Efficacy of Dimethomorph (CME 151) Against Downy Mildew of Grapevines

T. WICKS and B. HALL, South Australian Department of Agriculture, Box 1671, G.P.O., Adelaide, South Australia, 5001

ABSTRACT

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Leaf disks removed from sprayed vines were floated on water and inoculated with sporangia of *Plasmopara viticola* to test the efficacy of dimethomorph against downy mildew of grape. The formation of sporangia was inhibited completely on leaf disks that were inoculated 2, 8, and 15 days after applying dimethomorph at 50, 100, or 200 mg/L. Dimethomorph at 25 mg/L also inhibited the production of sporangia on leaves sprayed 9 days after inoculation. The protective and curative activity of dimethomorph was as good as or better than that achieved with a metalaxyl/mancozeb formulation. Systemic movement from sprayed to unsprayed leaves was demonstrated. Floating of leaf disks was shown to be a useful technique for evaluating downy mildew fungicides on grapes in areas and seasons where natural development of the disease is uncertain.

Downy mildew caused by *Plasmopara viticola* (Berk. & M. A. Curtis) Berl. & de Toni is a destructive disease of grapes (*Vitis vinifera* L.) in most of the world's viticultural areas (3). In Australia, downy mildew occurs sporadically. Because of this, conducting a field experiment in a vineyard that has previously been infected with downy mildew does not ensure that the disease will develop during the study and allow the collection of efficacy data adequate for registration of the test fungicides. The unreliability of natural infection has caused delays of 3–5 yr in the commercial release of fungicides for the control of grape downy mildew in Australia. To overcome the uncertainty of natural infection, we used the floating leaf disk technique (2,4) and artificial inoculation of vines as bioassays for evaluating the efficacy of fungicides applied to grapevines in the field. This paper describes the use of these techniques in evaluating dimethomorph as a suitable fungicide for the control of *P. viticola*.

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MATERIALS AND METHODS

Floating leaf disk technique. A floating leaf disk technique (2,4) was used to evaluate the efficacy of dimethomorph (CME 151), (E,Z)4-[3(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]-morpholine (1) derived from cinnamic acid and made available as a 10% emulsifiable concentrate by Shell Chemicals (Melbourne, Australia 3001). The technique involved removing disks (12-mm diameter) from expanding leaves on four field-grown or potted *V. vinifera* cultivars (Mataro, Shiraz, Sultana, and Taminga) and the interspecific hybrid LN-33. In most experiments, at least 10 leaves of similar maturity were sampled from each vine. Leaf disks taken from unsprayed vines were floated lower-surface-up on a fungicide suspension for preliminary screening; leaf disks from sprayed vines were floated similarly on deionized water.

In all experiments, at least 10 disks were floated in a petri dish containing 20 ml of a fungicide emulsion or water. Each treatment was replicated four or five times. An inoculum suspension was prepared by placing infected grape leaves (which had sporulated the previous night) into a container of deionized water, closing the container, and then shaking. The suspension was adjusted to approximately 10^4 *P. viticola* sporangia per milliliter; previous experience had shown that this inoculum concentration gave the most consistent level of infection. Each disk was inoculated with a 0.025-ml drop of the suspension. The lids of the petri dishes were removed 24 hr after inoculation to allow the inoculum to evaporate. Once this had occurred, the lids were replaced and the leaf disks incubated on a laboratory bench where temperatures ranged from 15 to 27 C.

Sporulation was assessed at least 2 days after it was first detected in the control disks, usually 9–14 days after inoculation. Sporulation was rated on a 0–4 scale in which 0 = no sporulation, 1 = less than 5% of the disk area covered with sporangia, 2 = 5–25% covered, 3 = 25–50% covered, and 4 = more than 50% covered.

In vitro screening. In vitro experiments were conducted with dimethomorph to determine the sensitivity of *P. viticola* to dilutions ranging from 1 to 200 mg of dimethomorph per liter. The procedure involved floating leaf disks from the cultivar Sultana on emulsions of fungicide and then inoculating them with *P. viticola*.

Protective activity. *Northfield experiments.* Two field experiments were undertaken on mature trellised grapevines grown at the Northfield Research Centre, Adelaide, South Australia. Due to the limited amount of fungicide available for testing, dimethomorph was applied with small hand-held sprayers to two vines per treatment for cultivars Mataro and LN-33. Vines were sprayed to runoff (250–500 ml per vine) with dimethomorph concentrations of 25, 50, or 100 mg/L. Fungicides were applied once during the period from 4 wk before to 2 wk after flowering. Treated vines were separated by unsprayed barrier vines.

In an experiment with LN-33 vines, metalaxyl at 250 mg/L was compared with dimethomorph. The youngest expanded leaves of LN-33 vines were

marked at the time of spraying to allow identification of sprayed and unsprayed leaves when leaf disks were taken. In most cases, leaf disks were inoculated within an hour of collection from the field.

Nuriootpa experiments. A large-scale field experiment was conducted at the Nuriootpa Research Station, 100 km north of Adelaide. In this experiment, the effects of dimethomorph solutions at 25 and 50 mg/L were compared with those of a formulated mixture of metalaxyl (0.112 g/L) and mancozeb (1.8 g/L). Each treatment was applied to a panel of four mature Taminga vines replicated three times and arranged with the unsprayed vines in a randomized block design. The fungicides were applied once with a hand wand delivering approximately 1,500 L/ha. Leaves were collected at random from sprayed vines at various times up to 4 wk after spraying. At least 25 leaves were collected from each treatment at each sampling; these were used to prepare 50 leaf disks per treatment. The efficacy of each treatment is expressed as the percentage by which sporulation was inhibited on sprayed leaves compared to the amount of sporulation present on unsprayed leaves.

Curative activity. *Glasshouse evaluation.* The curative activity of dimethomorph was evaluated on potted Shiraz and Sultana vines grown in a glasshouse maintained at approximately 25 C. Expanding leaves were inoculated by applying sporangia of *P. viticola* to the undersurface of five leaves at the tips of shoots. Inoculated shoots (one per vine) were enclosed in plastic bags for 12 hr. Chlorotic lesions were first noticed 6 days after inoculation, at which time all leaves were sprayed to runoff with water or with dimethomorph at 100 or 200 mg/L. Twelve days after inoculation, the inoculated leaves were removed from each shoot and placed into moistened plastic bags. The bags were then sealed and left overnight to induce sporulation (5). The curative activity of dimethomorph was assessed by estimating the percentage of leaf area covered with sporangia. The production of sporangia from the chlorotic lesions was rated on a 0–3 scale in which 0 = no sporangia and 3 = up to 600 sporangia per square millimeter of leaf tissue. This experiment was conducted once, with eight Sultana vines and four Shiraz vines in each treatment.

Field evaluation. This experiment was conducted on vines of *V. vinifera* cultivar Taminga at the Nuriootpa Research Centre. The experimental design and the spraying procedures were similar to those used in the protective experiment. Five shoots per vine were inoculated by spraying the shoots with 10^4 sporangia per milliliter and enclosing the shoots in a clear plastic bag overnight (5). Nine days after inoculation, either dimethomorph at 25 mg/L or the formulated mixture of metalaxyl (0.112 g/L) and mancozeb (1.8 g/L) was applied to inoculated vines.

Inoculated shoots were removed 6 days after spraying and all shoots were placed in moisted plastic bags overnight to induce further sporulation, although many leaves had sporulated naturally in the field (following rainfall that occurred 2–4 days after spraying). After incubating for 24 hr, the percentage of leaf area infected was estimated and the sporulation rated on a 0–6 scale, where 0 = no chlorosis and 6 = 50% or more of the leaf area was chlorotic. Sporulation was rated on a scale similar to that used for evaluating the leaf disks.

RESULTS

In vitro activity. Sporangia developed on 94% of inoculated Sultana leaf disks in the water check (Table 1). Inhibition of sporulation occurred on 74% of leaf disks at dimethomorph concentrations of 20 mg/L; at dimethomorph concentrations of 50–200 mg/L, the emulsion was phytotoxic to Sultana leaf disks.

Protective activity. *Northfield experiments.* No sporangia developed on Mataro leaf disks removed from vines 2, 8, or 15 days after treatment with dimethomorph at 50, 100, or 200 mg/L. Phytotoxicity was not detected on any vine. On all sampling dates, sporangia developed on more than 66% of inoculated leaf disks from unsprayed vines. Similar results were obtained when these experiments were repeated on LN-33 grapevines. Metalaxyl at 250 mg/L and dimethomorph at 100 mg/L completely inhibited the production of sporangia on leaves collected 8 days after spraying (Table 2). As no rain or dew had been recorded since spraying, the systemic activity of the fungicides was also evaluated by removing disks from distal leaves that had developed within 8 days after spraying. The production of sporangia on these leaves was inhibited by dimethomorph treatments at 50 and 100 mg/L, but the inhibition was significantly less ($P = 0.05$) than that achieved on the sprayed leaves.

Nuriootpa experiment. The metalaxyl/mancozeb formulation inhibited production of *P. viticola* sporangia on sprayed leaves for at least 21 days after application (Fig. 1). Dimethomorph at 50 mg/L also inhibited production of sporangia during this period, but at most times it appeared less effective than the formulated mixture of metalaxyl and mancozeb. Dimethomorph at 25 mg/L was effective for 7 days after spraying, but efficacy rapidly declined to less than 30% inhibition after 14 days.

Curative activity. *Glasshouse evaluation.* All inoculated Sultana leaves and 90% of inoculated Shiraz leaves were

Table 1. Production of *Plasmopara viticola* sporangia on leaf disks of *Vitis vinifera* (cultivar Sultana) floating on emulsions of dimethomorph

Fungicide concentration (mg/L)	Number of leaf disks assessed	Number of leaf disks in each sporulation category[y]				
		0	1	2	3	4
0 a[z]	50	3	2	0	5	40
1 b	50	5	13	15	17	0
5 c	50	27	16	5	2	0
10 cd	50	35	9	6	0	0
20 d	50	37	2	10	1	0

[y]Sporulation categories: 0 = no sporangia, 1 = less than 5% of leaf area covered with sporangia, 2 = 5–25% covered, 3 = 25–50% covered; 4 = more than 50% covered.
[z]Letters denote treatment difference ($P < 0.05$).

Table 2. Production of *Plasmopara viticola* sporangia on leaf disks of LN-33 inoculated after removal from leaves sprayed 8 days previously with either dimethomorph or metalaxyl

Fungicide concentration[a]	Leaf type[b]	Number of leaf disks in each disease category[c,d]				
		0	1	2	3	4
Control	us	4	6	12	25	3
	dus	4	4	13	18	11
Dimethomorph 25 mg/L	s	46	4	0	0	0
	dus	8	1	9	31	1
Dimethomorph 50 mg/L	s	49	1	0	0	0
	dus	16	9	15	9	1
Dimethomorph 100 mg/L	s	50	0	0	0	0
	dus	19	9	15	7	0
Metalaxyl 250 mg/L	s	50	0	0	0	0
	dus	4	7	20	19	0

[a]Applied 10 November.
[b]Leaf type: us = unsprayed, dus = distal unsprayed, s = sprayed.
[c]Inoculated 18 November and assessed 12 days later.
[d]Sporulation categories: 0 = no sporangia, 1 = less than 5% of leaf area covered with sporangia, 2 = 5–25% covered, 3 = 25–50% covered, 4 = more than 50% covered.

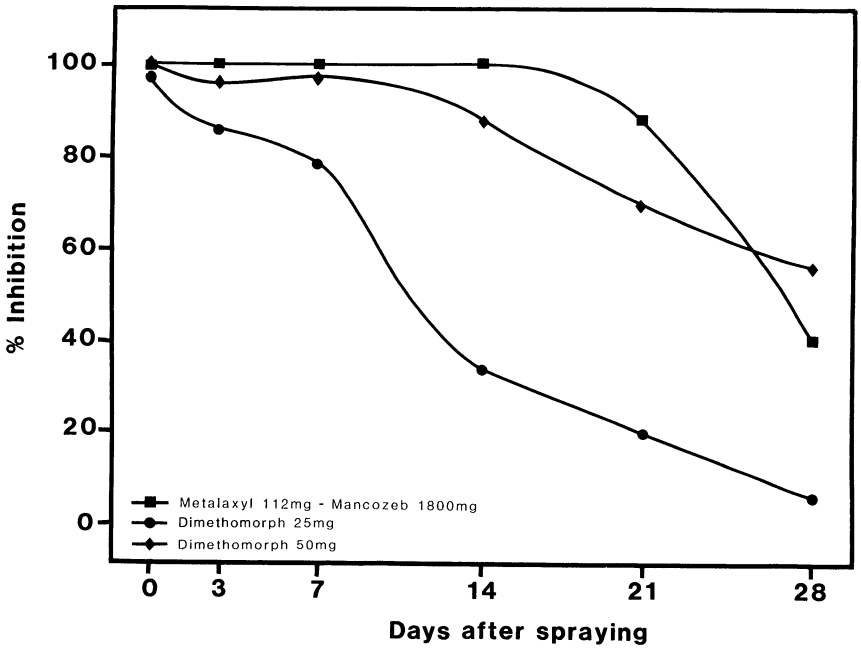

Fig. 1. The inhibition of sporulation on leaf disks removed from leaves of *Vitis vinifera* (cultivar Taminga) sprayed with fungicides and inoculated with *Plasmopara viticola* at various times after spraying.

Table 3. Effect of dimethomorph applied to *Vitis vinifera* cultivars Shiraz and Sultana 6 days after inoculation on the production of sporangia of *Plasmopara viticola*

	Area of leaf covered with sporangia (%)[y]		Sporulation rating[y,z]	
Fungicide	Shiraz	Sultana	Shiraz	Sultana
Dimethomorph 200 mg/L	2.5 a	7.1 a	0.04 a	0.09 a
Dimethomorph 100 mg/L	8.5 ab	8.4 a	1.20 ab	1.10 a
Control	22.0 b	52.1 b	2.30 b	2.90 b

[y]Different letters denote treatment differences ($P < 0.05$).
[z]Sporulation on 0–3 scale where 0 = no sporulation and 3 = maximum production (up to 600 sporangia per square millimeter).

Table 4. Sporulation on *Vitis vinifera* cultivar Taminga leaves sprayed with either dimethomorph or a mixture of metalaxyl and mancozeb 9 days after inoculation with *Plasmopara viticola*

Fungicide	Number of infected leaves per shoot	Rating of leaf area infected[a]	Sporulation rating[b]
Dimethomorph 25 mg/L	7.2	0.90	1.70
Metalaxyl 112 mg/L + mancozeb 1,800 mg/L	9.5	1.90	1.90
Unsprayed	16.7	2.90	2.80
LSD: $P = 0.05$	3.1	0.48	0.56

[a]On a 0–6 scale where 0 = no chlorosis and 6 = 50% or more of the leaf area was chlorotic.
[b]On a 0–3 scale where 0 = no sporulation and 3 = maximum production (up to 600 sporangia per square millimeter).

infected. The mean leaf area covered by sporangia was 52% for Sultana leaves and 22% for Shiraz leaves (Table 3). Sporangia production was significantly inhibited by dimethomorph concentrations of both 100 and 200 mg/L. On Sultana leaves, dimethomorph at 200 mg/L reduced sporulating leaf area to 7%; on Shiraz leaves, the sporulating leaf area was reduced to 2.5%. The same dimethomorph concentration inhibited the production of sporangia completely on 17% of infected Sultana leaves.

Field evaluation. When applied 9 days after infection, both dimethomorph and the metalaxyl/mancozeb formulation significantly reduced the number of infected leaves per shoot, the area of leaf affected, and the amount of sporulation compared to that on unsprayed leaves (Table 4).

DISCUSSION

Our study shows that the new fungicide dimethomorph warrants further evaluation, as it effectively controlled downy mildew of grape in all glasshouse and field experiments. Dimethomorph controlled downy mildew when applied before infection and showed significant curative activity (as well as some systemic activity) when applied up to 9 days after infection. It also compares favorably with metalaxyl for downy mildew control. Although our results showed dimethomorph concentrations of 50 mg/L or higher protected grape leaves for at least 15 days, further work is required to determine the most effective and economical concentration for use on grapevines.

Overall, our results confirm recent German studies (1) showing that dimethomorph has great potential for the control of downy mildew in grapevines. It was not phytotoxic to grape cultivars used in our studies or the German studies. Dimethomorph does not adversely affect fermentation or the taste of wine and it is effective against phenylamide-resistant isolates of *P. viticola* (1).

Our experiments show that removing leaf disks from sprayed vines and inoculating them with spores of *P. viticola* can be a useful screening technique when evaluating field applications of fungicides against downy mildew of grape. The technique is simple, reliable, and effective on different grape cultivars and types of fungicides. By using a standard level of inoculum, similar-sized leaf disks from leaves with the same degree of maturity, and a simple method of assessment, the floating-leaf technique allows fungicide treatments to be critically compared for activity, persistence, and systemicity.

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