The Generation and Quantification of Resistance to Dimethomorph in *Phytophthora infestans*

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ABSTRACT

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The generation of dimethomorph resistance in *Phytophthora infestans* was attempted using ethidium bromide/UV light mutagenesis and repeated culturing on dimethomorph-amended medium. Ethidium bromide/UV mutagenesis created two isolates of *P. infestans* with resistance factors for dimethomorph >20, i.e., the ratio of the 50% effective concentration (EC₅₀) of the mutant to that of the wild-type. With repeated culturing on dimethomorph-amended medium, the rate of growth (mm diameter/day) increased until the tenth subculture for most *P. infestans* isolates. Resistance factors generated from repeated culturing were <8 for all isolates. For most isolates, the generation of dimethomorph resistance resulted in reduced growth rates on non-amended medium, regardless of the level of resistance or induction treatment. Additionally, the frequency of infection of leaf disks and whole tubers was significantly reduced in >20% of the isolates repeatedly subcultured on dimethomorph-amended medium. Regardless of the induction treatment, reduced fitness was common for all *P. infestans* isolates, indicating a potential biological cost associated with dimethomorph resistance. Based on these results, the development of field resistance to dimethomorph in *P. infestans* is unlikely with the currently employed resistance management strategies.

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

Resistance to fungicides in fungal and Oomycete plant pathogens has become increasingly common following the release of systemic, single-mode-of-action fungicides in the 1960s (7). Field resistance to every major systemic fungicide class has occurred in at least one species of plant pathogenic fungi or Oomycetes (19,23), leading to drastic shifts in fungicide programs for many pathosystems, such as potato late blight. The migration of phenylamide insensitive populations of *Phytophthora infestans* (Mont.) de Bary, the causal agent of potato and tomato late blight, from Mexico to the rest of North America (12) necessitated cultural control methods and crop protection strategies that relied primarily on protectant foliar fungicide applications (22). 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* spp. (1). Cells of *P. infestans* lacking cell walls, such as zoospores and artificially generated protoplasts, were not affected by dimethomorph (2,24), 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 (16).

Laboratory resistance to dimethomorph was generated with UV light mutagenesis in one isolate of *P. parasitica* (6) and with chemical mutagenesis in one isolate of *P. capsici* (26). In both cases, an approximate 20-fold increase in the effective concentration required for a 50% reduction in colony diameter (EC₅₀) resulted. Virulence of dimethomorph resistant isolates of *P. parasitica* was found to be equal to, or less than, the wild-type. In addition, dimethomorph resistance in *P. parasitica* was stable in the absence of dimethomorph through several in vitro subcultures for 3 months. Conversely, attempts to generate resistance using mycelial selection in *P. infestans* and *P. capsici* resulted in small (less than twofold) decreases in sensitivity to dimethomorph (26). 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 (i) generate resistance in *P. infestans* to di-

methomorph using mycelial adaptation or ethidium bromide/UV mutagenesis, (ii) quantify the level of resistance generated, and (iii) determine if resistance to dimethomorph in *P. infestans* results in reduced virulence on potato foliage and tubers.

MATERIALS AND METHODS

Preparation of amended medium and fungicide stock solutions. Experiments involving in vitro hyphal growth were performed on modified rye B agar (2,5) consisting of the filtrate of pre-rinsed rye (*Secale cereale* L.) seed (100.0 g/liter) boiled for 1 h, deionized (d)H₂O added to a final volume of 1.0 liter, glucose (8.0 g/liter), β -sitosterol (0.05 g/liter), and Bacto agar (15.0 g/liter). All plates for each subculture on dimethomorph-amended medium, or run of the sensitivity assay were prepared from the same batch in order to limit variability.

Dimethomorph (BASF Corp., Research Triangle Park, NC) 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 medium 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- μ m syringe-driven filter (Millipore Corp., Bedford, MA) to sterilize. Medium for control plates received 10 ml of filter-sterilized ethanol per liter.

Resistance generation: mycelial selection. The wild-type of each isolate used in this study had been previously characterized (24) for mating type, genotype (11), and physiological race (3), was subcultured twice following re-isolation from inoculated potato tuber (*Solanum tuberosum* L. cv. Snowden) tissue, and was labeled "WT" (Table 1). Colonized agar plugs, 4 mm in diameter, were transferred from the margin of an actively growing culture of each isolate, mycelium-side down, onto modified rye B agar amended with 0.0 or 1.0 μ g/ml dimethomorph and incubated at 21°C in the dark with three replicate plates per concentration. The dimethomorph concentration used for selection was previously found to be highly inhibitory, yet sublethal for all isolates of *P. infestans* examined (24). Final colony diameter (FCD) was measured after 11 days. Isolates grown on control (0.0 μ g/ml)

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and dimethomorph-amended medium (1.0 µg/ml) were labeled "CT" and "DM", respectively. One plate from each treatment was then used to subculture the isolate onto medium amended with the same concentration of dimethomorph, for a total of 10 subcultures. Portions of the colony were chosen for subculturing in an attempt to retain the wild-type colony phenotype of each isolate, while selecting sectors with higher growth rates. In the absence of markedly accelerated growth, plugs were chosen randomly from the margin of the colony. The rate of colony growth (mm/day) for each replicate plate was calculated for each cycle number and normalized relative to the mean rate of growth for the control plates of that cycle number. The rates of colony growth were analyzed as described below. Following the tenth subculture, the EC₅₀ was calculated for each of the dimethomorph-exposed and control colonies, as described below.

Resistance generation: mutagenesis. Wild-type isolates of *P. infestans* (Table 1) for mutagenesis were grown on modified rye B agar at 21°C until the medium became completely colonized. Plates were flooded with 5.0 ml of a 1-µg/ml ethidium bromide (EtBr) solution (in sterile dH₂O) and allowed to dry in a biological hood for 45 min with the lids removed. Cultures were then exposed to 254-nm UV light (2.03 W/m²) for 5 min. From each isolate, four 4-mm-diameter colonized agar plugs were transferred to rye B agar amended with either 0.0 or 10.0 µg/ml dimethomorph. Cultures not treated with EtBr/UV were also transferred onto control and dimethomorph-amended rye B agar. Cultures were incubated in the dark at 21°C and examined at 7-day intervals for colony diameter and rapid growth or sectoring on the dimethomorph-amended medium. After 14 days, the fastest growing sector of each isolate from the fungicide-amended medium was used for further assays, and hereafter labeled "MU". The EC₅₀ was calculated for each as described below.

Calculation of EC₅₀ values and statistical analysis. To determine if the rate of growth for an isolate repeatedly cultured on dimethomorph-amended rye B agar increased significantly with culture number, the growth rates (mm/day) were analyzed with an analysis of variance calculated as a multifactorial, completely randomized design (Proc MIXED, SAS/Stat, SAS Institute, Cary, NC), with isolate, cycle number, and dimethomorph concentration in the medium as the main effects. Means were separated by Fisher's least significant difference (LSD) at *P* = 0.05.

The in vitro sensitivity of *P. infestans* isolates to dimethomorph was determined as described previously without modification (24). In summary, each isolate was cultured in triplicate on rye B agar amended with 0.0, 0.1, 1.0, or 10.0 µg of dimethomorph per ml. FCD was measured after 11 days, and the percent inhibition relative to the mean of the control plates was calculated for each concentration. Percent inhibition values were then transformed using probits and linearly regressed on the log₁₀ of concentration (9). The EC₅₀ for hyphal growth (diameter) was calculated based on regression parameter estimates (SigmaPlot, SPSS Inc., Chicago, IL) and back-transformed. Resistance factors were calculated as the ratio of the EC₅₀ for the dimethomorph-exposed, control, or EtBr/UV treated cultures of an isolate to the EC₅₀ of the wild-type. The assay was repeated three times, and the calculated EC₅₀ values for each isolate were used as replicate blocks in an analysis of variance calculated as a randomized complete block design (Proc MIXED, SAS/Stat). Means were separated by Fisher's least significant difference (LSD) at *P* = 0.05.

Virulence assays. Virulence, as defined by the frequency of successful infection of foliar disks or whole tubers, was assessed by removing fully expanded leaflets of similar age (main stem, leaf positions 10 to 12 above the soil line) from greenhouse-grown potato plants (cv. Snowden). Leaflets were surface-disinfested in 0.5% so-

dium hypochlorite in dH₂O (10% commercial bleach solution) for 1 min, rinsed three times in sdH₂O, allowed to dry, and cut into 20-mm-diameter leaf disks with a sterilized cork borer. Leaf disks were randomly assigned to treatments and placed on water agar (15.0 g/liter) amended with rifamycin (37.5 mg/liter), ampicillin (10 mg/liter), and nystatin (37.5 mg/liter). Sporangia/zoospore suspensions were prepared as previously described (24) for a final concentration of 1.0 × 10⁴ sporangia per ml. The sporangia/zoospore suspension from each isolate was applied to four leaf disks per replicate (50 µl/disk), with three replicates. The experiment was repeated twice. Inoculated leaf disks were incubated at 21°C light/18°C dark (12-h cycles) and examined with a dissecting microscope at 96 h after inoculation for symptoms and signs of infection by *P. infestans*, such as the formation of typical late blight lesions and sporulation. Pathogen identity was confirmed microscopically. Leaf disk inoculations were not performed with EtBr/UV-treated isolates, as sporulation was absent or highly disrupted for all isolates. An analysis of variance on the incidence of diseased leaf disks was performed as previously described.

Pathogen-free potato tubers (cv. Snowden) with an average diameter of 5 cm were surface-disinfested as before. Colonized portions of rye B agar, 1 cm², were excised from the margin of an actively growing colony of *P. infestans* and placed into a sterile 1-cc syringe with an 18½ gauge needle. A wound approximately 1 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 7 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 as before when applicable. Tuber inoculations were not performed on EtBr/UV-treated isolates. An analysis of variance on the incidence of tuber infection was performed as previously described.

RESULTS

Mycelial selection. Relative to the control (CT), the rate of growth for all isolates on medium amended with dimethomorph at 1.0 µg/ml (SL) increased with subculture number for all isolates, e.g., Pi671 (Fig. 1). With most isolates, the largest increase occurred between the initial and second subcultures, whereas changes at subsequent subcultures were generally smaller or absent. At the end of the tenth

Table 1. *Phytophthora infestans* isolate identification, year isolated, mating type, allozyme-based genotype, physiological race, and state in which isolated

Isolate	Year isolated	Mating type	Genotype ^x	Physiological race ^y	Origin ^z
Pi88	1995	A1	US1	3.6.7.10.11	ND
Pi95-5	1995	A1	US1	1.2.3.7.9	MI
Pi458	1998	A1	US17	1.2.3.4.5.6.7.9.11	ID
Pi670	1997	A2	US7	1.2.3.4.5.6.10.11	OR
Pi671	1997	A2	US14	1.10	WA
Pi213	1997	A2	US8	1.2.3.4.5.6.7.10.11	CO
Pi94-4	1994	A2	US8	1.3.5	MI
Pi95-7	1995	A2	US8	1.2.3.4.5.6.7.10.11	MI
Pi97-2	1997	A2	US8	1.3.4.5.8.11	MI
Pi98-1	1998	A2	US8	2.5.6.7.8.9.10.11	MI
Pi98-2	1998	A2	US8	1.2.3.4.5.7.10.11	MI

^x Based on allozyme banding patterns and mating type as described by Goodwin et al. (11).

^y Determined using inoculations onto potato lines with different R-genes as described by Al-Kherb et al. (3).

^z State of origin (United States). All isolates were obtained from single lesions on potato foliage.

subculture, the FCD of each DM isolate was from 1.7- to 5.5-fold larger than the FCD following the initial subculture.

The EC_{50} values for the WT and CT of any isolate were not significantly different (Table 2). Isolates Pi458, Pi670, Pi94-4, and Pi98-1 had significantly higher EC_{50} values for the DM than for WT or CT. For all isolates, except Pi98-2, the calculated resistance factor (RF) was numerically larger for the DM than for the CT. Of the four isolates with significantly different EC_{50} values, Pi94-4 had the largest RF value (6.45). However, the isolate with the largest RF, Pi95-5 (7.48), did not have significantly different EC_{50} values between DM and WT.

The ability to infect and cause symptoms on potato leaf disks was limited for most isolates examined, regardless of treatment, as only four of 11 WT isolates resulted in 75% or more leaf disks infected (Table 3). The WT of isolates Pi95-5, Pi671, Pi670, Pi94-4, Pi97-2, and Pi98-2

successfully infected 50% or less of leaf disks, indicating low virulence, whereas only the WT of Pi458 infected all leaf disks. In comparison to the WT, the DM had significantly reduced virulence for six of 11 isolates of *P. infestans*. In contrast, only the CT of isolates Pi458 and Pi95-7 infected significantly fewer leaf disks than the WT.

Infection and symptom development in whole tubers following inoculation with mycelium was more efficient than leaf disk inoculation with the WT of eight of 11 isolates causing symptoms in all tubers inoculated (Table 3). The DM cultures of seven isolates infected significantly fewer tubers compared with the WT. Four of 11 isolates subcultured on control medium (CT) infected significantly fewer tubers when compared with the corresponding WT.

Mutagenesis. Following the mutagenesis treatment, all isolates grew on non-amended rye B agar, except Pi88 (data not

shown). When cultured on nonamended medium, EtBr/UV-treated cultures exhibited disrupted colony morphology and slower growth rates compared with nonexposed cultures (data not shown). Of the isolates that grew following the mutagenesis treatment, only Pi458 and Pi98-2 failed to grow on medium amended with 10.0 µg/ml dimethomorph. However, most isolates required more than 14 days to develop sufficient growth for subculturing. Sectors with increased growth rates on dimethomorph-amended medium were common, but not always present. The EtBr/UV-treated colonies from all isolates had significantly higher EC_{50} values than did the corresponding WT (Table 4). The EtBr/UV-treated colony of isolate Pi94-4 had the highest EC_{50} value (9.92 µg/ml), and those of isolates Pi95-5 and Pi94-4 had the largest RF values at 23.17 and 22.45, respectively.

DISCUSSION

A significant decrease in sensitivity to dimethomorph (increase in the EC_{50} for in vitro growth) was demonstrated in *P. infestans* following repeated subculturing on medium amended with a sublethal concentration of dimethomorph in four of the 11 *P. infestans* isolates examined. Almost all isolates had a numerically increased EC_{50} for the dimethomorph-treated cultures compared with the WT. However, the resistance factor for most isolates was <3, which represents a relatively small change. The rapid increase in final colony diameter by the third subculture cycle on amended medium indicates a physiological adaptation to dimethomorph or selection for a mutation providing resistance. Culturing the isolates on nonamended medium prior to in vitro growth sensitivity assessments for EC_{50} calculation was employed to reduce the effects of physiological adaptation.

The development of resistance to dimethomorph was demonstrated in *P. infestans* following the EtBr/UV exposure of mycelium and resulted in two *P. infestans* isolates with resistance factor values >20. These values were similar to previously reported RF values for dimethomorph generated for *P. parasitica* using UV irradiation (6) and for *P. capsici* using chemical mutagenesis (26). In comparison, the resulting RF of *P. capsici* for the phenylamide fungicide metalaxyl was >100 in the latter study, and an RF value of 20 for dimethomorph was considered moderate. All isolates of *P. infestans* that survived the mutagenesis treatment had reduced growth rates on nonamended medium and disrupted colony morphology compared with the wild-type. On nonamended medium, both factors could be attributable to mutations unrelated to dimethomorph resistance negatively affecting growth or the dimethomorph resistance mechanism(s) itself. Particularly in the former situation,

Fig. 1. Rates of colony growth (mm/day) for *Phytophthora infestans* isolate Pi671 on rye B agar amended with dimethomorph (1.0 µg/ml) relative to the rate of growth of the same isolate on non-amended medium. Error bars represent Fisher's LSD ($P = 0.05$).

Table 2. Calculated EC_{50} ^x values and resistance factors for wild-type isolates of *Phytophthora infestans* and the same isolates repeatedly cultured on nonamended or dimethomorph-amended medium

Isolate	EC_{50}			Resistance factor ^y	
	WT ^z	CT	DM	CT	DM
Pi88	0.35 a	0.37 a	0.77 a	1.07	2.22
Pi95-5	0.11 a	0.19 a	0.80 a	1.74	7.48
Pi458	0.84 b	0.56 b	1.82 a	0.66	2.16
Pi670	0.62 b	0.54 b	1.49 a	0.88	2.42
Pi671	0.70 a	1.05 a	1.08 a	1.49	1.54
Pi213	0.68 a	0.87 a	0.98 a	1.28	1.44
Pi94-4	0.44 b	0.86 b	2.85 a	1.96	6.45
Pi95-7	0.61 a	0.62 a	0.74 a	1.01	1.21
Pi97-2	0.24 a	0.37 a	0.85 a	1.56	3.59
Pi98-1	0.56 b	0.53 b	1.65 a	0.96	2.96
Pi98-2	0.59 a	1.26 a	1.25 a	2.14	2.13

^x Effective concentration to reduce colony diameter to 50% of 0.0 µg/ml dimethomorph-amended control. Means in a row followed by the same letter are not significantly different within each isolate using Fisher's LSD ($P = 0.05$). Comparisons between treatments of different isolates are not shown.

^y Resistance factor = EC_{50} of manipulated isolate/ EC_{50} of WT. Resistance factor of WT not applicable.

^z WT = wild-type. CT and DM = subcultured 10 times on medium amended with 0.0 or 1.0 µg/ml dimethomorph, respectively.

one might have expected additional fitness reductions, including virulence, as mutations generally reduce the fitness of *Phytophthora* isolates (18). The loss of ability to sporulate supports this hypothesis.

The EC₅₀ value derived from in vitro colony growth, while commonly used in fungicide sensitivity studies (9,13,26), is sensitive to changes in the growth rate of the fungus because of the percent inhibition transformation relative to the control. A mutant isolate with a reduced growth rate may have the same colony diameter as the wild-type at nonlethal concentrations of the fungicide, and yet the smaller colony diameter on nonamended medium results in a lower percent inhibition due to the reduced slope of the regression. Therefore, when calculating the EC₅₀ value using percent inhibition versus concentration, a larger EC₅₀ is calculated. Similarly, the RF value, being calculated using the EC₅₀, is dependent on both reduced growth rates on nonamended medium and on extremely sensitive wild-type isolates. For example, the isolate Pi95-5 had reduced growth on nonamended medium for both repeated culturing on dimethomorph-amended medium and EtBr/UV-treated cultures and the lowest wild-type EC₅₀ of any isolate. The DM-treated colony of this isolate had an EC₅₀ that was not significantly different from either the WT or CT, and yet the isolate had the largest RF value.

The low frequency of infection by several WT isolates of *P. infestans* on leaf disks was not surprising because variable virulence has been documented, even on potato cultivars with no known R-genes (15). Possible reduction in efficiency of infection for the isolates subcultured on agar, regardless of fungicide amendment, was also expected, as *P. infestans* is known to lose virulence following long-term culturing in vitro (8). However, since the CT cultures of most isolates retained near-WT virulence, reduction in virulence of the DM cultures may have been associated with dimethomorph resistance. *P. infestans* requires the formation of viable sporangia for infection of foliar tissue, and any putative changes in cell wall formation resulting from the development of dimethomorph resistance or adaptation might disrupt sporangia formation. Additionally, cell wall components of *P. infestans* are known to induce resistance in potato (10), and it is possible that changes that occurred as a result of dimethomorph resistance allowed for the release of such compounds. Without detailed investigation of cell wall composition and formation, it is difficult to discern the true cause, and this phenomenon should be investigated further. In addition, excluding the chemical composition, little is known about the biochemistry involved with cell wall formation in *Phytophthora* (4), and therefore the genetic basis for resistance to dimethomorph may be difficult to discern.

The tuber inoculation test, while not biologically accurate because of the circumvention of the periderm, was performed because differences in foliar and tuber susceptibility have been noted and may be important (14,25). The lack of correlation between foliar and tuber inoculations was probably related to differences in the assays, in that the tuber inoculation did not require the development of viable sporangia 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 interaction.

The generation of resistance to dimethomorph in *P. infestans* was possible through both repeated selection on sublethal amended medium and chemical- or UV-induced mutation. The low amount of resistance that developed to dimethomorph, compared with previously published phenylamide resistance (6,26), indicates that the mechanism may be

quantitative and possibly multigenic, as with the demethylation inhibitor fungicides commonly used to control true fungi (20). If this hypothesis is true, one would expect resistance in the field to develop through directional selection and occur in small increments (21). Also, resistance management techniques such as block treatments and co-application of dimethomorph with protectant fungicides would likely be effective. Currently, the development of field resistance 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 multisite fungicide usage is restricted because of the greater environmental impact, dimethomorph usage becomes more likely. Additionally, dimethomorph is being studied for the control of other Oomycete plant pathogens (17), and active resistance management strategies should be developed and implemented where appropriate.

Table 3. Incidence of infection by *Phytophthora infestans* on potato leaf disks and tubers inoculated with wild-type isolates and the same isolates repeatedly subcultured on nonamended or dimethomorph-amended medium

Isolate	Leaf disks (%) ^y			Whole tubers (%)		
	WT ^z	CT	DM	WT	CT	DM
Pi88	58 a	50 a	58 a	100 a	33 b	22 b
Pi95-5	25 a	8 a	25 a	33 a	22 a	0 b
Pi458	100 a	8 b	25 b	100 a	89 a	44 b
Pi670	50 a	50 a	33 a	100 a	67 b	22 c
Pi671	50 a	50 a	17 b	100 a	100 a	100 a
Pi213	92 a	75 a	25 b	100 a	100 a	100 a
Pi94-4	42 a	17 ab	8 b	56 a	56 a	44 a
Pi95-7	92 a	58 b	42 b	100 a	78 b	44 c
Pi97-2	17 a	17 a	25 a	67 a	67 a	22 b
Pi98-1	92 a	75 a	8 b	100 a	56 b	33 c
Pi98-2	42 a	33 a	33 a	100 a	100 a	89 a

^y Determined using the percentage of leaf disks (out of four) or tubers (out of three) with symptoms and signs of infection by *P. infestans*. Leaf disks and tubers checked for infection at 96 and 168 h after inoculation, respectively. Means in a row followed by the same letter are not significantly different within each isolate using Fisher's LSD ($P = 0.05$). Comparisons between treatments of different isolates not shown.

^z WT = wild-type. CT and DM = subcultured 10 times on medium amended with 0.0 or 1.0 µg/ml dimethomorph, respectively.

Table 4. The EC₅₀^x values and resistance factors for wild-type isolates of *Phytophthora infestans* and the same isolates following treatment with ethidium bromide (EtBr)/UV light

Isolate	EC ₅₀		Resistance factor ^y
	WT ^z	MU	
Pi95-5	0.17 b	2.48 a	23.17
Pi670	0.62 b	1.21 a	1.97
Pi671	0.70 b	2.29 a	3.26
Pi213	0.68 b	4.37 a	6.42
Pi94-4	0.44 b	9.92 a	22.45
Pi95-7	0.61 b	4.43 a	7.23
Pi97-2	0.24 b	1.24 a	5.22
Pi98-1	0.56 b	1.98 a	3.56

^x Effective concentration to reduce colony diameter to 50% of 0.0 µg/ml dimethomorph-amended control. Means in a row followed by the same letter are not significantly different within each isolate using Fisher's LSD ($P = 0.05$). Comparisons between treatments of different isolates are not shown.

^y Resistance factor = EC₅₀ of manipulated isolate/EC₅₀ of WT. Resistance factor of WT not applicable.

^z WT = wild-type. MU = treated with EtBr/UV light.

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