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

J. M. Stein, Texas Agriculture Experiment Station, Bushland, TX 79012, and **W. W. Kirk**, Department of Plant Pathology, Michigan State University, East Lansing, MI 48824

Correspondence should be directed to:

William W. Kirk
Department of Plant Pathology
Michigan State University
East Lansing, MI 48824
(517) 353-4481
FAX (517) 353-1926
kirkw@msu.edu

ABSTRACT

Stein, J.M. and Kirk, W.W. Variations in the Sensitivity of *Phytophthora infestans* Isolates from Different Genetic Backgrounds to Dimethomorph.

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 EC_{50} values for most isolates <0.20 μg/ml, whereas direct sporangia germination and *in vitro* hyphal growth and sporulation were less sensitive (means of 0.45 and 0.22 μg/ml, respectively). Zoosporogenesis was not significantly inhibited at the maximum dimethomorph concentration examined, 10 μg/ml. Significant differences (Fisher's LSD, $P = 0.05$) 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 1000 μg/ml dimethomorph to potato leaf discs at 24 or 48 h before inoculation completely inhibited symptom incidence for most isolates, while 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 at 1000 μg/ml dimethomorph, even when symptom incidence was not significantly reduced.

Additional keywords: late blight, *Solanum tuberosum*, fungicide, Oomycete, antisporulation.

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 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 "antisporulation" 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), as these stages do not involve cell wall formation. The activity of dimethomorph on other *Phytophthora* species 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* species 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, nor antisporulation 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 antisporulant 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*) seeds (100 g/liter) boiled for 1 h, de-ionized (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, U.S.A.) 100X 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, U.S.A.) to sterilize. Control plates received 10 ml/liter of filter-sterilized ethanol.

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 µg/ml control replicates, within that experimental repeat. Percent inhibition values were then transformed using probits (12,22), i.e. the inverse of the standard normal distribution (16), and expressed as a function of the $\log_{10}$ of

concentration. Plot equation parameters were determined using linear regression (SigmaPlot, SPSS Inc., Chicago, IL, U.S.A.) and the EC_{50} for the inhibition of each variable (e.g. *in vitro* hyphal growth, zoospore encystment, etc.) 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 EC_{50} 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, U.S.A.). Means were separated by Fisher's LSD at P = 0.05.

Inhibition of hyphal growth and sporulation *in vitro*. Previously characterized isolates (Table 1) of *P. infestans* were sub-cultured once on modified rye B agar for 21 days at 18°C in the dark, following a single zoospore re-isolation from infected potato leaves. Isolates obtained prior to 1997 probably had not been exposed to dimethomorph as the fungicide was on limited emergency registration in much of the U.S.A. from 1995 to 1997. Those isolates obtained in 1997 and 1998 may have been exposed to dimethomorph, although the likelihood is small since 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 EC_{50} 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 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 ten plugs were then placed into a single 1.5 ml micro-centrifuge tube with 1.0 ml of sterile (s) dH_2O and agitated for 15 seconds using a laboratory vortex to dislodge the sporangia. Sporangia were quantified with a hemacytometer (average of four fields) and the number per colony area (cm^2) was calculated. The percent inhibition relative to the 0 μg/ml control and EC_{50} 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, potato tubers (cv. Russet Burbank) were surface disinfested with 0.5% sodium hypochlorite in dH_2O (10% commercial bleach solution) for 30 min, rinsed three times in sdH_2O, 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 cm^2 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 hour alternating cycles at 18°C (light) / 15°C (dark) until at least 50% of the tuber slice surface was covered by the mycelium (typically five days). Sporangia were harvested by gently removing the mycelium with a plastic culture spreader, transferred into sterile micro-centrifuge tubes, and 1.0 ml sdH_2O 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 1.0×10^4 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 ~100 sporangia (10μl) were added to 90μl sdH_2O in 96-well Costar culture plates (Corning Inc., Acton, MA, U.S.A.). Dimethomorph stock solutions were added for a final concentration of 0, 0.01, 0.1, 1, or 10 μg/ml dimethomorph, 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 were then 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 EC_{50} values were calculated, and an ANOVA was performed as described above.

Protectant, post infection, and antisporulant 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 hypochlorite in dH_2O (10% commercial bleach solution) for 1 min. Leaflets were rinsed three times in sdH_2O, 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 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 (~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°C light / 18°C dark (12 h alternating cycles) and were temporarily removed 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 1000 µg/ml as the formulated commercial product, Acrobat 50WP (BASF Corp, Research Triangle Park, NC, U.S.A.) using a CO_2-powered hand sprayer (344 kPa pressure) with a single XR11003VS spray nozzle (Spray Systems, Pomona, CA, U.S.A.).

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 were then placed in a 15 ml centrifuge tube containing 4.0 ml of dH_2O, agitated for 15 seconds using a laboratory vortex, and sporangia were quantified with a hemacytometer. The number of sporangia per leaf disc area (cm^2) was then calculated for each treatment replicate. The percent inhibition relative to the 0 µg/ml control and EC_{50} 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 0.01 µg/ml dimethomorph (Fig. 1A). At a concentration of 1 µg/ml dimethomorph, 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 EC_{50} values for inhibition of hyphal growth ranged from 0.13 to 0.80 µg/ml dimethomorph (Table 2). Five non-overlapping significance categories were detected, with the isolate Pi213 having a significantly higher EC_{50} 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 as isolate Pi95-5 was inhibited ~35% (Fig. 1B). Sensitivity of *in vitro* sporulation was more variable among isolates at 0.01 and 0.1 µg/ml dimethomorph than at 1 µg/ml and complete inhibition occurred at 10 µg/ml dimethomorph for all isolates. The calculated EC_{50} values ranged from 0.04 to 0.44 µg/ml dimethomorph (Table 2) and the isolate Pi213 had a significantly higher EC_{50} 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 at 0.01 µg/ml dimethomorph, 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 10 µg/ml dimethomorph was not always completely inhibitory. No significant differences were calculated between isolates for the EC_{50} of inhibition of direct sporangia germination and EC_{50} values ranged from 0.096 to 0.231, with a mean of 0.163 µg/ml dimethomorph (data not shown).

Zoosporogenesis was not significantly inhibited at any of the concentrations examined, up to 10 µg/ml dimethomorph (Fig. 1D). No significant differences in EC_{50} values were detected between isolates, and all exceeded 10 µg/ml dimethomorph (data not shown). Most isolates exhibited significant

inhibition of zoospore encystment (Fig. 1E) and cyst germination (Fig. 1F) at 0.01 µg/ml dimethomorph. Isolate Pi213 had a significantly higher EC_{50} value for zoospore encystment than Pi88, Pi95-5, Pi94-4, and Pi97-2 (Table 2). For cyst germination, the EC_{50} values for Pi670 and Pi88 were significantly higher than five of the 11 isolates, and not significantly different from the remaining isolates.

Protectant, post infection, and antisporulation activity of dimethomorph *in vivo*. Significant main effects of application timings, dimethomorph concentrations, and a significant timing x concentration interaction were detected for the inhibition of symptom incidence and sporulation following inoculation with *P. infestans*, while other factors and interactions were not significant (Table 3). The percent inhibition of symptom incidence values at each dimethomorph concentration were not significantly different between application timings on either side of the inoculation event, i.e. the 48 HBI and 24 HBI application timings were not significantly different but were significantly different from the 24 HAI 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 at 1 µg/ml dimethomorph. Complete inhibition of symptom incidence occurred at 1000 µg/ml dimethomorph for both HBI application timings, but was only inhibited to ~20% for both HAI timings.

The 24 HBI application timing had a significantly greater percent inhibition of sporulation than the 24 HAI timing at 10 µg/ml dimethomorph and all other application timings at 100 µg/ml dimethomorph (Fig. 2B). Because symptoms did not develop on leaf discs treated with 1000 µg/ml dimethomorph before inoculation, antisporulation could not be assessed at this concentration and application timing.

Significant main effects were detected with respect to the EC_{50} 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 x timing interaction was detected. For the inhibition of symptom incidence, EC_{50} values for both application timings before inoculation were not significantly different, but were significantly different from both timings after inoculation (Table 5). Mean EC_{50} values for the inhibition of symptom incidence were 36.18 and 62.45×10^4 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 EC_{50} value than both HAI timings, while the 48 HBI timing was not significantly different from any other timing (Table 5). EC_{50} 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 vs. 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. infestans* (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 EC_{50} values <0.10 µg/ml, whereas hyphal growth, sporulation, and direct sporangia germination were less sensitive with mean EC_{50} values 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 4 and 6-fold differences of EC_{50} 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* species (12,21). However, the cited studies included isolates with field resistance to phenylamides and exclusion of the phenylamide-resistant isolates from the sensitivity ranges results in a similar distribution to that of the isolates examined here to dimethomorph. *In vitro* sporulation, zoospore encystment and cyst germination all had larger ranges in sensitivity with >10-fold differences. The ranges in sensitivity measured in this study are probably indicative of natural variations in the sensitivity of fungal and Oomycete 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 as 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* have been found to vary in chemical composition and thickness (5) and it is possible that the biochemical basis of these variations may be associated with the different levels of sensitivity to dimethomorph. The true reason(s) for the variations in sensitivity will not be understood until the biochemical mode of action of dimethomorph is known.

The full label field rate (U.S.A.) of dimethomorph, assuming 234 liter/ha carrier volume, is ~1000 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 attributable 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 antisporulant activity. A previous study conducted by the authors examined sporulation under field conditions 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 antisporulation activity in field conditions when dimethomorph is applied within 48 h of the infection event is likely due to incomplete coverage, sub-efficacious 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 1000 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* species (7,27), however there has been no conclusive evidence of the development of practical resistance to dimethomorph in these pathogens. Nevertheless, resistance monitoring and management are important for this fungicide, and resistance development should be examined further.

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Table 1. *P. infestans* isolate identification, mating type, genotype, physiological race, state in which isolated (U.S.A.), and metalaxyl (phenylamide) sensitivity.

Isolate	Year Isolated	Mating Type	Genotype[a]	Physiological Race[b]	Origin[c]	Metalaxyl Sensitivity[d]
Pi88	1995	A1	US1	n/d [e]	ND	n/d
Pi95-5	1995	A1	US1	n/d	MI	S
Pi671	1997	A1	US14	n/d	WA	n/d
Pi458	1998	A2	US17	1.2.3.4.5.6.7.9.11	ID	n/d
Pi670	1997	A2	US7	1.2.3.4.5.6.10.11	OR	n/d
Pi213	1997	A2	US8	1.2.3.4.5.6.7.10.11	CO	R
Pi94-4	1994	A2	US8	1.3.5	MI	R
Pi95-7	1995	A2	US8	1.2.3.4.5.6.7.10.11	MI	R
Pi97-2	1997	A2	US8	1.3.4.5.8.11	MI	R
Pi98-1	1998	A2	US8	2.5.6.7.8.9.10.11	MI	S
Pi98-2	1998	A2	US8	1.2.3.4.5.7.10.11	MI	R

a. Allozyme-based genotype (13).
b. Determined using inoculations onto potato lines with different R-genes as described (3)
c. State of origin (U.S.A.). All isolates were obtained from potato.
d. Sensitivity to the fungicide metalaxyl assayed via the inhibition of *in vitro* hyphal growth: S = sensitive, R = resistant (11).
e. Not determined.

Table 2. The effective concentration of dimethomorph (µg/ml) required for a 50% reduction of *in vitro* hyphal growth (colony diameter), sporulation, zoospore encystment, and cyst germination for *P. infestans* isolates.

Isolate ID	EC_{50}[a] (mg/ml) Dimethomorph							
	Hyphal Growth[b]		Sporulation[c]		Zoospore[d] Encystment		Cyst Germination[e]	
Pi88	0.425	c[f]	0.199	abc	0.047	bc[d]	0.169	a
Pi95-5	0.131	e	0.036	c	0.016	bc	0.016	d
Pi671	0.625	b	0.320	ab	0.067	abc	0.117	ab
Pi458	0.585	b	0.203	abc	0.084	abc	0.047	bcd
Pi670	0.392	c	0.236	abc	0.036	bc	0.145	a
Pi213	0.800	a	0.437	a	0.136	a	0.114	abc
Pi94-4	0.270	d	0.247	abc	0.011	c	0.018	d
Pi95-7	0.533	b	0.171	abc	0.053	abc	0.042	bcd
Pi97-2	0.291	d	0.115	bc	0.033	bc	0.027	cd
Pi98-1	0.419	c	0.246	abc	0.098	ab	0.071	abcd
Pi98-2	0.429	c	0.163	bc	0.063	abc	0.046	bcd

a. Effective concentration for a 50% reduction, calculated using probit transformation of the percent inhibition relative to the untreated control.
b. Calculated from colony diameter on modified rye B media amended with dimethomorph.
c. Production of sporangia per cm^2 colony area on modified rye B media amended with dimethomorph.
d. Encystment of zoospores in sdH_2O amended with dimethomorph.
e. Germination of previously encysted zoospores in sdH_2O amended with dimethomorph.
f. Means followed by the same letter are not significantly different using Fisher's LSD ($P = 0.05$).

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

Source	Df[a]	Sum of Squares[b]	Mean Square	*F* Value	*P* Value[c]
A. 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[d]	3	50.52	16.84	128.53	< 0.0001
Isolate x Timing	30	0.92	0.03	0.23	0.9999
Conc.[e]	4	102.10	25.52	194.82	< 0.0001
Isolate x Conc.	40	1.11	0.03	0.21	0.9999
Timing x Conc.	12	40.49	3.37	25.75	< 0.0001
Isolate x Timing x Conc.	120	3.35	0.03	0.21	0.9999
Repeat x Isolate	20	3.76	0.19	1.43	0.0901
Repeat x Timing	6	0.90	0.15	1.15	0.3322
Repeat x Isolate x Timing	60	6.23	0.10	0.79	0.8735
Repeat x Conc.	8	0.63	0.08	0.60	0.7791
Repeat x Isolate x Conc.	80	6.15	0.08	0.59	0.9985
Repeat x Timing x Conc.	24	0.95	0.04	0.30	0.9996
Repeat x Isolate x Timing x Conc.	240	12.89	0.05	0.41	0.9999
Error	1320	173.94	0.13		
Total	1979	404.91			
B. 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 x Timing	30	2.39	0.08	1.35	0.0964
Conc.	4	331.39	82.85	1408.62	< 0.0001
Isolate x Conc.	40	2.57	0.06	1.09	0.323
Timing x Conc.	12	1.43	0.12	2.03	0.0188
Isolate x Timing x Conc.	120	4.52	0.04	0.64	0.9989
Repeat x Isolate	20	1.38	0.07	1.18	0.2672
Repeat x Timing	6	0.49	0.08	1.38	0.2191
Repeat x Isolate x Timing	60	4.48	0.07	1.27	0.0839
Repeat x Conc.	8	0.14	0.02	0.30	0.9662
Repeat x Isolate x Conc.	80	3.55	0.04	0.76	0.9458
Repeat x Timing x Conc.	24	0.60	0.03	0.43	0.9932
Repeat x Isolate x Timing x Conc.	240	9.81	0.04	0.70	0.9998
Error	1320	77.64	0.06		
Total	1979	442.24			

a. Degrees of freedom
b. Type III Sum of Squares
c. P = 0.05 indicates significance.
d. Application timings were 48 h before, 24 h before, 24 h after, or 48 h after inoculation with *P. infestans*.
e. Dimethomorph concentration

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

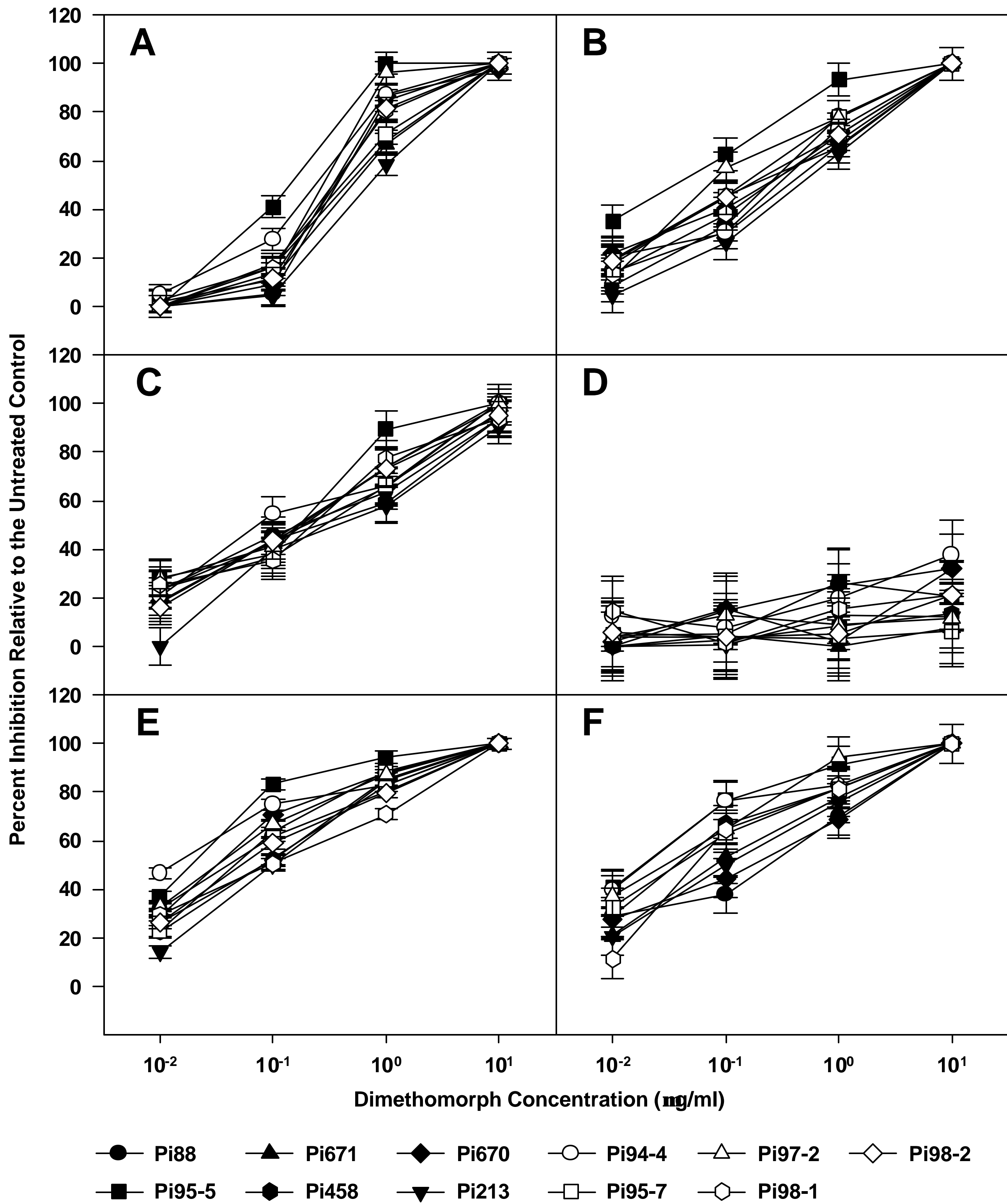
Source	Df[a]	Sum of Squares[b]	Mean Square	*F* Value	*P* Value[c]
A. Incidence of Symptom Development					
Isolate	10	9.58×10^{11}	9.58×10^{11}	0.66	0.7576
Repeat	2	5.35×10^{11}	2.67×10^{11}	1.84	0.1647
Timing[d]	3	1.29×10^{13}	4.29×10^{12}	29.57	< 0.0001
Isolate x Timing	20	2.44×10^{12}	8.14×10^{10}	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
B. Sporulation					
Isolate	10	9237.78	923.78	1.91	0.0551
Repeat	2	548.27	274.13	0.57	0.5702
Timing	3	6481.21	2160.40	4.46	0.0059
Isolate x Timing	20	16249.55	541.65	1.125	0.3373
Error	86	41690.82	484.78		
Total	131	74207.63			
Contrast for Timing	1	5083.88	5083.88	10.49	0.0017

a. Degrees of freedom
b. Type III Sum of Squares
c. P = 0.05 indicates significance.
d. Application timings were 48 h before, 24 h before, 24 h after, or 48 h after inoculation with *P. infestans.*

Table 5. 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 *P. infestans*.

Application Timing[a]	EC_{50}[b] (µg/ml) Dimethomorph			
	Symptom Incidence		Sporulation[c]	
48 HBI	39.62	b[d]	47.37	ab
24 HBI	32.73	b	39.37	b
24 HAI	$62.86x10^4$	a	53.51	a
48 HAI	$62.03x10^4$	a	59.05	a

a. Mean values pooled across isolates.
b. Effective concentration for a 50% reduction, calculated using probit transformation of the percent inhibition relative to the untreated control.
c. Production of sporangia per cm^2 leaf disc.
d. Means followed by the same letter are not significantly different using Fisher's LSD (P = 0.05).



A
B
C
D
E
F
120
100
80
60
40
20
0
Percent Inhibition Relative to the Untreated Control
10-2
10-1
100
101
Dimethomorph Concentration (mg/ml)
Pi88
Pi671
Pi670
Pi94-4
Pi97-2
Pi98-2
Pi95-5
Pi458
Pi213
Pi95-7
Pi98-1

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 *P. infestans*. Error bars represent Fisher's LSD (P = 0.05).

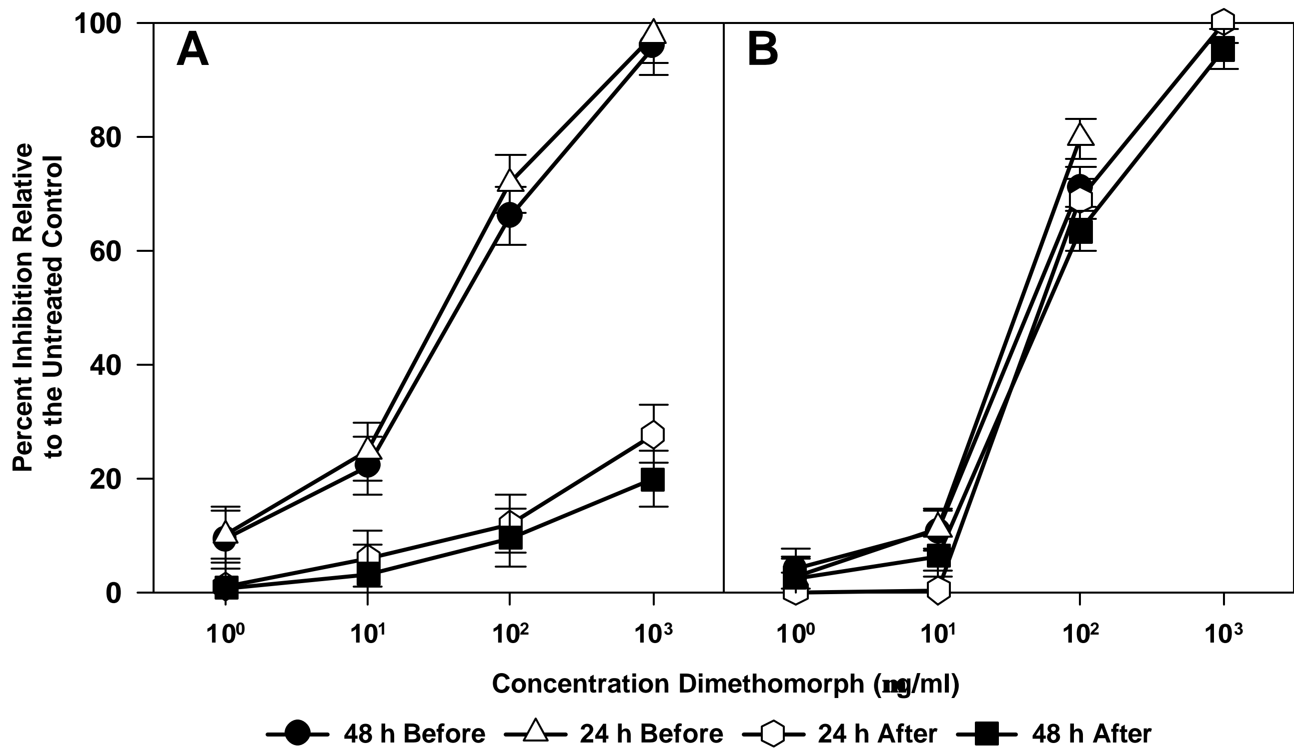

A
B
100
80
60
40
20
0
Percent Inhibition Relative
to the Untreated Control
10^0
10^1
10^2
10^3
Concentration Dimethomorph (µg/ml)
48 h Before
24 h Before
24 h After
48 h After

Fig. 2. Influence of dimethomorph concentration on (A) symptom incidence and (B) sporulation on potato leaf discs inoculated with *P. 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 LSD ($P = 0.05$).