# Effect of Fungicide Volatiles on Sporangial Production of *Plasmopara viticola*

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**ABSTRACT**

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The production of sporangia of *Plasmopara viticola* was almost completely inhibited by volatiles emanating from a 0.05-mg deposit of metalaxyl added to a 250-ml container of tissue-cultured grapevines 1 day after inoculation. Methyl-2-[*N*-phenylacetyl-*N*-(2,6-dimethylphenyl)amino]propanoate also inhibited sporulation but not to the same degree as metalaxyl. Phosethyl aluminum, mancozeb, and cyprofuram did not inhibit sporulation when added to containers 1 or 3 days after inoculation.

A dual-culture system for producing sporangia of *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni on tissue-cultured grapevines has recently been described and used to evaluate the curative properties of metalaxyl (2).

With this system the host, pathogen, and fungicide are maintained for several days in a closed but not airtight container. Because volatiles from metalaxyl have inhibited sporulation of *Peronospora viciae* in a closed container (3), we decided to investigate whether volatiles from metalaxyl and other fungicides for downy mildew also inhibited sporulation of *P. viticola*.

## MATERIALS AND METHODS

The fungicides used were metalaxyl (Ridomil, 250 g active ingredient [a.i.]/kg, Ciba-Geigy); phosethyl aluminum (Aliette, 800 g a.i./kg, Rhône-Poulenc); cyprofuram (Vinicur, 200 g a.i./kg, Schering); methyl-2-[*N*-phenylacetyl-*N*-(2,6-dimethylphenyl) amino]propanoate (Galben, 250 g a.i./kg, Montedison), and mancozeb (Dithane M-45, 800 g a.i./kg, Rohm and Haas).

Cultures of *Vitis vinifera* L. cv. Cabernet Franc were prepared. When plants had rooted and produced nine to 11 leaves, they were inoculated with *P. viticola* sporangia by the method previously described (2). One or 3 days after inoculation, fungicide was introduced into the culture tubes by the following method. Groups of 10 sterilized, single-ended cotton buds 50 mm long (Johnson & Johnson) were dipped singly in 1 ml of fungicide suspension so that the tips of each bud absorbed approximately 0.1 ml of the suspension. Two buds were inserted stem end into the agar of each tube such that the saturated cotton bud was at least 1 cm above the agar surface and not in contact with any plant tissue. As a check that the fungicide did not diffuse from the cotton bud down the stem into the agar, Congo red stain was added to the mancozeb suspension and also to the sterile distilled water treatments. No stain was detected in the agar of either treatment.

Ten tubes were used for each treatment (Table 1), and all tubes were incubated at 25 C.

Nine days after inoculation, sporangial production was assessed by rating all leaves into categories of 0–4, where 0 = no infection, 1 = 1–25% of the leaf area covered with sporangia, 2 = 26–50%, 3 = 51–75%, and 4 = >76%. The data were analyzed by a log linear model based on the assumption that there was a Poisson distribution of error. Different treatments were grouped according to whether treatments and disease ratings were independent (2).

The production of sporangia in each treatment was also measured; all sporulating leaves were removed from each plant and shaken in 50 ml of water. Spore numbers were then counted with a hemacytometer.

Two tissue-cultured plants were weighed individually both before and after they were dipped in a suspension of metalaxyl. This was done to determine the amount of fungicide remaining on grape shoots after they were dipped in fungicide.

## RESULTS AND DISCUSSION

Volatiles from metalaxyl inhibited the production of *P. viticola* sporangia when added to containers of tissue-cultured grapes 1 or 3 days after inoculation. The inhibition was greater 1 rather than 3 days after inoculation; at both times of inoculation, metalaxyl was more effective at 250 mg/L than at 50 mg/L (Table 1). The effect of volatiles was produced from

**Table 1.** Production of sporangia of *Plasmopara viticola* in the presence of fungicide volatiles added 1 or 3 days after inoculation

| Fungicide and rate (a.i.) | Leaves assessed (no.) | Leaves (no.) in disease category[y] 0 | 1 | 2 | 3 | 4 | | Sporangia ($\times 10^6$) |
|---|---|---|---|---|---|---|---|---|
| **After 1 day (except as noted)** | | | | | | | | |
| Control | 119 | 49 | 15 | 10 | 12 | 32 | a | 4.0 |
| Phosethyl Al, 2,000 mg/L | 114 | 40 | 15 | 13 | 16 | 30 | a | 5.7 |
| Mancozeb, 2,000 mg/L | 116 | 41 | 13 | 19 | 14 | 29 | a | 4.0 |
| Galben,[z] 250 mg/L | 136 | 65 | 29 | 9 | 9 | 24 | b | 2.3 |
| Galben, 250 mg/L (3 days) | 116 | 57 | 18 | 12 | 5 | 24 | a | 3.2 |
| Cyprofuram, 250 mg/L | 111 | 41 | 17 | 7 | 14 | 32 | a | 3.3 |
| Cyprofuram, 250 mg/L (3 days) | 119 | 44 | 21 | 18 | 16 | 20 | a | 3.2 |
| Metalaxyl, 50 mg/L | 110 | 77 | 14 | 6 | 8 | 5 | c | 2.2 |
| Metalaxyl, 250 mg/L | 103 | 96 | 6 | 0 | 0 | 1 | d | 0.023 |
| **After 3 days** | | | | | | | | |
| Control | 119 | 40 | 23 | 4 | 10 | 42 | a | 5.5 |
| Phosethyl Al, 400 mg/L | 97 | 31 | 15 | 8 | 15 | 28 | a | 8.9 |
| Phosethyl Al, 2,000 mg/L | 108 | 30 | 11 | 4 | 12 | 51 | b | 9.7 |
| Mancozeb, 400 mg/L | 110 | 39 | 14 | 13 | 5 | 39 | c | 5.3 |
| Mancozeb, 2,000 mg/L | 119 | 34 | 7 | 10 | 12 | 46 | d | 7.3 |
| Metalaxyl, 50 mg/L | 113 | 35 | 27 | 19 | 13 | 19 | e | 3.7 |
| Metalaxyl, 250 mg/L | 100 | 46 | 29 | 8 | 10 | 7 | f | 1.2 |

[y] Where 1 = 1–25% of the leaf area sporulating, 2 = 26–50, 3 = 51–75, and 4 = >76%. Treatments within each experiment with no letter in common are significantly different ($P = 0.05$) (log likelihood ratio test).

[z] Methyl-2-[*N*-phenylacetyl-*N*-(2,6-dimethylphenyl)amino]propanoate.

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low dosages of metalaxyl; approximately 0.2 ml of fungicide was added to each 250-ml container, and this quantity is equivalent to 0.05 and 0.01 mg of metalaxyl for the metalaxyl suspensions at 250 and 50 mg/L, respectively.

These results show that *P. viticola* is extremely sensitive to metalaxyl volatiles. Sporulation was almost completely inhibited despite the low dosage (0.05 mg) used and the low vapor pressure = ($2.9 \times 10^{-7}$ kPa at 20 C) of metalaxyl.

Other fungi are also sensitive to metalaxyl volatiles. A 0.1-mg deposit of metalaxyl produced volatiles that completely inhibited the growth of *Pythium splendens* in vitro (1), and *Peronospora viciae* sporulation was completely inhibited by volatiles emanating from a reservoir of metalaxyl at 200 mg/L (3).

Of the other fungicides tested, methyl-2-[*N*-phenylacetyl-*N*-(2,6-dimethylphenyl) amino]propanoate at 250 mg/L was the only one that produced volatiles that inhibited the production of *P. viticola* sporangia. The inhibition, however, was not as great as that achieved with the equivalent rate of metalaxyl.

The inability of phosethyl aluminum, mancozeb, and cyprofuram to inhibit sporulation in this experiment suggests that volatiles were either not produced from these fungicides or that, if present, they had little effect on sporulation.

The amount of fungicide deposited on grape shoots after they were dipped in a fungicide suspension was approximately 0.3 ml. This amount is similar to that introduced into the containers on cotton buds; therefore, the suppression of *P. viticola* sporangia reported in our previous experiment (2) could have been caused by volatiles emanating from metalaxyl deposited on leaves as well as metalaxyl distributed systemically. In treatments where metalaxyl was incorporated into the agar, volatiles may also have inhibited sporulation.

Although metalaxyl volatiles have been demonstrated to be fungitoxic in laboratory experiments, it is uncertain whether they are of practical significance in field situations. This emphasizes the importance of field testing because the use of in vitro and other closed systems for screening could favor fungicides that have high vapor activity but may not be effective in the field.

**LITERATURE CITED**


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