

Variations in the Sensitivity of *Phytophthora infestans* isolates from Several Genetic Backgrounds to Dimethomorph.

5 **J.M. Stein and W.W. Kirk**, Department of Plant Pathology, Michigan State University, East Lansing, MI 48824

Correspondence should be directed to:

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

ABSTRACT

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

5 The sensitivities of 11 isolates of *Phytophthora infestans* to dimethomorph at all stages of the asexual life cycle and when inoculated onto potato leaf disks were examined. Zoospore encystment and cystospore germination were both highly sensitive to dimethomorph with EC₅₀ values for most isolates <0.20 µg ml⁻¹, while direct sporangia germination and *in vitro* hyphal growth and sporulation were less so. Zoosporogenesis was not significantly inhibited at the
10 maximum dimethomorph concentration examined, 10.0 µg ml⁻¹. Significant differences (Fischer's LSD, α = 0.05) in the EC₅₀ values were present between isolates for all stages of the asexual life cycle, except direct sporangia germination and zoosporogenesis. The degree of dimethomorph sensitivity in all stages of the asexual life cycle was positively and significantly correlated, except cystospore germination. Sensitivity ratios between the least sensitivity and
15 most sensitive isolates were 6.1, 12.1, 8.5, and 10.4 for hyphal growth, sporulation, zoospore encystment, and cystospore germination, respectively. Application of 1000.0 µg ml⁻¹ dimethomorph to potato leaf disks at 24 or 48 hours before inoculation completely inhibited symptom development for most isolates, while application after inoculation generally was not significantly different from the untreated control, regardless of concentration. Sporulation from
20 leaf disks treated with dimethomorph at 24 or 48 hours after inoculation was completely inhibited at 1000.0 µg ml⁻¹. The range in sensitivities of the isolates of *P. infestans* examined to dimethomorph was small in comparison to other fungicides used to control *P. infestans* and indicates a sensitive population.

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

Dimethomorph, a cinnamic acid derivative, was one of the fungicides released in response to the migration (10) or spontaneous development (20) of phenylamide resistant strains of *Phytophthora infestans*. Initial studies with dimethomorph demonstrated a specificity of activity towards the genus *Phytophthora* and certain members of the Peronosporaceae (1).

5 Dimethomorph was most effective when used as a protectant fungicide, however a degree of curative activity also occurred and a moderate amount of translaminar and acropetal systemicity was noted (1,6). One of the most interesting aspects of dimethomorph was the inhibition of *P. infestans* sporangia formation when applied to normally developing lesions under controlled conditions (2,6), also known as “antisporeulation” activity.

10 The specific biological mode of action of dimethomorph has not yet been elucidated but a disruption of cell wall formation, specifically the organization and not the synthesis of wall components, was described (1,12). Dimethomorph disrupted all stages of the asexual life cycle of *P. infestans* except zoosporogenesis, zoospore release and motility, as these stages do not involve cell wall biosynthesis (2,6,12). The activity of dimethomorph on other *Phytophthora*
15 species was examined (13) and found to be similar to that in *P. infestans*. However, differences in the effective concentration for a 50% reduction relative to the untreated control (EC₅₀) for mycelial growth and cystospore germination were apparent between species.

The majority of studies examining dimethomorph activity against *Phytophthora* species typically consisted of only one isolate per species. The single study that compared the sensitivity
20 of several isolates of *P. infestans* to dimethomorph examined only the *in vivo* activity and not the effects on the different stages of the asexual life cycle, nor antisporeulation activity (6). Varying baseline sensitivity to fungicides is present in other Oomycete plant pathogens (19) and it is possible that such variation is present in *P. infestans* to dimethomorph.

The objective of this study was to examine the sensitivity of isolates of *P. infestans* from various genetic backgrounds to dimethomorph i) at multiple stages in the potato late blight disease cycle (asexual life cycle of *P. infestans*) and ii) protectant, curative, and antispore activity of dimethomorph when inoculated onto potato leaves.

5

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 (2,3) consisting of the filtrate of pre-rinsed rye (*Secale cereale* L.) seeds (100.0 g L⁻¹) boiled for one hour, de-ionized (di) H₂O added to a final volume of 1.0 L, glucose (8.0 g L⁻¹), β-sitosterol (0.05 g L⁻¹) and agar (15.0 g L⁻¹). All plates for each replication of the experiment were prepared from the same batch of media in order to limit variability.

A dimethomorph stock solution was prepared by dissolving technical grade (95% pure) dimethomorph into 95% ethanol and performing serial dilutions as required. For solid media, the fungicide stock solutions were added to molten media at 10.0 ml L⁻¹ when the temperature was 55°C. Sterility was obtained by filter sterilizing the fungicide solution through a 0.22 μm syringe-driven filter (Millipore Corp., Bedford, MA, U.S.A.).

Production of viable sporangia. To produce viable sporangia of similar age, potato tubers (cv. Russet Burbank) were surface disinfested with 0.5% sodium hypochlorite in diH₂O (10% commercial bleach solution) for 30 minutes, rinsed three times in sterile diH₂O, and allowed to dry. Tubers were sliced into 7.0 mm sections and placed into sterile 150 mm plastic petri dishes on top of 1.0 cm² agar sections previously colonized by *P. infestans*. Plates were sealed with Parafilm and incubated at 18°C / 15°C (12 hours light / 12 hours dark) until at least 50% of the

tuber 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 microcentrifuge tubes, and 1.0 ml sterile diH₂O was added. Sporangia were counted with a hemacytometer and the concentration was adjusted to 1.0 x 10⁴ sporangia ml⁻¹.

5 **Inhibition of hyphal growth and sporulation *in vitro*.** Previously characterized (Table 1) isolates of *P. infestans* that had been sub-cultured once after re-isolation from infected potato leaves were cultured on rye B agar for 21 days. Colonized agar plugs, 4.0 mm diameter, were transferred from the margin of the colony onto fungicide amended rye B media in 60 mm diameter plastic petri dishes and incubated at 21°C. The fungicide concentrations used were 0.0, 10 0.01, 0.1, 1.0, 10.0 µg ml⁻¹, with three replicate plates per concentration. Colony diameter was measured 11 days after inoculation (DAI). Percent inhibition of radial growth was calculated with respect to the mean colony diameter of the non-amended plates within each isolate. Percent inhibition values were then transformed using probits, i.e. the inverse of the standard normal distribution (11), and expressed as a function of the log₁₀ of concentration (8). Plot equation 15 parameters were then determined using linear regression (SigmaPlot, SPSS Inc., Chicago, IL, U.S.A.) and the EC₅₀ for hyphal growth (diameter) was calculated and reverse transformed for each isolate. The experiment was repeated three times and the EC₅₀ values for each isolate were used as replicates for an analysis of variance (Proc GLM - SAS/Stat, SAS Institute, Cary, NC, U.S.A.) at α = 0.05 by pair-wise comparisons using Fisher's LSD.

20 The effects of dimethomorph on sporangia production *in vitro* were examined by using a modification of a previously described method of sporangia quantification (3). Ten colonized agar plugs, 0.1 mm diameter, were randomly excised from each replicate plate of the *in vitro* sensitivity assay, five plugs 2.0 mm from the colony margin, and five 2.0 mm from the initially

transferred inoculum. The plugs were then placed into a 1.5 ml micro-centrifuge tube with 1.0 ml of sterile de-ionized H₂O (diH₂O) and agitated to dislodge the sporangia. Sporangia were counted with a hemacytometer and the number per colony area (cm⁻²) was calculated. Percent inhibition and EC₅₀ values were calculated and analyzed as described.

5 **Inhibition of direct and indirect germination.** To assess the effects of dimethomorph on direct germination, aliquots of the previously prepared sporangial suspensions from inoculated tuber slices were transferred to 96 well polystyrene culture plates (Costar, Corning Incorporated) and dimethomorph stock solutions were added for a final concentration of 0.0, 0.01, 0.1, 1.0, and 10.0 µg ml⁻¹ dimethomorph, in each of three replicate wells per isolate. Sporangia/fungicide
10 solutions were incubated for 72 hours at 21°C and the numbers of total and germinated sporangia were counted. To examine indirect germination and zoospore encystment, sporangia/fungicide solutions were incubated at 8°C for five hours to induce zoosporogenesis and the number of motile zoospores was counted. The solutions were then incubated at 21°C and the number of total cystospores was counted after 24 hours.

15 To examine cystospore germination, zoospore suspensions were prepared as described and incubated at 21°C for 24 hours to allow for zoospore encystment. Fungicide solutions were added, the solutions were incubated at 21°C for 48 hours, and the number of total and germinated cystospores were counted. Percent inhibition and EC₅₀ values were calculated and analyzed as described.

20 **Isolate sensitivity ranking and correlations of the asexual life cycle stages.** To compare the overall sensitivity of each isolate to dimethomorph, the isolates were ranked within each stage of the asexual life cycle using the least significant mean EC₅₀ values generated from each analysis of variance. An analysis of variance on ranks (11) was performed from these values and

a mean rank of sensitivity was generated for each isolate and compared. Zoosporegenesis was not included in this analysis because of the lack of sensitivity of this process to dimethomorph. Pearson's Product Moment Correlation (11) was used to examine the correlation of the asexual life cycle stages of the *P. infestans* isolates used to detail any trends between stages.

5 **Protectant, curative, and antisporeulation activity of dimethomorph *in vivo*.** The *in vivo* activity of dimethomorph was assessed relative to the inoculation event by removing fully expanded leaflets of similar age from greenhouse grown potato plants (cv. Snowden) and surface disinfecting them with 0.50% sodium hypochlorite in diH₂O (10% commercial bleach solution) for one minute. Leaflets were then rinsed three times in sterile diH₂O, allowed to dry, and cut
10 into 20 mm diameter leaf disks with a sterilized core borer. Leaf disks were placed onto water agar (15.0 g l⁻¹) amended with rifamycin (37.5 mg l⁻¹), ampicillin (10 mg l⁻¹), and nystatin (37.5 mg l⁻¹) which was previously dissolved in 1.0 ml dimethylsulfoxide, stored frozen in the dark, and added to the molten media following sterilization. Leaf disks were temporarily removed from the agar for treatment and dimethomorph was applied until run-off using the formulated
15 commercial product (Acrobat 50WP, BASF Corp.) at 0.0, 1.0, 10.0, 100.0, and 1000.0 µg ml⁻¹ at 24 or 48 hours before (HBI) or after (HAI) inoculation. Twelve leaf disks were inoculated per experiment, and the experiment was repeated three times. Following inoculation, leaf disks were incubated at 21°C light / 18°C dark (12 hour cycles) and assessed at 96 hours after inoculation for symptoms and signs of infection by *P. infestans*, such as necrosis and sporulation. To assay
20 for the inhibition of sporulation, the four leaf disks from each replicate were placed into a 15.0 ml centrifuge tube containing 4.0 ml of diH₂O, agitated, and sporangia were counted with a hemacytometer. Percent inhibition and EC₅₀ values were calculated and analyzed as described for both incidence of symptom development and the number of sporangia produced per leaf disk.

RESULTS

For all isolates, no significant inhibition of hyphal growth (Fischer's LSD, $\alpha = 0.05$) was observed on media amended with $0.01 \mu\text{g ml}^{-1}$ dimethomorph (Fig. 1). At $0.1 \mu\text{g ml}^{-1}$ dimethomorph, all isolates exhibited less than 20% inhibition of hyphal growth except Pi94-4 and Pi95-5, which were inhibited to 28% and 41%, respectively. At $1.0 \mu\text{g ml}^{-1}$ dimethomorph, more than 50% inhibition of hyphal growth occurred in all isolates, with Pi95-5 being completely inhibited, while $10.0 \mu\text{g ml}^{-1}$ dimethomorph completely inhibited hyphal growth of all isolates. Most isolates had a sigmoidal shaped sensitivity curve when percent inhibition was plotted against the \log_{10} of concentration. The calculated EC_{50} values for inhibition of hyphal growth ranged from 0.131 to $0.800 \mu\text{g ml}^{-1}$ dimethomorph (Table 2). Five non-overlapping significance categories were present with the isolate Pi213 having a significantly higher EC_{50} value than all others.

Regardless of the concentration of dimethomorph in amended media, almost all isolates produced significantly less sporangia per colony area (cm^{-2}) than the untreated control (Fig. 1) and Pi95-5 exhibited a 35% inhibition at a concentration of only $0.01 \mu\text{g ml}^{-1}$ dimethomorph. Sensitivity of *in vitro* sporulation was more variable between isolates at 0.01 and $0.1 \mu\text{g ml}^{-1}$ dimethomorph and response curves tended to be more linear than sigmoidal, in comparison to those of hyphal growth. The calculated EC_{50} values ranged from 0.036 to $0.437 \mu\text{g ml}^{-1}$ dimethomorph (Table 2) and the isolate Pi213 had a significantly higher EC_{50} than Pi95-5, Pi97-2, and Pi98-2.

For all isolates except Pi213, $0.01 \mu\text{g ml}^{-1}$ dimethomorph significantly inhibited direct sporangia germination in comparison to the untreated control (Fig. 1). For most isolates, inhibition trends were similar to those of *in vitro* sporulation and sensitivity curves were

primarily linear. 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⁻¹ dimethomorph.

Zoosporogenesis was not significantly inhibited at any of the concentrations examined, up to 5 10.0 µg ml⁻¹ dimethomorph (Fig. 1) and no significant differences were measured in EC₅₀ values between isolates, and all exceeded 10.0 µg ml⁻¹ dimethomorph (data not shown). Zoospore encystment and cystospore germination inhibition trends were generally similar and most isolates exhibited significant inhibition of both factors at 0.01 µg ml⁻¹ dimethomorph (Fig. 1). For both factors, sensitivity trends were generally linear. The isolate Pi213 had a significantly 10 higher EC₅₀ value for zoospore encystment, than Pi88, Pi95-5, Pi94-4, and Pi97-2, while other distinct trends in sensitivity and genetic background were not apparent (Table 2). For cystospore germination, the EC₅₀ values for Pi670 and Pi88 were significantly higher than five of the 11 isolates.

No correlations were measured between EC₅₀ value and isolate mating type, genotype, or 15 location of isolation. The most sensitive isolate overall was Pi95-5, an A1/US1 strain (Table 2). However, the sensitivity of the other A1/US1 isolate, Pi88, was near the average of the isolates examined. The isolate with the lowest overall sensitivity was Pi213. This isolate, Pi458, and Pi671 had significantly lower mean sensitivity than all other isolates, except Pi98-1. All stages of the asexual life cycle were positively and significantly correlated with each other except 20 cystospore germination, which was not significantly correlated with inhibition of any other stage (Table 3).

Within each application timing, no significant difference between isolates was measured for the percent inhibition of the incidence of leaf disk symptom development at each dimethomorph

concentration (data not shown). The inhibition of symptom development sensitivity curves for dimethomorph application prior to inoculation were sigmoidal and complete inhibition of symptom development occurred at 1000.0 $\mu\text{g ml}^{-1}$ dimethomorph (Fig 2a.). No significant inhibition of symptom development occurred when dimethomorph was applied after inoculation, regardless of timing, although the incidence of symptom development for most isolates was inhibited to approximately 20% at 1000.0 $\mu\text{g ml}^{-1}$ dimethomorph.

Within each application timing, the EC_{50} values for the incidence of symptom development were not significantly different among isolates (data not shown). Mean EC_{50} values for symptom development were not significantly different between application timings before inoculation, nor for application timings after inoculation. The mean EC_{50} values for the inhibition of the incidence of symptom development for dimethomorph application before and after inoculation were 36.2 ± 11.5 and $>1000.0 \mu\text{g ml}^{-1}$ dimethomorph, respectively, with the latter being significantly larger.

For all isolates, no significant difference in the percent inhibition of sporulation was present between application timings at each concentration of dimethomorph (Fig. 2b). No significant inhibition of sporulation occurred at 1.0 or 10.0 $\mu\text{g ml}^{-1}$ dimethomorph, while 100.0 and 1000.0 $\mu\text{g ml}^{-1}$ dimethomorph significantly inhibited sporulation, relative to the untreated control, to 70.7% and 98.4%, respectively. The lack of symptom development for leaf disks treated with 1000.0 $\mu\text{g ml}^{-1}$ dimethomorph before inoculation did not allow for assessment of antsporulation. No significant differences between isolates, timings, or dimethomorph application relative to inoculation were present for the EC_{50} values of sporulation inhibition (data not shown). The mean EC_{50} value for the inhibition of sporulation was $49.4 \pm 15.7 \mu\text{g ml}^{-1}$ dimethomorph.

DISCUSSION

The mean concentrations required for the inhibition of *P. infestans in vitro* hyphal growth, direct sporangia germination, zoospore encystment, cystospore germination, and inhibition of symptom development and sporulation *in vivo* were similar to those previously reported for *P. infestans* (1,2,6,12,14). Zoosporogenesis was not sensitive to the concentrations of dimethomorph examined, while *in vitro* hyphal growth and sporulation, and direct sporangia germination were moderately sensitive to inhibition by dimethomorph, with mean EC₅₀ values of 0.45, 0.22, and 0.19 µg ml⁻¹. In contrast, zoospore encystment and cystospore germination were both highly sensitive to dimethomorph with EC₅₀ values for most isolates <0.10 µg ml⁻¹ dimethomorph.

The higher sensitivity of the zoospore encystment and cystospore stages of the asexual life cycle may be related to the physiology of *Phytophthora* at those stages. Specifically, disrupting the extension of hyphal tips or sporangiophore formation will not be immediately lethal to a colony and it is possible that physiological changes in the cytoplasm could temporarily or partially off-set the disruption of cell wall formation. In contrast, disruption of the *de novo* synthesis of the cell wall, as in zoospore encystment, would be lethal in a much shorter time period as zoospores must actively offset osmotic pressure and have comparatively limited energy reserves (7). As with zoospores, cystospores have relatively limited energy reserves, saprophytic ability, and longevity (5) and the inhibition of germ tube formation would be lethal to each cyst while a sporangium might be able to attempt multiple germinations. It is possible that direct sporangia germination was less susceptible because of the larger physical size and energy reserves of the sporangium in comparison to a zoospore or cystospore.

Direct sporangia germination and *in vitro* hyphal growth had the smallest ranges in sensitivity between isolates, with >4 and >6-fold differences of EC₅₀ values between the most and least insensitive isolates, respectively. *In vitro* sporulation, zoospore encystment and cystospore germination all had larger ranges in sensitivity with >10-fold differences. The ranges of dimethomorph sensitivity are much smaller than those reported for phenylamide sensitivity in *P. infestans* (18) and other *Phytophthora* species (8,15). However, these studies included isolates with field resistance to phenylamides, and exclusion of those isolates results in a similar sensitivity distribution. The sensitivity ranges determined from disease incidence for *Plasmopara viticola* to the strobilurin fungicide azoxystrobin were similar to those presented here (19).

The positive correlation between the sensitivities of most stages of the asexual life cycle indicates that all genetically unique individuals of *P. infestans* should have similar relative sensitivities between stages. Therefore, future dimethomorph sensitivity surveys in *P. infestans*, and likely other *Phytophthora* spp., could rely on hyphal growth on dimethomorph amended media only. Such a reduction in the number of assays required would simplify studies.

Application of 1000.0 µg ml⁻¹ dimethomorph at 24 or 48 hours before inoculation almost completely inhibited symptom development incidence in inoculated leaf disks, while application within 48 hours 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 (1,6,16). The EC₅₀ values for the inhibition of *in vivo* symptom development were much larger than those at the specific stages in the asexual life cycle of *P. infestans*. While dimethomorph is considered to be slightly systemic, it is also known to have a high resistance to washing off with water following application (6). These results, and the fact

that dimethomorph has low solubility in water, indicates that dimethomorph probably has a high affinity for the cuticle of the leaf. Once applied, only a small percentage of dimethomorph is likely to dissolve into any free water on the leaf surface, therefore requiring larger concentrations of dimethomorph for *in vivo* efficacy to offset the low solubility.

5 Inhibition of sporulation of *P. infestans* occurred when dimethomorph was applied between 48 hours before and 48 hours after inoculation at 100.0 $\mu\text{g ml}^{-1}$ or higher. These results are similar to those previously reported (6). Outside of the examined time frame and under field conditions, dimethomorph may not have the same level of ant sporulation activity. However, a previous study examining ant sporulation under field conditions failed to demonstrate any
10 inhibition of sporulation following dimethomorph, or other fungicides, applications (17). 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 ant sporulation activity in the field is due to either incomplete coverage leaving unprotected foliage, sub-
efficacious concentrations of dimethomorph on the leaf surface, or a combination of the two.

15 Dimethomorph at low concentrations is highly inhibitory to most stages of the asexual portion of the *P. infestans* life cycle. Frequent protectant applications of dimethomorph at concentrations exceeding 1000.0 $\mu\text{g ml}^{-1}$ would likely inhibit infection by *P. infestans* in the field and possibly reduce sporulation from previously established infections, but not cure them. The *P. infestans* isolates examined all had similar sensitivity to dimethomorph in the assays
20 performed. However, low levels of resistance to dimethomorph have been generated *in vitro* for *P. infestans* (20) and other *Phytophthora* species (4,20), and therefore should be examined further in order to manage resistance development.

LITERATURE CITED

1. Albert, G., J. Curtze, and C.A. Drandarevski. 1988. Dimethomorph (CME 151), a novel curative fungicide. *Proc. Brighton Crop Prot. Conference - Pests and diseases* 1:17-24.
- 5 2. Albert, G., A. Thomas, and M. Guhne. 1991. Fungicidal activity of dimethomorph on different stages in the life cycle of *Phytophthora infestans* and *Plasmopara viticola*. at ANPP - Third International Conference on Plant Diseases, at Bordeaux, FR. 17-24
3. Caten, C.E., and J.L. Jinks. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variations. *Can. J. Bot.* 46:329-348.
- 10 4. Chabane, K., P. Leroux, and G. Bompeix. 1993. Selection and characterization of *Phytophthora parasitica* mutants with ultraviolet-induced resistance to dimethomorph or metalaxyl. *Pestic. Sci.* 39:325-329.
5. Coffey, M.D., and R. Gees. 1991. The cytology of development. In *Advances in Plant Pathology. Phytophthora infestans, the Cause of Potato Late Blight*, edited by D.S. Ingram and P.H. Williams. San Diego, CA USA: Academic Press Limited.
- 15 6. Cohen, Y., A. Balder, and B.H. Cohen. 1995. Dimethomorph activity against Oomycete fungal plant pathogens. *Phytopathology* 85:1500-1506.
7. Erwin, D.C., and O.K. Ribeiro. 1996. *Phytophthora Diseases Worldwide*. St. Paul, MN: APS Press.
- 20 8. Ferrin, D.M., and J.N. Kabashima. 1991. *In vitro* insensitivity to metalaxyl of isolates of *Phytophthora citricola* and *P. parasitica* from ornamental hosts in southern California. *Plant Dis.* 75:1041-1044.
9. Goodwin, S.B., R.E. Schneider, and W.E. Fry. 1995. Use of cellulose-acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Dis.* 79:1181-1185.
- 25 10. Goodwin, S.B., L.S. Sujkowski, and W.E. Fry. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and Western Canada. *Phytopathology* 86:793-800.

11. Kuehl, R.O. 1994. *Statistical principles of research design and analysis*. Belmont, USA: Duxbury Press.
12. Kuhn, P.J., D. Pitt, S.A. Lee, G. Wakley, and A.N. Sheppard. 1991. Effects of dimethomorph on the morphology and ultrastructure of *Phytophthora*. *Mycological Research* 95:333-340.
13. Matheron, M.E., and H. Porchas. 2000. Comparison of five fungicides on development of root, crown, and fruit rot of chile pepper and recovery of *Phytophthora capsici* from soil. *Plant Dis.* 84:1038-1043.
14. Matheron, M.E., and M. Porchas. 2000. Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl-al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. *Plant Dis.* 84:454-458.
15. Parra, G., and J.B. Ristaino. 2001. Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing *Phytophthora* blight of bell pepper. *Plant Dis.* 85:1069-1075.
16. Stein, J.M., and W.W. Kirk. 2002. Containment of existing potato late blight (*Phytophthora infestans*) foliar epidemics with fungicides. *Crop Prot.* 21:575-582.
17. Stein, J.M., W.W. Kirk, and B.A. Niemira. 1998. The role of select fungicides within a potato late blight program: Antisporulation and tuber blight aspects. at The Annual Meeting of the Potato Association of America, at Somerset, USA. 45.
18. Sujkowski, L.S., B.A. Fry, R.J. Power, S.B. Goodwin, T.L. Peever, R.A. Hamlen, and W.E. Fry. 1995. Sensitivities of Mexican isolates of *Phytophthora infestans* to chlorothalonil, cymoxanil, and metalaxyl. *Plant Dis.* 79:1117-1120.
19. Wong, F.P., and W.F. Wilcox. 2000. Distribution of baseline sensitivities to azoxystrobin among isolates of *Plasmopara viticola*. *Plant Dis.* 84 (3):275-281.
20. Young, D.H., S.L. Spiewak, and R.A. Slawewski. 2001. Laboratory studies to assess the risk of development of resistance to zoxamide. *Pest Manag. Sci.* 57:1081-1087.

Table 1. *P. infestans* Isolate ID, mating type, genotype, and State in which isolated (U.S.A.).

Isolate ID	Mating Type	Genotype^a	Origin
Pi88	A1	US1	ND
Pi95-5	A1	US1	MI
Pi671	A1	US14	WA
Pi458	A2	US17	ID
Pi670	A2	US7	OR
Pi213	A2	US8	CO
Pi94-4	A2	US8	MI
Pi95-7	A2	US8	MI
Pi97-2	A2	US8	MI
Pi98-1	A2	US8	MI
Pi98-2	A2	US8	MI

a. Allozyme-based genotype (9).

Table 2. The effective concentration of dimethomorph ($\mu\text{g ml}^{-1}$) required for a 50% reduction of *in vitro* hyphal growth (colony diameter), sporulation, zoospore encystment, cystospore germination, and mean rank of isolate sensitivity for *P. infestans* isolates.

Isolate ID	<i>in vitro</i> EC ₅₀ (mg ml ⁻¹) ^a				Median Rank of Isolate Sensitivity ^f
	Hyphal Growth ^b	Sporangia Production ^c	Zoospore ^d Encystment	Germination Cystospore ^e	
Pi88	0.425 c ^g	0.199 abc	0.047 bc ^d	0.169 a	6 b
Pi95-5	0.131 e	0.036 c	0.016 bc	0.016 d	1 d
Pi671	0.625 b	0.320 ab	0.067 abc	0.117 ab	9 a
Pi458	0.585 b	0.203 abc	0.084 abc	0.047 bcd	9 a
Pi670	0.392 c	0.236 abc	0.036 bc	0.145 a	7 b
Pi213	0.800 a	0.437 a	0.136 a	0.114 abc	11 a
Pi94-4	0.270 d	0.247 abc	0.011 c	0.018 d	2 cd
Pi95-7	0.533 b	0.171 abc	0.053 abc	0.042 bcd	6 bc
Pi97-2	0.291 d	0.115 bc	0.033 bc	0.027 cd	3 d
Pi98-1	0.419 c	0.246 abc	0.098 ab	0.071 abcd	8 ab
Pi98-2	0.429 c	0.163 bc	0.063 abc	0.046 bcd	5 bc

- Effective concentration for a 50% reduction, calculated using probit transformation of the percent inhibition relative to the untreated control.
- Calculated from colony diameter on rye B media amended with 0, 0.01, 0.1, 1.0, or 10 $\mu\text{g ml}^{-1}$ dimethomorph.
- Production of sporangia per cm² colony area on rye B media amended with 0, 0.01, 0.1, 1.0, or 10 $\mu\text{g ml}^{-1}$ dimethomorph.
- Encystment of zoospores in sterile diH₂O amended with 0, 0.01, 0.1, 1.0, or 10 $\mu\text{g ml}^{-1}$ dimethomorph.
- Germination of previously encysted zoospores in sterile diH₂O amended with 0, 0.01, 0.1, 1.0, or 10 $\mu\text{g ml}^{-1}$ dimethomorph.
- Sensitivity ranking of isolates is from most sensitivity (lowest) to least sensitivity (highest) and was calculated using an Analysis of Variance on Ranks.
- Means followed by the same letter are not significantly different using Fischer's LSD ($\alpha = 0.05$).

Table 3. Correlation of the stages of the asexual life cycle of *P. infestans* examined and the P-value for each, excluding zoosporogenesis, analyzed with Pearson's Product Moment Correlation.

Variable	Correlation Coefficient ¹ and (P-value) of EC ₅₀ Values			
	<i>in vitro</i> Sporulation	Sporangia Germination	Zoospore Encystment	Cystospore Germination
Hyphal Growth	0.811 (0.002)	0.779 (0.005)	0.847 (>0.001)	0.458 (0.157)
<i>in vitro</i> Sporulation	n/a ²	0.769 (0.006)	0.706 (0.015)	0.529 (0.094)
Sporangia Germination	n/a	n/a	0.815 (0.002)	0.314 (0.347)
Zoospore Encystment	n/a	n/a	n/a	0.320 (0.338)

1. Pearson Product Moment Correlation Coefficient, where >0.50 (P-value >0.05) indicates a significant positive correlation between variables, while a coefficient <0.50 (P-value >0.05) indicates a significant negative correlation.
2. A non-applicable self-comparison or displayed in another cell of the matrix.

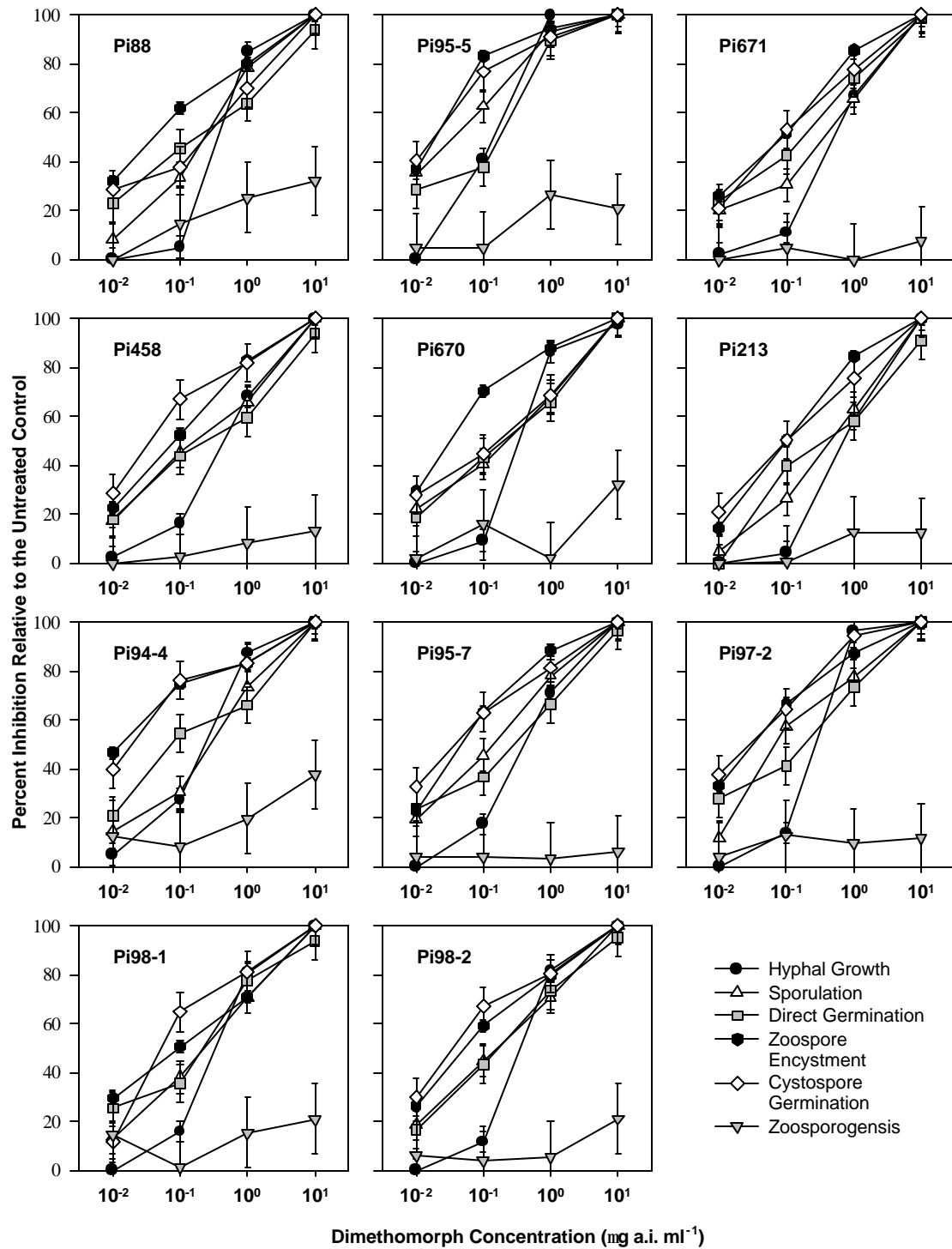


Fig. 1. Influence of dimethomorph concentration on *in vitro* hyphal growth and sporulation, direct sporangia germination, zoospore encystment and cystospore germination for isolates of *P. infestans* isolates examined. Error bars represent Fischer's LSD ($\alpha = 0.05$).

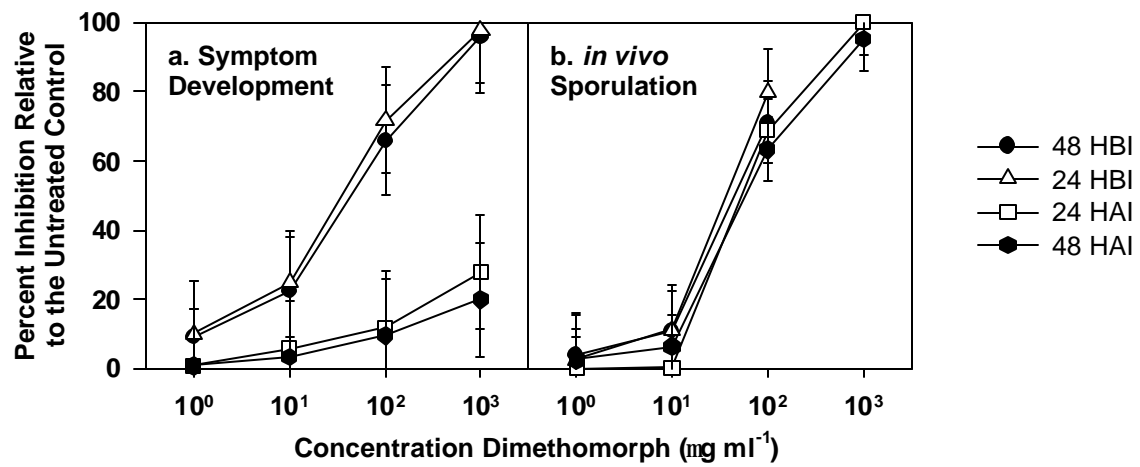


Fig. 2 Influence of dimethomorph concentration on a) symptom development and b) *in vivo* sporulation of potato leaf disks inoculated with *P. infestans*. Plots represent the mean of all isolates when dimethomorph was applied at 48 and 24 hours before and 24 and 48 hours after inoculation. Error bars represent Fischer's LSD ($\alpha = 0.05$).