediate between the two. Clearly, azoxystrobin does not provide sufficient postinfection control of lesion development that it should be used as a curative fungicide by design, e.g., in response to a recently concluded infection period identified by a disease forecasting model. However, its pronounced antispore activities may contribute to control of secondary disease cycles and attendant epidemic development, thereby augmenting its protective activity when used within a disease management program. For instance, in a recent field trial (22), azoxystrobin and mancozeb each provided vir-

tually complete control of cluster infections when applied four times at 2-week intervals throughout the period of susceptibility to *P. viticola*. However, control (relative to the untreated check) was reduced to 83 and 29%, respectively, when the first and final sprays were omitted, perhaps reflecting, in part, the relative abilities of these two fungicides to suppress secondary disease spread.

The azoxystrobin-*P. viticola* interaction described by this study is the first detailed investigation of the physical mode of action of a strobilurin fungicide against an intercellular Oomycete pathogen and may serve as a model for other interactions involving similar fungicides and pathogens. A similar study of physical modes of action for kresoxim-methyl against *Puccinia recondita*, *U. necator*, and *Venturia inaequalis* (8) indicated clear differences in activity against these three pathogens. Thus, it would be prudent to understand a range of fungicide-pathogen interactions and to utilize investigations on fungicide physical modes of action to optimize their use in disease management programs.

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Fig. 5. Effects of azoxystrobin, mancozeb, and metalaxyl on *Plasmopara viticola* oospore formation. *Vitis vinifera* cv. Riesling seedlings, inoculated with isolates DM-15 and AR-1, were treated with fungicides or H₂O 3 days after inoculation (postinfection) or 1 day after sporulation (postsymptom). Oospore formation was evaluated 14 days after sporangia production, following incubation at 12°C. For each test, six individual seedlings were used for each fungicide treatment, with the test performed three times.

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