Variations in the Sensitivity of *Phytophthora infestans* Isolates from Different Genetic Backgrounds to Dimethomorph

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ABSTRACT

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The sensitivities of 11 isolates of *Phytophthora infestans* to dimethomorph were examined at all stages of the asexual life cycle and when inoculated onto potato leaf discs. In vitro zoospore encystment and cyst germination were highly sensitive to dimethomorph with 50% reduction of mycelial growth and cyst germination (EC_{50}) values for most isolates <0.20 $\mu\text{g/ml}$, whereas direct sporangia germination and in vitro hyphal growth and sporulation were less sensitive (means of 0.45 and 0.22 $\mu\text{g/ml}$, respectively). Zoosporogenesis was not significantly inhibited at the maximum dimethomorph concentration examined, 10 $\mu\text{g/ml}$. Significant differences (Fisher's least significant difference, $P = 0.05$) in the EC_{50} values were present between isolates for all stages of the asexual life cycle, except direct sporangia germination and zoosporogenesis. Sensitivity ratios between the least- and most-sensitive isolates were 6.11, 12.14, 12.36, and 10.56 for hyphal growth, in vitro sporulation, zoospore encystment, and cyst germination, respectively. Application of dimethomorph at 1,000 $\mu\text{g/ml}$ to potato leaf discs at 24 or 48 h before inoculation completely inhibited symptom incidence for most isolates, whereas application after inoculation generally was not significantly different from the untreated control, regardless of concentration. Sporulation from leaf discs treated with dimethomorph at 24 or 48 h after inoculation was completely inhibited for all isolates with dimethomorph at 1,000 $\mu\text{g/ml}$, even when symptom incidence was not significantly reduced.

Additional keywords: antisporeulation, fungicide, Oomycete, *Solanum tuberosum*

Dimethomorph, a cinnamic acid derivative, was one of the fungicides released for use in the United States following the migration (14) or spontaneous development (27) of phenylamide-resistant strains of *Phytophthora infestans*. Initial studies with dimethomorph demonstrated specific activity toward the genus *Phytophthora* and certain members of the family *Peronosporaceae* (1). Dimethomorph has a moderate amount of translaminar and acropetal systemicity (1,9) and is most effective when used as a protectant fungicide; however, it has some degree of post-infection activity. One of the most interesting aspects of dimethomorph activity is its "antisporeulation" activity; dimethomorph inhibits *P. infestans* sporangia formation (sporulation) when applied to inoculated potato (*Solanum tuberosum*) plants (9) or

leaf discs (2,9) prior to symptom development.

The biochemical mode of action of dimethomorph has not been elucidated but a disruption of cell wall formation, specifically the organization and not the synthesis of wall components, has been described (1,17). Dimethomorph disrupts all stages of the asexual life cycle of *P. infestans* except zoosporogenesis, zoospore release, and zoospore motility (2,9,17), because these stages do not involve cell wall formation. The activity of dimethomorph on other *Phytophthora* spp. has been examined and found to be similar to that in *P. infestans* (19). However, differences in the effective concentration for a 50% reduction of mycelial growth and cyst germination relative to the untreated control (EC_{50}), were apparent among species.

The majority of studies examining dimethomorph activity against *Phytophthora* spp. typically consisted of only one isolate per species (2,4,17,18). The single study that compared the sensitivity of eight isolates of *P. infestans* to dimethomorph examined only the in vivo sensitivity range and not differences in sensitivities at various stages of the asexual life cycle or antisporeulation activity (9). Variations in the sensitivity of *P. infestans* to dimethomorph

under field conditions has been noted (10); however, comparisons of the sensitivity of isolates from different genetic backgrounds is lacking.

The objectives of this study were to determine (i) the in vitro variation in dimethomorph sensitivity of 11 isolates of *P. infestans* from different genetic backgrounds at multiple stages in the asexual life cycle of *P. infestans* and (ii) the protectant, post-infection, and antisporeulant activity of dimethomorph on these isolates when inoculated onto potato leaves.

MATERIALS AND METHODS

Preparation of amended media and fungicide stock solutions. Assessment of the inhibition of in vitro hyphal growth rate and sporulation was performed on modified rye B agar (2,6) consisting of the filtrate of prerinsed rye (*Secale cereale*) seed (100 g/liter) boiled for 1 h, deionized (d) H_2O added to a final volume of 1.0 liter, glucose (8.0 g/liter), β -sitosterol (0.05 g/liter), and agar (15.0 g/liter). All plates for each of the experiments were prepared from the same batch of media in order to reduce variability.

Dimethomorph (BASF Corp., Research Triangle Park, NC) 100 $\times$ stock solutions were prepared by dissolving technical grade (95% active) dimethomorph into 95% ethanol and performing serial dilutions as required in 95% ethanol. The stock solutions were added to molten media at 10 ml/liter when the temperature was cooled to approximately 55°C in a water bath. Fungicide solutions were passed through a 0.22- μm syringe-driven filter (Millipore Corp., Bedford, MA) to sterilize. Control plates received filter-sterilized ethanol at 10 ml/liter.

Calculation of EC_{50} values and statistical analysis. Each dimethomorph concentration (treatment) was replicated three times and each experiment was repeated three times for each variable examined. The percent inhibition relative to the control for an isolate was calculated for each replicate of a dimethomorph concentration with respect to the mean of the 0 $\mu\text{g/ml}$ control replicates, within that experimental repeat. Percent inhibition values then were transformed using probits (12,22) (i.e., the inverse of the standard normal distribution; 16), and expressed as a function of the

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log₁₀ of concentration. Plot equation parameters were determined using linear regression (SigmaPlot; SPSS Inc., Chicago) and the EC₅₀ for the inhibition of each variable (e.g., in vitro hyphal growth, zoospore encystment, and so on) was calculated and reverse transformed for every isolate examined. All measurements, calculations, transformations, and regressions were performed independently within each repeat of the experiment, and the EC₅₀ values generated for each isolate from each repeat of an experiment were used as replicates for an analysis of variance (ANOVA) calculated as a randomized complete block design (Proc GLM, SAS/Stat; SAS Institute, Cary, NC). Means were separated by Fisher's least significant difference (LSD) at *P* = 0.05.

Inhibition of hyphal growth and sporulation in vitro. Previously characterized isolates (Table 1) of *P. infestans* were subcultured once on modified rye B agar for 21 days at 18°C in the dark, following a single zoospore reisolation from infected potato leaves. Isolates obtained prior to 1997 probably had not been exposed to dimethomorph because the fungicide was on limited emergency registration in much of the United States from 1995 to 1997. Those isolates obtained in 1997 and 1998 may have been exposed to dimethomorph, although the likelihood is small because adoption by growers was minimal until 1999. Colonized 4-mm-diameter agar plugs were transferred from the margin of the colony onto dimethomorph-amended modified rye B media in 60-mm-diameter plastic petri dishes and incubated at 21°C. The dimethomorph concentrations used were 0, 0.01, 0.1, 1, and 10 µg/ml. Colony diameter was measured 11 days after transferring to media. The percent inhibition relative to the 0 µg/ml control and EC₅₀ values were calculated, and an ANOVA was performed as described above.

The effect of dimethomorph on in vitro sporulation was examined using a modification of a previously described method of sporangia quantification (6). Ten colonized agar plugs, 1 mm in diameter, were excised

from each replicate plate of every dimethomorph concentration from the in vitro hyphal growth sensitivity assay. Five of the plugs were harvested at approximately 2 mm in from the colony margin and five approximately 2 mm out from the edge of the initial inoculum plug. All 10 plugs then were placed into a single 1.5-ml microcentrifuge tube with 1.0 ml of sterile (s)dH₂O and agitated for 15 s using a laboratory vortex to dislodge the sporangia. Sporangia were quantified with a hemacytometer (average of four fields) and the number per square centimeter of colony area was calculated. The percent inhibition relative to the 0 µg/ml control and EC₅₀ values were calculated, and an ANOVA was performed as described above.

Production of viable sporangia for germination studies and inoculations. To produce viable sporangia of similar age (11), potato tubers (cv. Russet Burbank) were surface disinfested with 0.5% sodium hypochlorite in dH₂O (10% commercial bleach solution) for 30 min, rinsed three times in sdH₂O, and allowed to dry. Tubers were sliced into 7-mm sections and placed into sterile 150-mm (diameter) plastic petri dishes on top of 1 cm² of modified rye B agar sections previously colonized by individual isolates of *P. infestans*. Plates were sealed with Parafilm and incubated under fluorescent lighting with 12-h alternating cycles at 18°C (light) and 15°C (dark) until at least 50% of the tuber slice surface was covered by the mycelium (typically 5 days). Sporangia were harvested by gently removing the mycelium with a plastic culture spreader, transferred into sterile microcentrifuge tubes, and 1.0 ml of sdH₂O was added. Tubes were agitated using a laboratory vortex to dislodge sporangia and filtered through four layers of sterile cheesecloth to remove most of the mycelial fragments. Sporangia were quantified with a hemacytometer as described above and the concentration was adjusted to 1.0 × 10⁴ sporangia/ml.

Inhibition of direct sporangia germination, zoosporogenesis, zoospore encystment, and cyst germination in vitro.

To assess the effects of dimethomorph on direct sporangia germination, aliquots from the previously prepared suspension that contained approximately 100 sporangia (10 µl) were added to 90 µl of sdH₂O in 96-well Costar culture plates (Corning Inc., Acton, MA). Dimethomorph stock solutions were added for a final concentration of dimethomorph at 0, 0.01, 0.1, 1, or 10 µg/ml, in each of three replicate wells per concentration, per *P. infestans* isolate. Sporangia–fungicide suspensions were incubated for 72 h in the dark at 21°C and the numbers of total and germinated sporangia per well were quantified with a microscope. To examine zoosporogenesis and zoospore encystment, sporangia–fungicide suspensions were incubated in the dark at 8°C for 5 h to induce zoosporogenesis and the number of motile zoospores was quantified with a hemacytometer as described above. The zoospore suspensions then were incubated in the dark at 21°C and the number of total cysts per well was quantified microscopically after 24 h. The number of cysts per well was adjusted based upon the number of zoospores removed during quantification. To examine cyst germination, zoospore suspensions were prepared as described above and incubated in the dark at 21°C for 24 h to allow for zoospore encystment. Fungicide solutions were added, the solutions were incubated in the dark at 21°C for 48 h, and the number of total and germinated cysts was quantified with a hemacytometer. The percent inhibition relative to the 0 µg/ml control and EC₅₀ values were calculated, and an ANOVA was performed as described above.

Protectant, post-infection, and antiporulant activity of dimethomorph in vivo. The in vivo activity of dimethomorph on the *P. infestans* isolates examined in the previous studies was measured with respect to the inoculation event. Fully expanded leaflets of similar age and from leaf positions 10 to 12 (mainstem, above the soil line) were excised from greenhouse-grown potato plants (cv. Snowden) and surface disinfested in 0.5% sodium hypo-

Table 1. *Phytophthora infestans* isolate identification, year isolated, mating type, genotype, physiological race, state in which isolated (United States), and metalaxyl (phenylamide) sensitivity

Isolate	Year isolated	Mating type	Genotype ^w	Physiological race ^x	Origin ^y	Metalaxyl sensitivity ^z
Pi88	1995	A1	US1	3.6.7.10.11	North Dakota	nd
Pi95-5	1995	A1	US1	1.2.3.7.9	Michigan	S
Pi671	1997	A2	US14	1.10	Washington	nd
Pi458	1998	A1	US17	1.2.3.4.5.6.7.9.11	Idaho	nd
Pi670	1997	A2	US7	1.2.3.4.5.6.10.11	Oregon	nd
Pi213	1997	A2	US8	1.2.3.4.5.6.7.10.11	Colorado	R
Pi94-4	1994	A2	US8	1.3.5	Michigan	R
Pi95-7	1995	A2	US8	1.2.3.4.5.6.7.10.11	Michigan	R
Pi97-2	1997	A2	US8	1.3.4.5.8.11	Michigan	R
Pi98-1	1998	A2	US8	2.5.6.7.8.9.10.11	Michigan	S
Pi98-2	1998	A2	US8	1.2.3.4.5.7.10.11	Michigan	R

^w Allozyme-based genotype (13).

^x Determined using inoculations onto potato lines with different *R* genes as described (3).

^y State of origin. All isolates were obtained from potato.

^z Sensitivity to the fungicide metalaxyl assayed via the inhibition of in vitro hyphal growth: nd = not determined, S = sensitive, R = resistant (23).

chlorite in dH₂O (10% commercial bleach solution) for 1 min. Leaflets were rinsed three times in sdH₂O, allowed to dry, and cut into 20-mm-diameter leaf discs with a sterilized cork borer. Leaf discs were pooled prior to being randomly assigned to treatments and placed onto water agar (15.0 g/liter) amended with rifamycin (37.5 mg/liter), ampicillin (10 mg/liter), and nystatin (37.5 mg/liter) which were previously dissolved in 1.0 ml of dimethylsulfoxide, stored frozen (–20°C) in the dark, and added to the molten media immediately prior to pouring. At 48 h after being placed onto agar, all leaf discs were inoculated with 50 µl (approximately 500 sporangia) of a single-isolate *P. infestans* sporangia suspension that had been incubated in the dark at 8°C for 5 h to induce zoosporogenesis. The zoospore number was not quantified, but the presence of active zoospores was confirmed microscopically. Four leaf discs were inoculated per treatment replicate, with three replicates of each dimethomorph concentration, and the experiment was repeated three times. Following inoculation, leaf discs were incubated at 21 and 18°C (light and dark, respectively in 12-h alternating cycles) and were removed temporarily from the agar at 48 h before inoculation (HBI), 24 HBI, 24 h after inoculation (HAI), or 48 HAI for treatment. Dimethomorph was applied until run-off at 0, 1, 10, 100, or 1,000 µg/ml as the formulated commercial product, Acrobat 50WP (BASF Corp.) using a CO₂-powered hand sprayer (344 kPa pressure) with a single XR11003VS spray nozzle (Spray Systems, Pomona, CA).

Leaf discs were assessed at 96 h after inoculation for symptoms and signs of infection by *P. infestans*, such as necrosis and sporulation. The mean incidence of infection of the four leaf discs (percentage that were symptomatic) was determined for each treatment replicate. The four leaf discs then were placed in a 15-ml centrifuge tube containing 4.0 ml of dH₂O and agitated for 15 s using a laboratory vortex, after which sporangia were quantified with a hemacytometer. The number of sporangia per square centimeter of leaf disc area then was calculated for each treatment replicate. The percent inhibition relative to the 0 µg/ml control and EC₅₀ values were calculated, and an ANOVA was performed as described above. The contrast function in SAS/Stat was used to compare application timings before inoculation with those after inoculation for the inhibition of in vivo symptom incidence and sporulation.

RESULTS

Inhibition of hyphal growth and sporulation in vitro. For all isolates, no significant inhibition of hyphal growth (Fisher's LSD, $P = 0.05$) was observed on media amended with dimethomorph at 0.01 µg/ml (Fig. 1A). At a dimethomorph con-

centration of 1 µg/ml, more than 50% inhibition of hyphal growth occurred in all isolates, with Pi95-5 being completely inhibited. At a concentration of 10 µg/ml, dimethomorph completely inhibited hyphal growth of all isolates. The calculated EC₅₀ values for inhibition of hyphal growth by dimethomorph ranged from 0.13 to 0.80 µg/ml (Table 2). Five nonoverlapping significance categories were detected, with the isolate Pi213 having a significantly higher EC₅₀ value than all others for in vitro hyphal growth.

In contrast to in vitro hyphal growth, dimethomorph at 0.01 µg/ml had a pronounced effect on in vitro sporulation because isolate Pi95-5 was inhibited approximately 35% (Fig. 1B). Sensitivity of in vitro sporulation was more variable among isolates with dimethomorph at 0.01 and 0.1 µg/ml than at 1 µg/ml, and complete inhibition occurred at 10 µg/ml for all isolates. The calculated EC₅₀ values ranged from 0.04 to 0.44 µg/ml (Table 2) and the isolate Pi213 had a significantly higher EC₅₀ value than isolates Pi95-5, Pi97-2, and Pi98-2.

Inhibition of direct sporangia germination, zoosporogenesis, zoospore encystment, and cyst germination in vitro. Direct sporangia germination was not inhibited in isolate Pi213 with dimethomorph at 0.01 µg/ml, whereas the other 10 isolates were inhibited from 16 to 29% at this concentration (Fig. 1C). For most isolates, direct germination inhibition trends were similar to those of in vitro sporulation, except that dimethomorph at 10 µg/ml was not always completely inhibitory. No significant differences were calculated between isolates for the EC₅₀ of inhibition of direct sporangia germination and EC₅₀ values ranged from 0.096 to 0.231, with a mean of 0.163 µg/ml (*data not shown*).

Zoosporogenesis was not significantly inhibited at any of the dimethomorph concentrations examined, up to 10 µg/ml (Fig. 1D). No significant differences in EC₅₀ values were detected between isolates, and all exceeded 10 µg/ml (*data not shown*). Most isolates exhibited significant inhibition of zoospore encystment (Fig. 1E) and cyst germination (Fig. 1F) at 0.01 µg/ml. Isolate Pi213 had a significantly higher EC₅₀ value for zoospore encystment than did Pi88, Pi95-5, Pi94-4, and Pi97-2 (Table 2). For cyst germination, the EC₅₀ values for Pi670 and Pi88 were significantly higher than 5 of the 11 isolates, and not significantly different from the remaining isolates.

Protectant, post-infection, and antispore activity of dimethomorph in vivo. Significant main effects of application timings, dimethomorph concentrations, and a significant timing × concentration interaction were detected for the inhibition of symptom incidence and sporulation following inoculation with *P. infestans*, whereas other factors and inter-

actions were not significant (Table 3). The percent inhibition of symptom incidence values at each dimethomorph concentration was not significantly different between application timings on either side of the inoculation event (i.e., the 48- and 24-HBI application timings were not significantly different but were significantly different from the 24- and 48-HAI timings, and visa versa) (Fig. 2A). For application timings before inoculation, increasing the dimethomorph concentration resulted in significantly greater inhibition of symptom incidence. Application of dimethomorph after inoculation with *P. infestans* resulted in significantly lower values for the inhibition of symptom incidence at each concentration than the corresponding HBI timings, except for dimethomorph at 1 µg/ml. Complete inhibition of symptom incidence occurred at 1,000 µg/ml for both HBI application timings, but was only inhibited to approximately 20% for both HAI timings.

The 24-HBI application timing had a significantly greater percent inhibition of sporulation than the 24-HAI timing for dimethomorph at 10 µg/ml and all other application timings at 100 µg/ml (Fig. 2B). Because symptoms did not develop on leaf discs treated before inoculation with dimethomorph at 1,000 µg/ml, antispore activity could not be assessed at this concentration and application timing.

Significant main effects were detected with respect to the EC₅₀ values for the application timing factor of the incidence of symptom development and sporulation in vivo, but not the isolate factor (Table 4). No isolate × timing interaction was detected. For the inhibition of symptom incidence, EC₅₀ values for both application timings before inoculation were not significantly different, but were significantly different from both timings after inoculation (Table 5). Mean EC₅₀ values for the inhibition of symptom incidence were 36.18 and 62.45 × 10⁴ µg/ml for the before and after inoculation timings, respectively. For the inhibition of *P. infestans* sporulation by dimethomorph, the 24-HBI application timing had a significantly lower EC₅₀ value than both HAI timings, while the 48-HBI timing was not significantly different from any other timing (Table 5). The EC₅₀ values for the inhibition of sporulation ranged from 39.37 to 59.05 µg/ml. For both symptom incidence and sporulation the, contrast function comparing dimethomorph application before versus after inoculation was significant (Table 4).

DISCUSSION

The mean concentrations required for the inhibition of *P. infestans* in vitro hyphal growth, direct sporangia germination, zoospore encystment, cyst germination, and in vivo inhibition of symptom incidence and sporulation were similar to those previously reported for single isolates of *P. in-*

festans (1,2,9,17,19). The different stages of the *P. infestans* asexual life cycle showed a range of sensitivities to dimethomorph in vitro. Zoospore encystment and cyst germination appeared to be most sensitive to dimethomorph with mean EC₅₀ values <0.10 µg/ml, whereas hyphal growth, sporulation, and direct sporangia germination were less sensitive with mean

EC₅₀ values of 0.45, 0.22, and 0.19 µg/ml, respectively.

Direct sporangia germination and in vitro hyphal growth had the smallest ranges in sensitivity between isolates, with four- and sixfold differences in EC₅₀ values between the most- and least-sensitive isolates, respectively. The range of dimethomorph sensitivity for in vitro hyphal

growth reported here is much smaller than those reported for phenylamide sensitivity in *P. infestans* (26) and other *Phytophthora* spp. (12,21). However, the cited studies included isolates with field resistance to the phenylamides. Recalculating the sensitivity range using only the phenylamide-sensitive isolates from these studies results in a sensitivity distribution for phenyla-

Fig. 1. Influence of dimethomorph concentration on in vitro **A**, hyphal growth and **B**, sporulation; **C**, direct sporangia germination; **D**, zoosporogenesis; **E**, zoospore encystment; and **F**, cyst germination for 11 isolates of *Phytophthora infestans*. Error bars represent Fisher's least significant difference ($P = 0.05$).

mides similar to the one reported here for *P. infestans* to dimethomorph. In vitro sporulation, zoospore encystment, and cyst germination all had larger ranges in sensi-

tivity, with >10-fold differences. The ranges in sensitivity measured in this study probably are indicative of natural variations in the sensitivity of fungal and Oo-

mycete plant pathogens, as noted with other fungicides (15,20,26).

The higher dimethomorph sensitivity of the zoospore encystment and cyst germination stages of the *P. infestans* asexual life cycle may be related to the physiology of those stages. Exposure to dimethomorph has been shown to cause aberrant cell wall formation and thickening, eventually resulting in burst hyphal tips (17). Disruption of the de novo construction of the cell wall by dimethomorph, as in zoospore encystment, could be lethal in a short time period because zoospores have comparatively limited carbohydrate reserves to actively offset osmotic pressure and for use in cell wall formation (5). As with zoospores, cysts have relatively limited reserves, saprophytic ability, and longevity (8) and the inhibition of germ tube formation for penetration into the host plant cell would be lethal. The sporangial and hyphal stages are less sensitive, possibly because of their larger carbohydrate reserves and ability to tolerate multiple attempts at hyphal tip formation. Alternatively, the cell walls of the different stages of *Phytophthora* spp. have been found to vary in chemical composition and thickness (5), and it is possi-

Table 2. Effective concentration of dimethomorph required for a 50% reduction of in vitro hyphal growth (colony diameter), sporulation, zoospore encystment, and cyst germination for *Phytophthora infestans* isolates

Isolate ID	EC ₅₀ dimethomorph (µg/ml) ^v			
	Hyphal growth ^w	Sporulation ^x	Zoospore encystment ^y	Cyst germination ^z
Pi88	0.43 c	0.20 abc	0.05 bc	0.17 a
Pi95-5	0.13 e	0.04 c	0.02 bc	0.02 d
Pi671	0.63 b	0.32 ab	0.07 abc	0.12 ab
Pi458	0.59 b	0.20 abc	0.08 abc	0.05 bcd
Pi670	0.39 c	0.24 abc	0.04 bc	0.15 a
Pi213	0.80 a	0.44 a	0.14 a	0.11 abc
Pi94-4	0.27 d	0.25 abc	0.01 c	0.02 d
Pi95-7	0.53 b	0.17 abc	0.05 abc	0.04 bcd
Pi97-2	0.29 d	0.12 bc	0.03 bc	0.03 cd
Pi98-1	0.42 c	0.25 abc	0.10 ab	0.07 abcd
Pi98-2	0.43 c	0.16 bc	0.06 abc	0.05 bcd

^v Effective concentration for a 50% reduction, calculated using probit transformation of the percent inhibition relative to the untreated control. Means followed by the same letter are not significantly different using Fisher's least significant difference ($P = 0.05$).

^w Calculated from colony diameter on modified rye B media amended with dimethomorph.

^x Production of sporangia per square centimeter of colony area on modified rye B media amended with dimethomorph.

^y Encystment of zoospores in sdH₂O-amended with dimethomorph.

^z Germination of previously encysted zoospores in sdH₂O-amended with dimethomorph.

Table 3. Summary of the analysis of variance of the incidence of symptom development or sporulation following inoculation of potato leaf discs by *Phytophthora infestans* for all 11 isolates, five dimethomorph concentrations applied at one of four application timings, and with three experimental repeats

Source ^w	df ^x	Sum of squares ^y	Mean square	F value	P value ^z
Incidence of symptom development					
Isolate	10	0.50	0.05	0.38	0.9540
Repeat	2	0.46	0.23	1.77	0.1702
Timing	3	50.52	16.84	128.53	<0.0001
Isolate × timing	30	0.92	0.03	0.23	0.9999
Concentration	4	102.10	25.52	194.82	<0.0001
Isolate × concentration	40	1.11	0.03	0.21	0.9999
Timing × concentration	12	40.49	3.37	25.75	<0.0001
Isolate × timing × concentration	120	3.35	0.03	0.21	0.9999
Repeat × isolate	20	3.76	0.19	1.43	0.0901
Repeat × timing	6	0.90	0.15	1.15	0.3322
Repeat × isolate × timing	60	6.23	0.10	0.79	0.8735
Repeat × concentration	8	0.63	0.08	0.60	0.7791
Repeat × isolate × concentration	80	6.15	0.08	0.59	0.9985
Repeat × timing × concentration	24	0.95	0.04	0.30	0.9996
Repeat × isolate × timing × concentration	240	12.89	0.05	0.41	0.9999
Error	1,320	173.94	0.13	...	...
Total	1,979	404.91	...	...	...
Sporulation					
Isolate	10	0.90	0.09	1.54	0.1196
Repeat	2	0.03	0.02	0.28	0.7544
Timing	3	0.90	0.30	5.10	0.0016
Isolate × timing	30	2.39	0.08	1.35	0.0964
Concentration	4	331.39	82.85	1,408.62	<0.0001
Isolate × concentration	40	2.57	0.06	1.09	0.323
Timing × concentration	12	1.43	0.12	2.03	0.0188
Isolate × timing × concentration	120	4.52	0.04	0.64	0.9989
Repeat × isolate	20	1.38	0.07	1.18	0.2672
Repeat × timing	6	0.49	0.08	1.38	0.2191
Repeat × isolate × timing	60	4.48	0.07	1.27	0.0839
Repeat × concentration	8	0.14	0.02	0.30	0.9662
Repeat × isolate × concentration	80	3.55	0.04	0.76	0.9458
Repeat × timing × concentration	24	0.60	0.03	0.43	0.9932
Repeat × isolate × Timing × concentration	240	9.81	0.04	0.70	0.9998
Error	1,320	77.64	0.06	...	...
Total	1,979	442.24	...	...	...

^w Timing = application timings 48 h before, 24 h before, 24 h after, or 48 h after inoculation with *P. infestans*. Concentration = dimethomorph concentration.

^x Degrees of freedom.

^y Type III sum of squares.

^z Significance indicated by $P \leq 0.05$.

Fig. 2. Influence of dimethomorph concentration on **A**, symptom incidence and **B**, sporulation on potato leaf discs inoculated with *Phytophthora infestans*. Plots represent the mean of all 11 isolates when dimethomorph was applied at 48 h before, 24 h before, 24 h after, or 48 h after inoculation with *P. infestans*. Error bars represent Fisher's least significant difference ($P = 0.05$).

Table 4. Summary of the analysis of variance of the effective concentration of dimethomorph (µg/ml) required for a 50% reduction of in vivo symptom incidence and sporulation from potato leaf discs, when applied at 48 h before, 24 h before, 24 h after, or 48 h after inoculation with *Phytophthora infestans*

Source ^w	df ^x	Sum of squares ^y	Mean square	F value	P value ^z
Incidence of symptom development					
Isolate	10	9.58×10^{11}	9.58×10^{11}	0.66	0.7576
Repeat	2	5.35×10^{11}	2.67×10^{11}	1.84	0.1647
Timing	3	1.29×10^{13}	4.29×10^{12}	29.57	<0.0001
Isolate $\times$ timing	20	2.44×10^{12}	8.14×10^{10}	0.56	0.9622
Error	86	1.25×10^{13}	1.45×10^{11}	...	...
Total	131	2.93×10^{13}	...	...	...
Contrast for timing	1	1.29	1.29	88.69	<0.0001
Sporulation					
Isolate	10	9,237.78	923.78	1.91	0.0551
Repeat	2	548.27	274.13	0.57	0.5702
Timing	3	6481.21	2,160.40	4.46	0.0059
Isolate $\times$ timing	20	16,249.55	541.65	1.125	0.3373
Error	86	41,690.82	484.78	...	...
Total	131	74,207.63	...	...	...
Contrast for timing	1	5,083.88	5,083.88	10.49	0.0017

^w Timing = application timings 48 h before, 24 h before, 24 h after, or 48 h after inoculation with *P. infestans*.

^x Degrees of freedom.

^y Type III sum of squares.

^z Significance indicated by $P \leq 0.05$.

ble that the biochemical basis of these variations may be associated with the different levels of sensitivity to dimethomorph. The true reason or reasons for the variations in sensitivity will not be understood until the biochemical mode of action of dimethomorph is known.

The full-label field rate (United States) of dimethomorph, assuming a carrier volume of 234 liter/ha, is approximately 1,000 µg/ml. Application of the full rate of dimethomorph at 24 or 48 h before inoculation almost completely inhibited symptom

incidence in inoculated leaf discs, whereas application within 48 h after inoculation failed to offer significant inhibition. When used to control *P. infestans* in the field, dimethomorph should be applied in a protectant fashion, reinforcing previously reported results (1,9,24). The EC_{50} values for the inhibition of in vivo symptom incidence were much larger than those at specific stages in the asexual life cycle of *P. infestans* in vitro, as has been noted previously with *P. infestans* (23), and is most likely attribut-

able to the dilution or metabolism of dimethomorph in planta.

Inhibition of sporulation of *P. infestans* occurred when dimethomorph was applied between 48 h before and 48 h after inoculation at concentrations of 100 µg/ml or higher. These results are similar to those previously reported (9). Outside of the examined time frame and under field conditions, dimethomorph may not have the same level of antispore activity. A previous study conducted by the authors examined sporulation under field conditions

Table 5. Effective concentration of dimethomorph required for a 50% reduction of in vivo symptom incidence and sporulation from potato leaf discs, when applied at 48 h before, 24 h before, 24 h after, or 48 h after inoculation with *Phytophthora infestans*

Application timing ^y	EC ₅₀ dimethomorph (µg/ml) ^x	
	Symptom incidence	Sporulation ^z
48 HBI	39.62 b	47.37 ab
24 HBI	32.73 b	39.37 b
24 HAI	62.86 × 10 ⁴ a	53.51 a
48 HAI	62.03 × 10 ⁴ a	59.05 a

^x Effective concentration for a 50% reduction, calculated using probit transformation of the percent inhibition relative to the untreated control. Means followed by the same letter are not significantly different using Fisher's least significant difference ($P = 0.05$).

^y Mean values pooled across isolates. HBI = hours before inoculation and HAI = hours after inoculation.

^z Production of sporangia per square centimeter of leaf disc.

and failed to detect any inhibition of sporulation following dimethomorph applications (25). Application of fungicides under field conditions is unlikely to confer the same homogeneity of leaf coverage as application under controlled conditions. Thus, the lack of antispore activity in field conditions when dimethomorph is applied within 48 h of the infection event is likely due to incomplete coverage, subefficacious concentrations through dilution or metabolism in planta, or a combination of these factors.

The results of the in vitro assays demonstrated that dimethomorph is most active against zoospore encystment and cyst germination in addition to having an effect on hyphal growth, sporangia formation, and germination. Frequent protectant applications of dimethomorph at concentrations of 1,000 µg/ml would likely inhibit infection by *P. infestans* in the field and possibly reduce sporulation from previously established infections, but not eliminate them. The inhibition of *P. infestans* zoospore encystment and cyst germination by dimethomorph, in combination with a reduction in sporulation from infected foliar tissue, will likely have a larger impact upon epidemic development than the inhibition of the infectious stages of the asexual lifecycle alone. The *P. infestans* isolates examined all had similar sensitivity to dimethomorph in the assays performed. Isolates exhibiting low levels of resistance to dimethomorph have been generated in vitro for *P. infestans* (27) and other *Phytophthora* spp. (7,27); however there has been no conclusive evidence of the development of practical resistance to dimethomorph in *P. infestans*. Nevertheless, resistance monitoring and management are important for this fungicide, and resistance development should be examined further.

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