The Generation, Quantification, and Characterization of Dimethomorph Insensitivity in *Phytophthora infestans* and other *Phytophthora* species.

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ABSTRACT

The Generation, Quantification, and Characterization of Dimethomorph Insensitivity in *Phytophthora infestans* and other *Phytophthora* species.

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The generation of dimethomorph insensitive strains of *Phytophthora infestans* was attempted using ethidium bromide / ultra-violet light mutagenesis and repeated culturing on amended media. Ethidium bromide / UV mutagenesis created two strains of *P. infestans* with resistance factors >20, i.e. the ratio of the EC_{50} of the mutant strain to that of the wild-type. The 10 rate of growth (mm diameter day^{-1}) on dimethomorph amended media increased until the fourth sub-culture for most *P. infestans* isolates. Resistance factors for strains generated from repeated culturing were <8. For most isolates of *P. infestans*, dimethomorph insensitive strains had significantly reduced growth rates on un-amended media, regardless of the level of insensitivity and induction treatment. Additionally, virulence on leaf disks and in whole tubers was 15 significantly reduced in >20% of the isolates examined. Insensitivity generation in several other *Phytophthora* species was attempted with repeated culturing on amended media and one strain of *P. erythroseptica* occurred through colony sectoring with a resistance factor >7. However, colony morphology and fitness of this strain were altered in comparison to the wild-type strain. Regardless of the induction treatment, reduced fitness was common for all *Phytophthora* spp., 20 indicating a biological cost associated with dimethomorph insensitivity. Based on these results, the development of field resistance to dimethomorph of *P. infestans* and other species is unlikely with the currently employed dimethomorph resistance management schemes.

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

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Resistance to fungicides in fungal and Oomycete plant pathogens has become increasingly more common following the release of systemic, single mode of action fungicides in the 1960's (8). Field resistance to every major systemic fungicide class has occurred in at least one species of plant pathogenic fungi or Oomycetes (21,26), leading to drastic shifts in fungicide programs for many pathosystems, such as potato late blight (13). The migration of phenylamide insensitive populations of *Phytophthora infestans*, the causal agent of potato and tomato late blight, from Mexico to the rest of North America (13), necessitated cultural control methods and crop protection strategies that relied primarily on protectant foliar fungicide applications (24). Concurrently, the agrichemical industry developed and released systemic fungicides with novel modes of action in an attempt to replace the phenylamides.

Dimethomorph, one of the fungicides released in response to phenylamide resistance, is a cinnamic acid derivative with a high specificity to certain members of the Peronosporaceae, including *Phytophthora* (1). Cells of *P. infestans* lacking cell walls, such as zoospores and artificially generated protoplasts, were not affected by dimethomorph (2) and it was concluded that dimethomorph disrupted cell wall formation by interfering with the molecular arrangement of cell wall components and not the inhibition of component synthesis (18).

Insensitivity to dimethomorph was generated with ultraviolet (UV) light mutagenesis in one isolate of *P. parasitica* (5) and with chemical mutagenesis in one isolate of *P. capsici* (28). In both cases, an approximate 20-fold decrease in sensitivity resulted. Virulence of dimethomorph insensitive *P. parasitica* strains was equal to, or less than, the wild-type. In addition, insensitivity in *P. parasitica* was stable in the absence of dimethomorph through several *in vitro* sub-cultures for three months. Conversely, attempts to generate insensitivity using mycelial adaptation in *P. infestans* and *P. capsici* resulted in small (<2-fold) decreases in sensitivity to

dimethomorph (28). The use of only one culture of *P. infestans* and *P. capsici* in the latter experiment may have limited the results as genetic variability was absent.

The objectives of this study were to generate strains of *P. infestans* insensitive to dimethomorph using ethidium bromide / UV mutagenesis or mycelial adaptation and determine
5 the level of resistance generated and examine virulence of *P. infestans* insensitive strains on potato foliage and tubers. Insensitivity generation was also attempted using mycelial adaptation using three other *Phytophthora* species.

MATERIALS AND METHODS

10 **Media preparation.** Experiments involving *in vitro* hyphal growth were performed on modified rye B agar (2,4) consisting of the filtrate of pre-rinsed rye (*Secale cereale*) seed (100.0 g l^{-1}) boiled for one hour, de-ionized (di) H_2O 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}) because of the relatively rapid growth compared to synthetic media. All plates for each sub-culture on dimethomorph amended media or run of the
15 baseline sensitivity assay were prepared from the same batch of media in order to reduce variability within each run of an experiment.

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

Insensitivity generation: mycelial selection. Using previously determined effective concentration for a 50% reduction in colony diameter (EC_{50}) values for the inhibition of *in vitro*

growth by dimethomorph (27), culturing of *Phytophthora sp.* on media amended with a highly inhibitory (1.0 µg ml^{-1}), sub-lethal concentration of dimethomorph was performed to select for insensitivity in cultures. Initial wild-type isolates were previously characterized (Table 1), two sub-cultures from re-isolation from infected potato tuber tissue, and labeled strain "WT", e.g. Pi95-7_{WT}. Colonized agar plugs, 4.0 mm in diameter, from the margin of an actively growing culture were transferred mycelium-side down onto modified rye B media amended with 0.0 or 1.0 µg ml^{-1} dimethomorph and incubated at 21°C in the dark with three replicate plates per concentration. Colony diameter was measured after 11 days for *P. infestans*, and for other *Phytophthora* species at five and 11 days for 0.0 and 1.0 µg ml^{-1}, respectively. Isolates grown on control (0.0 µg ml^{-1}) and sub-lethal dimethomorph amended media (1.0 µg ml^{-1}) were labeled strains "CT" and "SL", respectively, e.g. Pi95-7_{CT} and Pi95-7_{SL}.

After colony diameter was measured, one plate from each concentration was used to sub-culture the strain onto media with the same concentration of dimethomorph, for ten sub-cultures total. Portions of the colony were chosen for sub-culturing in an attempt to retain the wild-type colony phenotype of each isolate, while selecting sectors with higher growth rates. In the absence of accelerated growth on dimethomorph, plugs were chosen randomly from the margin of the colony. Following the tenth sub-culture, the cultures were examined for baseline sensitivity and virulence.

Insensitivity generation: mutagenesis. Cultures of *P. infestans* for mutagenesis were grown on modified rye B agar at 21°C until complete colonization of the media. Plates were flooded with 5.0 ml of a 1 µg ml^{-1} ethidium bromide solution (in sterile diH_2O), allowed to dry, and exposed to 254 nm ultraviolet (UV) light (2.03×10^3 ergs cm^{-2} s^{-1}) for five minutes. From each isolate, four 4 mm diameter colonized agar plugs were transferred to rye B media amended

with 0.0 or 10.0 μg ml^{-1} dimethomorph. Non-exposed cultures were also transferred onto control and amended rye B media. Cultures were incubated in the dark and examined on seven-day intervals for colony diameter and rapid growth or sectoring on the dimethomorph amended media. The fastest growing sector of each isolate from the fungicide amended media was used
5 for further assays, and hereafter labeled strain "UV", e.g. Pi95-7_{UV}.

Dimethomorph sensitivity assays. Prior to performing baseline sensitivity assays, all cultures were grown on non-amended media. Colonized agar plugs, 4 mm diameter, were transferred from the margin of the colony onto dimethomorph amended rye B media in 60 mm diameter plastic petri dishes and incubated at 21°C. The concentrations used were 0.0, 0.1, 1.0,
10 and 10.0 dimethomorph μg ml^{-1}, with three replicate plates per concentration. Final colony diameter (FCD) was measured 11 days after inoculation (DAI). Percent inhibition of radial growth was calculated with respect to the mean final colony diameter on non-amended media within each strain. Percent inhibition values were then transformed using probits, i.e. the inverse of the standard normal distribution (17), and expressed as a function of the $\log_{10}$ of concentration
15 (10). Plot equation parameters were then determined using linear regression (SigmaPlot, SPSS Inc., Chicago, IL, U.S.A.) and the EC_{50} for hyphal growth (diameter) was calculated and reverse transformed for each strain of each isolate. Resistance factors were calculated for all strains using the following formula:

$$RF = \frac{EC_{50}X}{EC_{50}WT}$$

where $EC_{50}X$ is the EC_{50} value of the strain being examined, and $EC_{50}WT$ is the EC_{50} value of
20 the wild-type strain of that isolate.

The assay was repeated three times and the FCD and EC_{50} values for each strain were used as replicates for an analysis of variance (Proc GLM - SAS/Stat, SAS Institute, Cary, NC, U.S.A.)

at $\alpha = 0.05$ by pair-wise comparisons using Fisher's LSD. Prior to the combined ANOVA, each run was analyzed separately and the homogeneity of variance was confirmed using the F Max test (14).

Virulence assays. Foliar virulence was assessed by removing fully expanded leaflets of similar age from greenhouse grown potato plants (cv. Snowden) and surface disinfesting them with 0.5% sodium hypochlorite in diH_2O (10% commercial bleach solution) for one minute. Leaflets were then rinsed three times in sterile diH_2O, allowed to dry, and cut into 20 mm diameter leaf disks with a sterilized core borer. Leaf disks were then placed onto water agar (15.0 g l^{-1} Bacto) 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. Sporangia/zoospore suspensions were prepared as previously described (27) for a final concentration of 1.0×10^4 sporangia ml^{-1}. The sporangia/zoospore suspension from each strain of every isolate was applied to four leaf disks per replicate (50 µl $disk^{-1}$), with three replicates. The experiment was repeated twice. Inoculated leaf disks were incubated at 21°C light / 18°C dark (12 hour cycles) and examined with a dissecting microscope at 96 hours after inoculation for symptoms and signs of infection by *P. infestans*, such as sporulation. Pathogen identity was confirmed with a compound microscope. Leaf disk inoculations were not performed with ethidium bromide / UV generated strains as sporulation was absent or highly disrupted for all isolates. An analysis of variance on the incidence of leaf disks infection was performed as described.

Pathogen free potato tubers (cv. Snowden), were surface disinfested with 0.5% sodium hypochlorite in diH_2O (10% commercial bleach solution) for 30 minutes, rinsed three times in sterile diH_2O, and allowed to dry. Colonized portions of rye B agar, 1.0 cm^2, were excised from

5 the margin of an actively growing colony of *P. infestans* and placed into a sterile 1.0 ml syringe with an 18½ gauge needle. A wound approximately 1.0 cm from the apex of the tuber and 0.5 cm deep into the tissue was created by stabbing the tuber with a pair of flame-sterilized forceps. Three tubers per replicate were inoculated by extruding approximately 0.1 ml of macerated colonized agar through the needle into the wound and placed into a covered plastic container. There were three replicates per run and the experiment was repeated twice. The tubers were incubated in the dark for seven days at 18°C and tuber surface and cross-sections were evaluated for symptoms and signs of infection by *P. infestans*. Pathogen presence was verified with a compound microscope when applicable. Tuber inoculations were not performed on ethidium bromide / UV generated strains. An analysis of variance on the incidence of tuber infection was performed as described.

RESULTS

Insensitivity generation. Following ethidium bromide / UV light treatment, all isolates except Pi88 grew on non-amended media rye B media (data not shown). When cultured on non-amended media, treated cultures exhibited disrupted colony morphology and lower growth rates compared to untreated cultures. Of the isolates that grew following ethidium bromide / UV light treatment, only Pi458 and Pi98-2 failed to grow on media amended with 10.0 µg ml^{-1} dimethomorph. However, most isolates required more than 14 days to develop sufficient growth for sub-culturing. Sectors with increased growth rates on dimethomorph amended media were common, but not always present.

For all isolates, regardless of species, the rate of growth on media amended with 1.0 µg ml^{-1} dimethomorph (strain SL) increased with sub-culture number, e.g. Pi213 (Figure 1). With most

isolates, the largest increase occurred between the initial and second sub-cultures, while changes at subsequent sub-cultures were smaller or absent. A fast growing sector appeared from one isolate of *P. erythroseptica*, Pe00-1, following sub-culture five on dimethomorph amended media, hereafter denoted strain “RS”. Pe00-1_{RS} exhibited different colony morphology than all other strains of Pe00-1, and consistently died when cultured on non-amended media, or stored for more than 21 days at 18°C.

Dimethomorph sensitivity. The final colony diameter (FCD) of the sub-cultured control strain of each *P. infestans* isolate was either similar to or significantly larger (Fischer’s LSD, α = 0.05), than the corresponding wild-type strain on non-amended media, while the ethidium bromide / UV generated strain had the smallest FCD (Figure 2). The sub-cultured on dimethomorph amended media strains of Pi88, Pi95-5, and Pi97-2 had a significantly smaller FCD than both wild-type and control sub-cultured strains on non-amended media. On media amended with 0.1 $\mu g\ ml^{-1}$ dimethomorph, differences in FCD between wild-type and strains sub-cultured on control or dimethomorph amended media became smaller, or non-significant, while the ethidium bromide / UV generated strains had the lowest FCD values. At 1.0 $\mu g\ ml^{-1}$ dimethomorph, the sub-cultured on dimethomorph amended media strain of each isolate typically had a significantly larger FCD than all other strains. If growth occurred on media amended with 10.0 $\mu g\ ml^{-1}$ dimethomorph, it was always a strain sub-cultured on dimethomorph amended media or ethidium bromide / UV generated, with the latter typically having the larger FCD.

The FCD curves for the other *Phytophthora* species examined, were similar to those of *P. infestans*, and wild-type and sub-cultured on non-amended media strains had similar FCD values at each concentration (Figure 3). The strains sub-cultured on dimethomorph amended media

typically exhibited larger FCD values at 1.0 µg ml^{-1} dimethomorph, but not other concentrations. The only strains that grew at 10.0 µg ml^{-1} dimethomorph were the sub-cultured on dimethomorph amended media of strains Pcap A2 and Pe96-2, Pcap $A2_{SL}$ and Pe96-2_{SL}, and the strain that spontaneously sectored from Pe00-1_{SL}, Pe00-1_{RS}.

5 The *P. infestans* isolates Pi458, Pi94-4, and Pi98-1 had significantly larger EC_{50} values for the strains sub-cultured on dimethomorph amended media than wild-type and sub-cultured on control media strains (Table 2). The calculated EC_{50} values for *in vitro* growth of sub-cultured on dimethomorph amended media strain were numerically larger than those of the wild-type and sub-cultured on control media strains for nine of 11 isolates of *P. infestans*, indicating a decrease
10 in sensitivity to dimethomorph through sub-culturing. The isolates Pi94-4 and Pi98-2 showed large, but not significant, increases in EC_{50} of the strain sub-cultured on control media in comparison to the wild-type strain. The ethidium bromide / UV generated strains from five of the eight isolates had significantly higher EC_{50} values than the other strains within each isolate. For all isolates of *P. infestans*, except Pi98-2, the calculated resistance factor (RF) was larger for
15 the sub-lethal generated strain than the control strain. The RF values for the ethidium bromide / UV generated strains were larger than all other strains for seven of the eight isolates with such strains. The isolates Pi95-5 and Pi94-4 had the largest RF factors for sub-lethal and ethidium bromide / UV generated strains with 7.5 and 23.2, and 6.5 and 22.5, respectively.

The strains sub-cultured on dimethomorph amended media of the other *Phytophthora*
20 species examined had significantly larger EC_{50} values compared to both the wild-type and the strains sub-cultured on control media within isolates (Table 3). Within the isolate Pe00-1, the RS strain had a significantly larger EC_{50} than the other strains. Pe00-1_{RS} also had the largest resistance factor.

Virulence. The ability to infect and cause symptoms on potato leaf disks (cv. Snowden) was limited for most isolates of *P. infestans*, regardless of strain, as only four of 11 wild-type strains resulted in 75% or more leaf disks infected (Table 4). The wild-type strains of isolates, Pi95-5, Pi671, Pi670, Pi94-4, Pi97-2, and Pi98-2 successfully infected 50% or less of leaf disks,
5 indicating low virulence of the wild-type strain, while only the wild-type strain of Pi458 infected all leaf disks. In comparison to the wild-type strains, the strains sub-cultured on dimethomorph amended media had significantly reduced percent leaf disks infected for five of 11 isolates of *P. infestans*. However, the strains sub-cultured on control media of isolates $Pi458_{CT}$ and $Pi95\text{-}7_{CT}$ infected significantly fewer leaf disks than the wild-type strain.

10 Infection and symptom development in whole tubers following inoculation of mycelium was more efficient than leaf disk inoculation with the wild-type strains of eight of 11 isolates causing symptoms in all tubers inoculated. The strains sub-cultured on dimethomorph amended media of seven isolates infected significantly less tubers compared to the wild-type strain of each isolate. Some of the strains sub-cultured on control media infected significantly lower numbers of tubers
15 when compared to the corresponding wild-type strains.

DISCUSSION

The development of insensitivity to dimethomorph was demonstrated in *P. infestans* following ethidium bromide / UV light exposure of mycelium. Mutagenesis created strains of
20 two *P. infestans* isolates with resistance factor (RF) values >20. These values were similar to previously reported RF values generated for *P. parasitica* using UV irradiation (5), and *P. capsici* using chemical mutagenesis (28). In comparison, the resulting RF of *P. capsici* to the phenylamide fungicide metalaxyl was >100 in the latter study, and a RF value of 20 for

dimethomorph was considered moderate (28). All isolates of *P. infestans* that survived the mutagenesis treatment had reduced growth rates on non-amended media and disrupted colony morphology compared to the wild-type. On non-amended media, both factors could be attributable to mutations unrelated to dimethomorph insensitivity negatively affecting growth or that the dimethomorph insensitivity mechanism(s) themselves disrupted growth. Particularly in the former situation, one might have expected additional fitness reductions, including virulence as mutations generally reduce the fitness of an organism (20). The loss of the ability to sporulate supports this hypothesis. Excluding the composition of the cell wall, little is known about the biochemistry involved with cell wall formation in *Phytophthora* (3) and therefore the genetic basis for insensitivity to dimethomorph may be difficult to discern.

The development of insensitivity to dimethomorph was demonstrated in *P. infestans* and other *Phytophthora* species following repeated sub-culturing on media amended with a sub-lethal concentration of dimethomorph. A significant decrease in sensitivity (*in vitro* growth) occurred in three of 11 isolates of *P. infestans* and all isolates of the other *Phytophthora* species examined. Almost all isolates had numerically decreased sensitivity compared to the wild-type. However, the resistance factor for most isolates was less < 3, which represents a relatively small change. The only strain generated from a distinct sectoring of the colony was formed from the *P. erythroseptica* isolate Pe00-1.

The rapid increase in final colony diameter by the third sub-culture cycle on amended media indicates a physiological adaptation to *in vitro* growth on dimethomorph or the selection of the genetic factors responsible for insensitivity following the *de novo* development of nuclear or cytoplasmic insensitivity. Culturing the isolates on non-amended media prior to the *in vitro* growth sensitivity assessments was used to reduce the effects of physiological adaptation, but

without detailed investigation of cell wall composition and formation it is difficult to discern the true cause and should be further investigated.

The EC_{50} metric of *in vitro* colony growth, while commonly used in fungicide sensitivity studies (10,15,28), is sensitive to changes in the growth rate of the fungus because of the
5 percentage inhibition transformation relative to the control colonies. A mutant strain with a reduced growth rate may have the same colony diameter as the wild-type at all concentrations of fungicide and yet the smaller colony diameter on non-amended media results in a lower percent inhibition. Therefore, when calculating the EC_{50} value using percent inhibition versus concentration, a larger EC_{50} is calculated. Similarly, the RF value, being calculated using the
10 EC_{50}, is dependent on both reduced growth rates on non-amended media and to extremely sensitive wild-type isolates. For example, the isolate Pi95-5 had reduced growth on non-amended media for both repeated culturing on dimethomorph amended media and ethidium bromide / UV generated strains and the lowest wild-type EC_{50} of any isolate. The Pi95-5_{SL} strain of this isolate had an EC_{50} that was not significantly different from either the wild-type or
15 repeated culturing on control media strains, and yet had the largest RF value of the SL strain type for all *P. infestans* isolates. As an alternative to EC_{50}, the growth of an isolate at a pre-determined (discriminating) concentration or inoculation of fungicide treated plant tissue would likely be better indicators of sensitivity, as previously described (25). In the case of dimethomorph sensitivity in *Phytophthora* a concentration of 10.0 $\mu g\ ml^{-1}$ dimethomorph
20 amended media would likely serve as an effective discriminating dose to differentiate between sensitive and moderately insensitive isolates.

The low virulence of several wild-type strains of *P. infestans* on leaf disks was not surprising as variable virulence (16) and pathogenic specialization (19) have been documented,

even on potato cultivars with no known R-genes. Possible reductions in virulence of the strains generated by repeated sub-culturing, regardless of fungicide amendments, was expected as *P. infestans* is known to lose virulence following long-term *in vitro* culturing (9). However, since most isolates sub-cultured on non-amended media retained near wild-type virulence, any reduction in virulence of the strains sub-cultured on dimethomorph amended media was probably caused by the mechanism(s) responsible for dimethomorph insensitivity. *P. infestans* requires the formation of viable sporangia for pathogenicity on foliar tissue, and any putative changes in cell wall formation resulting from the development of dimethomorph insensitivity might be disruptive to sporangia formation. Additionally, cell wall components are known to induce resistance in potato (11) and it is possible that any changes which occurred as a result of dimethomorph insensitivity allowed for the release of such compounds.

The tuber inoculation test, while not biologically accurate, was performed because differences in foliar and tuber susceptibility have been noted and may be important (6,7). The lack of correlation between the foliar and tuber inoculations is probably related to the differences in the assays in that the tuber inoculation did not require the development of viable sporangia and/or zoospores for infection. Instead, it only required the pathogen to overcome any tuber defenses, and since the periderm was eliminated, the major tuber defense mechanism was absent. *In situ* foliar and tuber infection studies would be required to more fully evaluate this relationship.

The generation of insensitivity to dimethomorph in *Phytophthora* was possible through both repeated selection on sub-lethal amended media and chemical or UV induced mutation. The low amount of insensitivity that developed indicates that resistance may be quantitative and possibly multigenic, as with the dimethylation inhibitor fungicides commonly used to control true fungi

(22). If this hypothesis is true, one would expect resistance in the field to develop through directional selection and occur in small increments (23). Also, resistance management techniques such as block treatments and co-application of dimethomorph with protectant fungicides would likely be effective. Currently, the development of insensitivity to dimethomorph in *P. infestans* is unlikely for most potato growing regions of the United States because growers rely primarily on protectant fungicide applications and the use of systemic fungicides, including dimethomorph, typically is limited. However, as use of protectant fungicides is restricted because of their larger environmental impact and higher rates required for control in comparison to many systemic fungicides, dimethomorph usage may increase and active resistance management strategies may require implementation.

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Table 1. Isolate ID, *Phytophthora* species, mating type (if applicable), genotype (if applicable), and State in which isolated (U.S.A.).

Isolate ID	***Phytophthora* Species**	**Mating Type**	**Genotype[a]**	**Origin**
Pi88	*P. infestans*	A1	US1	ND
Pi95-5	*P. infestans*	A1	US1	MI
Pi671	*P. infestans*	A1	US14	WA
Pi458	*P. infestans*	A2	US17	ID
Pi670	*P. infestans*	A2	US7	OR
Pi213	*P. infestans*	A2	US8	CO
Pi94-4	*P. infestans*	A2	US8	MI
Pi95-7	*P. infestans*	A2	US8	MI
Pi97-2	*P. infestans*	A2	US8	MI
Pi98-1	*P. infestans*	A2	US8	MI
Pi98-2	*P. infestans*	A2	US8	MI
Pcap	*P. capcisi*	A2	n/a	MI
PcacX	*P. cactorum*	H[b]	n/a	MI
PcacY	*P. cactorum*	H	n/a	MI
Pe96-2	*P. erythroseptica*	H	n/a	MI
Pe00-1	*P. erythroseptica*	H	n/a	ID

a. Allozyme-based genotype (12).

5 b. Homothallic species designated by “H”.

Table 2. Calculated EC_{50} for in vitro hyphal growth (diameter) and resistance factors for all strains of *Phytophthora infestans* isolates examined. Statistical comparisons shown between strains within each isolate using Fischer's LSD (α= 0.05).

Isolate	*In vitro* EC_{50} for Hyphal Growth ($\mu g\ ml^{-1}$)[a]				Resistance Factor[b]		
	WT[c]	CT	SL	UV	CT	SL	UV
Pi88	0.35 a[d]	0.37 a	0.77 a	n/a[e]	1.07	2.22	n/a
Pi95-5	0.11 b	0.17 b	0.80 b	2.48 a	1.74	7.48	23.17
Pi671	0.70 b	1.05 b	1.08 b	2.29 a	1.49	1.54	3.26
Pi458	0.84 b	0.56 b	1.82 a	n/a	0.66	2.16	n/a
Pi670	0.62 ab	0.54 b	1.49 a	1.21 ab	0.88	2.42	1.97
Pi213	0.68 b	0.87 b	0.98 b	4.37 a	1.28	1.44	6.42
Pi94-4	0.44 c	0.87 c	2.85 b	9.92 a	1.96	6.45	22.45
Pi95-7	0.61 b	0.62 b	0.74 b	4.43 a	1.01	1.21	7.23
Pi97-2	0.24 b	0.37 ab	0.85 ab	1.24 a	1.56	3.59	5.22
Pi98-1	0.56 b	0.54 b	1.65 a	1.98 a	0.96	2.96	3.56
Pi98-2	0.59 a	1.26 a	1.25 a	n/a	2.14	2.13	n/a

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a. Effective concentration to reduce colony diameter to 50% of 0.0 $\mu g\ ml^{-1}$ dimethomorph amended control.

b. Resistance factor = EC_{50} of manipulated strain / EC_{50} of WT strain. RF of WT strains not shown.

c. WT = wild-type, or CT and SL = sub-cultured ten times on media amended with 0.0 or 1.0 $\mu g\ ml^{-1}$ dimethomorph, respectively, or UV = treated with ethidium bromide and UV light.

d. Means followed by the same letter are not significantly different within each isolate using Fischer's LSD (α = 0.05). Comparisons between strains of different isolates not shown.

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e. Attempted mutagenesis was lethal with this isolate or no growth occurred on 10.0 $\mu g\ ml^{-1}$ dimethomorph following treatment.

Table 3. Calculated EC_{50} for in vitro hyphal growth (diameter) and resistance factor, for all strains of *Phytophthora capcisi* (Pcap), *P. cactorum* (Pcac), *and P. erythroseptica* (Pe) isolates examined. Statistical comparisons shown between strains within each isolate using Fischer's LSD ($\alpha = 0.05$).

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Isolate	***In vitro* EC_{50} for Hyphal Growth ($\mu g\ ml^{-1}$)[a]**				**Resistance Factor[b]**		
	WT[c]	**CT**	**SL**	**RS**	**CT**	**SL**	**RS**
Pcap A2	0.23 b[d]	0.27 b	1.00 a	n/a	1.15	4.29	n/a
Pcac X	0.25 b	0.24 b	0.66 a	n/a	0.95	2.68	n/a
Pcac Y	0.26 b	0.25 b	0.70 a	n/a	0.96	2.70	n/a
Pe96-2	0.23 b	0.26 b	0.65 a	n/a	1.13	2.77	n/a
Pe00-1	0.18 c	0.20 c	0.66 b	1.253 a	1.14	3.76	7.12

a. Effective concentration to reduce colony diameter to 50% of 0.0 $\mu g\ ml^{-1}$ dimethomorph amended control.

b. Resistance factor = EC_{50} of manipulated strain / EC_{50} of WT strain.

c. WT = wild-type, or CT and SL = sub-cultured 10 times on media amended with 0.0 or 1.0 $\mu g\ ml^{-1}$ dimethomorph, respectively or RS = fast growing sector of *P. erythroseptica* that spontaneously appeared.

10 d. Means followed by the same letter are not significantly different within each isolate using Fischer's LSD ($\alpha = 0.05$). Comparisons between strains of different isolates not shown.

Table 4. The inhibition of the incidence of infection by *Phytophthora infestans* isolates and strains, expressed as a significant reduction in comparison to the wild-type (WT) control strain using Fischer's LSD (α = 0.05). *P. infestans* strains were inoculated onto both excised leaf disks (20 mm diameter) and into whole tubers (>4.0 cm, <6.5 cm) and the percent infection incidence determined. Leaf disks and tubers were assessed for infection at 96 and 168 hours after inoculation, respectively.

Isolate	Leaf Disks[a]			Whole Tubers		
	%WT[b]	CT[c]	SL[d]	%WT	CT	SL
Pi88	58	-[e]	-	100	*	*
Pi95-5	25	-	-	33	*	*
Pi671	50	-	*	100	-	-
Pi458	100	*	*	100	-	*
Pi670	50	-	-	100	*	*
Pi213	92	-	*	100	-	-
Pi94-4	42	-	-	56	-	-
Pi95-7	92	*	*	100	-	*
Pi97-2	17	-	-	67	-	*
Pi98-1	92	-	*	100	*	*
Pi98-2	42	-	-	100	-	-

a. Determined using the percentage of leaf disks (out of four) or tubers (out of three) with symptoms and signs of infection by *P. infestans*. Percentage of symptomatic leaf disks
b. Percent of Wild-Type (WT) leaf disks or tubers showing infection following inoculation.
c. Strains CT and SL = sub-cultured 10 times on media amended with 0.0 (ConTrol) or 1.0 (Sub-Lethal) µg ml^{-1} dimethomorph, respectively.
d. "*" indicates a significant reduction of the incidence of infection using Fischer's LSD (α = 0.05) relative to the wild-type (WT) strain, while "-" indicates no significant difference.

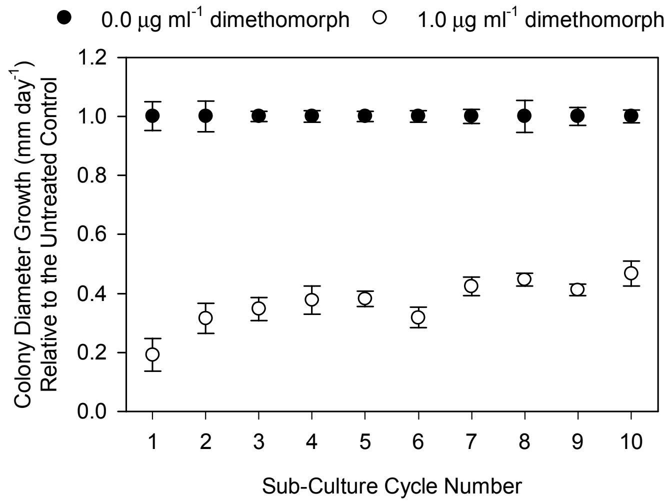

Fig. 1. Representative colony growth, relative to the untreated control, in diameter (mm) day^{-1} vs. cycle number for *Phytophthora infestans* isolate Pi213 when grown on rye B media amended with 0.0 (●) or 1.0 (○) µg ml^{-1} dimethomorph. Error bars represent one standard deviation for replicate plates.

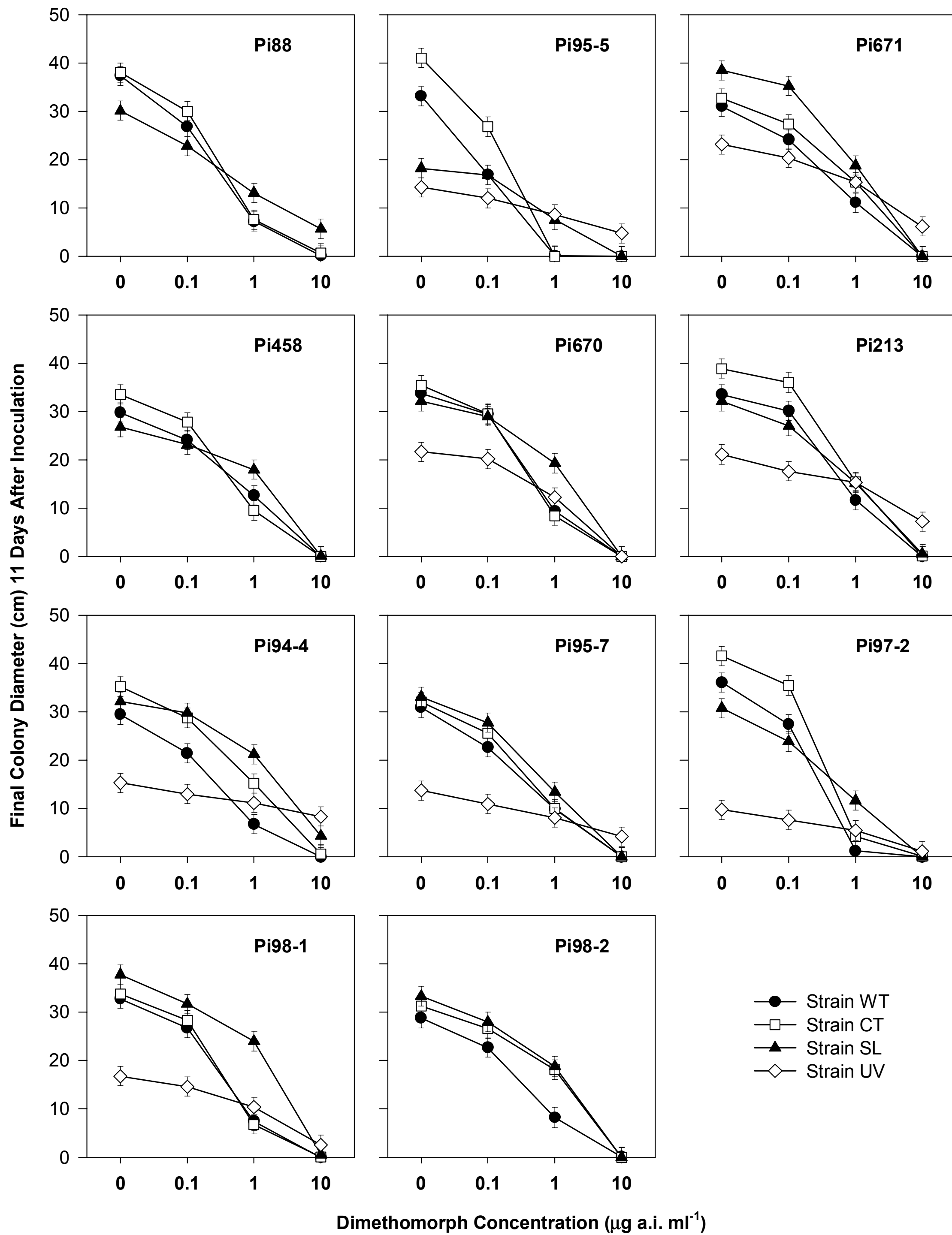

Fig. 2. Final colony diameter of *Phytophthora infestans* isolates for the wild-type strains (●), repeated culturing control strains (□), repeated culturing on 1.0 µg ml^{-1} dimethomorph amended media strains (▲), and UV / ethidium bromide generated strains (◊) at 11 days after inoculation.
5 Isolates were grown on rye B media amended with 0.0, 0.1, 1.0, and 10.0 µg ml^{-1} dimethomorph. Error bars represent Fischer's LSD ($\alpha = 0.05$).

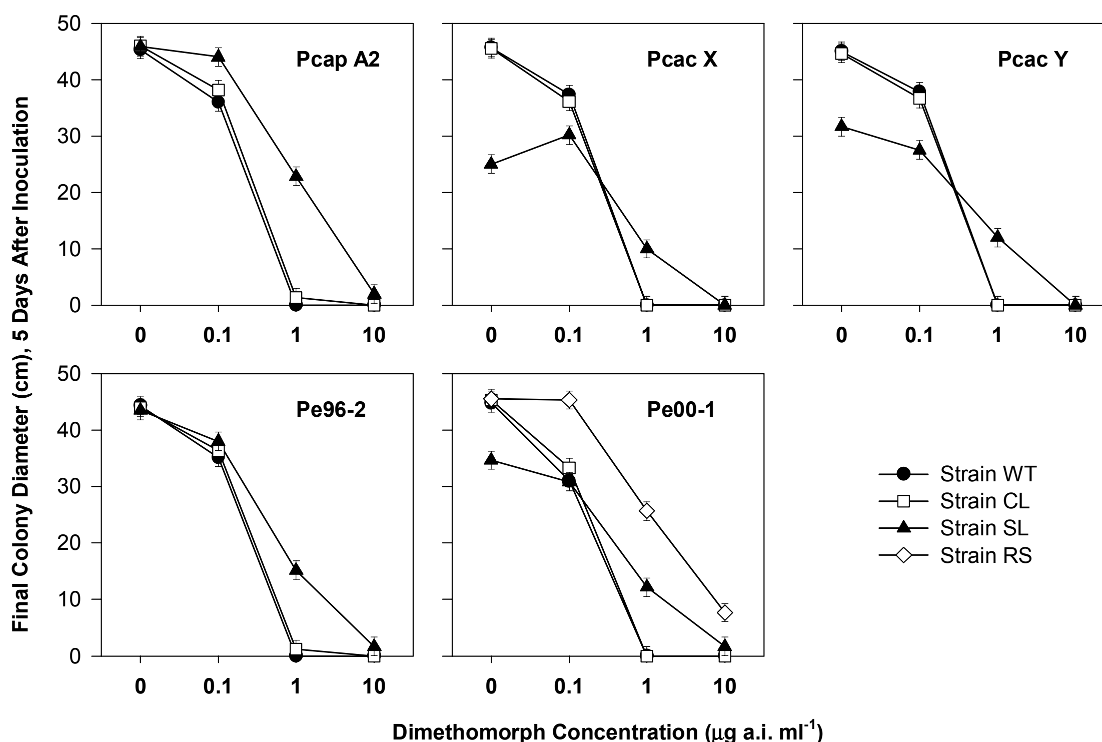

Fig. 3. Final colony diameter at 5 days after inoculation of the wild-type strains (●), repeated culturing control strains (□), repeated culturing on 1.0 µg ml^{-1} dimethomorph amended media strains (▲), and fast growing sector of *Phytophthora erythroseptica* that spontaneously appeared (◊) for *P. capcisi (Pcap), P. cactorum* (Pcac), or *P. erythroseptica* (Pe). Isolates were grown on rye B media amended with 0.0, 0.1, 1.0, and 10.0 µg ml^{-1} dimethomorph. Error bars represent Fischer's LSD ($\alpha = 0.05$).