Evaluation of Fungicides Applied After Infection for Control of *Plasmopara viticola* on Grapevine

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ABSTRACT

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Sporulation of *Plasmopara viticola* was inhibited when tissue-cultured grapevine shoots were dipped in one-quarter the recommended rate of either metalaxyl, cyprofuram, phosethyl aluminum, milfuram, or cymoxanil 3 days after inoculation. Metalaxyl, cyprofuram, and phosethyl aluminum almost completely inhibited sporangial production when applied to grape leaves in the field 4 days after inoculation. Cymoxanil and milfuram both inhibited sporulation in the field but not to the same extent as the other systemic fungicides. The protectant fungicides mancozeb, copper oxychloride, and folpet did not inhibit sporulation. Metalaxyl and cyprofuram were the most effective fungicides to suppress sporulation from lesions sprayed 17 days after natural infection and to reduce germination of these sporangia.

Systemic fungicides specific to the phycomycete fungal pathogens have recently been developed (9). Although these fungicides enable diseases such as downy mildews to be controlled when applied to plants already infected (12), there is little information on this aspect. Most work has been with metalaxyl (1,3,4,11), and there are few published reports on the curative properties of other systemic materials.

This study compared the curative action of a number of fungicides in vitro and in vivo on grapevine downy mildew caused by *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni.

MATERIALS AND METHODS

Fungicides. The fungicides and formulations used were a formulated mixture of metalaxyl (150 g active ingredient [a.i.] per kilogram) and copper oxychloride (350 g a.i./kg) (Ridomil Plus); a formulated mixture of phosethyl aluminum (440 g a.i./kg; Aliette) and mancozeb (260 g a.i./kg) (Mikal M); a formulated mixture of milfuram (60 g a.i./L) and folpet (450 g a.i./L) (Caltan); cyprofuram [3-chloro-*N*-(2-oxoperhydro-3-fryl)cyclopropanecarboxanilide; 200 g a.i./kg; Vinicur, SN 78314]; cymoxanil (800 g a.i./kg; Curzate); copper oxychloride (500 g a.i./kg); mancozeb (800 g a.i./kg); and folpet (500 g a.i./kg). Cyprofuram and cymoxanil were mixed with copper oxychloride in experiment A and with mancozeb in experiment B because they are unlikely to be marketed as single products for use on vines.

Laboratory experiments with dual cultures. Dual cultures of *P. viticola* and grapevines (7) were used to compare the curative properties of fungicides in the laboratory. Shoots of tissue-cultured plants *Vitis vinifera* L. cv. Cabernet Franc were dipped into a suspension of approximately 2×10^3 spores per milliliter of *P. viticola* sporangia and then incubated for 3 days at 25 C before being dipped in a fungicide suspension. Up to 10 plants were used for each treatment.

All operations except incubation were carried out in a laminar flow cabinet.

Except for milfuram, stock suspensions of all fungicides were prepared by dissolving the fungicides in 4 ml of acetone and then diluting with sterile distilled water. Milfuram was diluted only with sterile distilled water because the formulation would not dissolve in acetone. The rates of fungicides used in this test were approximately one-quarter of those recommended for use on vines, because previous work has demonstrated that some fungicides were effective at this concentration (7). Twelve days after inoculation, leaves were rated according to the amount of *P. viticola* sporulation on a scale of 0–4, where 0 = no sporulation, 1 = 1–25% of the leaf area covered with sporangia, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%.

The data were analyzed by a log linear model based on the assumption that there was a Poisson distribution of error; different fungicide treatments were grouped according to whether treatments and disease ratings were independent.

Field experiments with inoculated leaves. In this experiment, the youngest six leaves on shoots of 40-yr-old *V. vinifera* cv. Biancone (syn. White Grenache) were inoculated with a suspension of 1.6×10^3 spores per milliliter of *P. viticola*. The inoculum was prepared by shaking naturally infected leaves in deionized water. Within 2 hr of preparation, the inoculum was applied with a small hand-sprayer to the undersurface of leaves. After a small hole was punched in the youngest inoculated leaf on each shoot, the inoculated leaves were immediately enclosed in clear plastic bags that were sealed by being tied to the shoot. The bags were applied once 1 hr or less before sunset and were removed at sunrise. Four days later, the fungicides at the rates shown in Table 1 were applied with a hand-sprayer to eight shoots per treatment randomized along two rows of vines. The sprayed rows were separated by an unsprayed row, and individual, sprayed shoots were separated by at least 2 m to minimize spray drift between treatments. Nineteen days after inoculation, six leaves below the marked leaf were removed from the shoot. Because no sporulation was detected in the field, the leaves were incubated in the dark for 18 hr at 22 C in plastic bags containing moistened tissue paper. Previous experience had shown that this method induced downy mildew sporulation on grapevine leaves (12). Each leaf was rated according to the leaf area covered with sporangia, and the ratings were analyzed as in the previous experiment. Leaf wetness data

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were collected on a Dewitt Leaf Wetness Recorder installed in the vine canopy; temperature and rainfall were recorded at a meteorologic station situated 50 m from the trial site.

Field experiments with naturally infected leaves. The effect of fungicides on well-established lesions was determined by spraying a fungicide on nonsporulating chlorotic lesions on naturally infected leaves. Each fungicide was applied separately to eight mature leaves, each having one to three discrete chlorotic lesions. Leaves were removed from the field 19 days after applying the fungicide, and *P. viticola* was induced to sporulate as above. Spore production from lesions was measured by removing pieces of sporulating leaf tissue with a cork borer, shaking them in deionized water, and measuring numbers of sporangia in a hemacytometer. Disks (1 cm diameter) of sporulating leaf tissue were removed from eight lesions and bulked for each treatment. Sporangiophores and sporangia were picked from sporulating lesions with fine-pointed tweezers and teased into a drop of deionized water in a hollow, ground glass slide. Three replicates of each treatment were incubated on the laboratory bench at approximately 23 C for 4 hr before germination was assessed by counting the number of empty sporangia.

Table 1. Production of *Plasmopara viticola* sporangia on tissue-cultured grapevine shoots dipped in fungicides 3 days after inoculation

Fungicide (rate of a.i. per 100 L)	Leaves assessed (no.)	Leaves in each disease category (no.)[y]					
		0	1	2	3	4	
Control: water	101	65	17	5	8	6	a[z]
Mancozeb (40 g)	97	49	13	7	18	10	a
Copper oxychloride (12.5 g)	53	46	3	3	1	0	b
Phosethyl aluminum (37 g) + mancozeb (22 g)	90	83	7	0	0	0	c
Milfuram (6 g) + folpet (45 g)	64	61	1	0	2	0	d
Cymoxanil (4.8 g) + copper oxychloride (12.5 g)	47	45	2	0	0	0	d
Cyprofuram (6 g) + copper oxychloride (12.5 g)	60	59	1	0	0	0	d
Metalaxyl (4 g) + copper oxychloride (10 g)	75	75	0	0	0	0	d

[y]0 = No sporangia, 1 = 1–25% of the leaf area covered with sporangia, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%.
[z]Treatments with no letter in common are significantly different at the 5% probability level (log likelihood ratio test).

Table 2. Production of *Plasmopara viticola* sporangia on grapevine leaves sprayed with fungicides 4 days after inoculation

Fungicide (rate of a.i. per 100 L)	Leaves assessed (no.)	Leaves in each disease category (no.)[y]					
		0	1	2	3	4	
Control (no spray)	48	2	23	3	9	11	a[z]
Mancozeb (160 g)	48	0	15	15	9	9	b
Copper oxychloride (50 g)	48	0	14	12	2	20	b
Folpet (200 g)	30	0	10	8	5	7	b
Milfuram (24 g) + folpet (180 g)	46	1	21	14	10	0	c
Cymoxanil (20 g) + mancozeb (160 g)	46	2	21	14	9	0	c
Phosethyl aluminum (198 g) + mancozeb (117 g)	48	16	31	1	0	0	d
Cyprofuram (24 g) + mancozeb (160 g)	48	34	14	0	0	0	e
Metalaxyl (22.5 g) + copper oxychloride (52.5 g)	48	46	2	0	0	0	f

[y]0 = No sporangia, 1 = 1–25% of the leaf area covered with sporangia, 2 = 26–50%, 3 = 51–75%; 4 = 76–100%.
[z]Treatments with no letter in common are significantly different at the 5% probability level (log likelihood ratio test).

Table 3. Production and viability of *Plasmopara viticola* spores from lesions on the leaves sprayed with fungicides

Fungicide (rate of a.i. per 100 L)	Spore production per cm²[y] (mean no. × 10³)	Spore germination (%)[z]
Control (no spray)	164	97
Cymoxanil (20 g) + mancozeb (160 g)	156	95
Copper oxychloride (50 g)	154	73
Mancozeb (160 g)	132	83
Milfuram (24 g) + folpet (180 g)	114	54
Phosethyl aluminum (198 g) + mancozeb (160 g)	86	86
Cyprofuram (24 g) + mancozeb (160 g)	52	42
Metalaxyl (22.5 g) + Copper oxychloride (52.5 g)	15	22
LSD ($P = 0.05$)	48	

[y]Mean of three replicates each of eight leaf disks per treatment.
[z]Calculated from 500 sporangia per treatment.

RESULTS

Laboratory experiments with dual cultures. Sporangia of *P. viticola* developed on 36% of leaves that were dipped in water 3 days after inoculation (Table 1). Compared with the water treatment, phosethyl aluminum, milfuram, cymoxanil, cyprofuram, and metalaxyl severely inhibited sporulation when applied 3 days after inoculation. Metalaxyl was the only fungicide that inhibited sporulation completely, but this effect was not significantly different from that achieved with the other systemic materials except for phosethyl aluminum.

Mancozeb did not suppress sporulation when compared with the control. Sporulation was inhibited by copper oxychloride but not to the same extent as by the systemic fungicides. Marginal leaf necrosis occurred on the leaves of many plants dipped in fungicides containing copper oxychloride.

Field experiments with inoculated leaves. No lesions were detected on leaves before inoculation, and weather data showed that conditions suitable for *P. viticola* infection had not occurred in the 12 days prior to inoculation. After the inoculated leaves were incubated, however, *P. viticola* sporangia developed on more than 95% of the unsprayed leaves and on leaves sprayed with either mancozeb, copper oxychloride, or folpet (Table 2). On many leaves sporulation was profuse, covering most of the leaf surface. Although milfuram and cymoxanil suppressed sporulation compared with that on the unsprayed leaves and those sprayed with either mancozeb, copper oxychloride, or folpet, neither was as effective as metalaxyl, cyprofuram, and phosethyl aluminum.

The suppression obtained with metalaxyl, cyprofuram, and phosethyl aluminum was even greater than indicated in Table 2 because sporulation occurred on less than 5% of the leaf area of the leaves in category 1 of these treatments.

Field experiments with naturally infected leaves. Leaf wetness and rainfall data indicated that the sprayed chlorotic lesions originated from infections that occurred 17 days prior to the application of fungicides. Compared with unsprayed lesions, metalaxyl, cyprofuram, phosethyl

aluminum, and milfuram significantly reduced the production of sporangia (Table 3).

Metalaxyl was the most effective fungicide inhibiting the production and germination of sporangia. Copper oxychloride, mancozeb, and cymoxanil did not significantly affect the production of sporangia or have a marked effect on their germination.

DISCUSSION

Fungicides formulated with either metalaxyl, cyprofuram, or phosethyl aluminum are suitable for use as curative sprays to control *P. viticola* and should substantially reduce the number of sprays normally required to control grapevine downy mildew.

Cymoxanil and milfuram were less effective than the other systemic fungicides when applied in the field 4 days after infection and are therefore less likely to be used as curative sprays.

In glasshouse experiments, Klopping and Delp (6) found cymoxanil effective up to 3 days after inoculation, which explains why this fungicide performed well in our laboratory experiments only.

Of the fungicides tested, metalaxyl appeared the most effective because it inhibited sporulation and reduced germination of sporangia more than other fungicides. In previous experiments (Wicks, *unpublished*), it retarded lesion expansions of grapevine downy mildew. Similar effects of metalaxyl have been observed on other pathogens (1,3,8,10). This explains the dramatic inhibition of downy mildew epidemics following the application of metalaxyl in South Australian vineyards. No comparisons were made between field experiments, but the results and our observations in commercial vineyards suggest that the systemic materials are more effective when applied 4 days rather than 17 days after inoculation. Therefore, maximum benefit from curative sprays would be achieved by applying the fungicides within a few days after infection. Such a procedure requires accurate determination of conditions suitable for downy mildew sporulation as well as infection. Facilities for doing this are not available in most grape-growing areas of Australia. In Europe, however, where the main grape-growing areas have spray warning services based on infection periods (5), the use of curative sprays should have a major impact on the control of grapevine downy mildew.

Although Bruck et al (2) reported sporulation of *Phytophthora infestans* to be inhibited by mancozeb applied to potato leaves up to 5 days after infection, we were unable to detect any inhibitory effect of mancozeb, copper oxychloride, or folpet on the sporulation of *P. viticola* in the field. In dual culture experiments, copper oxychloride inhibited sporulation, but this may have been associated with the phytotoxicity that it caused on some of the plants.

The suppression of sporulation by all systemic fungicides used at low rates on plants in the dual culture system shows that the technique may be useful for initial screening of potential fungicides for systemic and curative properties on downy mildew diseases.

Singh and Dickinson (10) reported that volatiles from metalaxyl solutions inhibit sporulation of pea downy mildew. It is thus possible that with some of the fungicides tested, sporulation in the dual culture system could have been inhibited by volatiles originating from fungicide leaf deposits rather than from the systemic ingredient of the fungicide. This aspect is being investigated.

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