Dimethomorph Activity Against Oomycete Fungal Plant Pathogens

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ABSTRACT

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Dimethomorph (DMM) was effective in controlling late blight in potato and tomato caused by either metalaxyl-sensitive (MS) or metalaxyl-resistant (MR) field isolates of *Phytophthora infestans* and downy mildew in cucumbers and melons caused by MS or MR isolates of *Pseudoperonospora cubensis*. The fungicide did not affect zoospore discharge from sporangia of *P. infestans* but strongly inhibited zoospore encystment, cystospore germination, and mycelial growth in vitro. DMM showed translaminar activity and local systemic activity in intact plants but failed to translocate from one leaf to another in either acropetal or basipetal direction. DMM applied as a soil drench strongly protected tomatoes against late blight but failed to protect cucumbers against downy mildew. When applied in lanolin paste to the stem surface, but not when applied to the hypocotyl, it provided excellent control of both diseases in the foliage. DMM applied 1 day postinoculation partially protected potato against late blight and cucumber against downy mildew, and in both systems reduced sporulation of the pathogen. DMM diminished the sporulation of *P. infestans* and *P. cubensis* when applied to normally developed infected tissue. In *P. cubensis* it induced enhanced callose-encasement of haustoria. It showed a remarkable persistence on foliage subsequent to excessive washing with water as well as a high residual activity lasting for 9 days. DMM seems to be a good candidate for the control of oomycete diseases in the field especially in growing areas where phenylamide-resistant fungal populations prevail.

Additional keyword: phenylamide fungicides.

Dimethomorph (DMM) was introduced in 1988 (1) as a novel systemic fungicide with protectant, curative, and antisporulant activities against members of the *Peronosporaceae* and the genus *Phytophthora* (but not *Pythium*). Dose-response studies carried out with vine leaf disks inoculated with *Plasmopara viticola* (Berk. & M. A. Curtis) Berk. & De Toni in Sacc. revealed low ED_{95} values of 0.25 to 1.15 μg of active ingredient (a.i.) per ml (3) and a prolonged residual activity of 15 days (11,16). Attempts to elucidate the primary mode of action of DMM remained inconclusive but several studies (2,10,15) indicated that DMM interferes with the assembly of wall polymers in the fungal cell. As a result, zoospores fail to produce cell walls and hyphae stop expanding.

Several features made DMM worth evaluating against late blight in potatoes and downy mildew in cucurbits, two major oomycete diseases worldwide. First, it has no cross-resistance to phenylamide fungicides (1), which lost their efficacy in the control of some diseases due to resistance (5,8,9); second, dimethomorph possesses systemic, curative, and antisporulant attributes, which may ensure prolonged efficacy of after-infection sprays (16); and third, the fungicide is highly effective at relatively low doses (3,10). We describe here the efficacy of DMM in controlling late blight in potato and tomato, and downy mildew of cucumber and melon.

METHODS AND MATERIALS

Plants. The potato (*Solanum tuberosum* L.) cv. Alpha, and the tomato (*Lycopersicon esculentum* Mill.) F_1 hybrid Baby were used for studying the effects of DMM on late blight. The cucumber (*Cucumis sativus* L.) cv. Dalila and the melon (*Cucumis melo* L.) cv. Ein-Dor were used for downy mildew studies. Plants were grown in the greenhouse (20 to 30°C). Potatoes were grown from tubers in pots (1 tuber per 1-liter pot) and used at 6 weeks after planting after plants had developed 9 to 11 compound leaves. Tomatoes, cucumbers, and melons were grown from seeds, one plant per 0.1-liter pot. Tomato plants were used 4 weeks after sowing, when five leaves had fully developed; cucumber and melon plants at 3 weeks after sowing, when two fully expanded leaves had developed. One experiment was conducted with tobacco (*Nicotiana tabacum* L.) cv. Ky-16 at the six-leaf stage.

Pathogens. Late blight of potato and tomato was established in most experiments with *Phytophthora infestans* (Mont.) de Bary isolates MS-315 (metalaxyl-sensitive, A_2 mating type from Japan) and MR1 (metalaxyl-resistant, A_2 mating type from Israel) (8). Some experiments were done with the metalaxyl-sensitive A_1 isolate S49 (Sandoz collection). Six other isolates, two metalaxyl sensitive (MS), one metalaxyl intermediate (MI), and three metalaxyl resistant (MR), were used to obtain information on the sensitivity distribution to DMM in *P. infestans* populations. Downy mildew in cucumbers was tested with two isolates of *Pseudoperonospora cubensis* (Berk. & M. A. Curtis) Rostovzev.: an MS isolate collected in 1992 and an MR isolate collected in nature in 1991. Downy mildew in tobacco was induced with *Peronospora tabacina* Adam collected in 1990.

Fungicide. Dimethomorph, (E,Z)4-[3-(4-chlorophenyl-3-(3,4-dimethoxyphenyl)acryloyl] morpholine, (Shell WL127294, CME151) technical grade (95.5%) was dissolved in ethanol for mycelial growth studies on rye liquid medium. A 25WP formulation was used for spray application with potato, tomato, tobacco, cucumber and melon. Some experiments were conducted with a 15DC (dispersive concentrate) formulation. Spraying was done using a glass atomizer operated with air pressure of 10 kPa. All concentrations of fungicides used are given as active ingredients (a.i.).

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Inoculum production and inoculation. *Phytophthora infestans* was propagated on potato tuber slices (cv. Alpha) at 15°C in the dark. Freshly produced sporangia were harvested (at 6 days after inoculating the slices) with cold (4°C) glass-distilled water and their concentration adjusted to 2,500 sporangia/ml with the aid of a hemacytometer. Inoculum suspension was sprayed onto the adaxial (upper) or abaxial (lower) leaf surfaces of potato or tomato plants using a glass atomizer at about 10 ml/plant.

Pseudoperonospora cubensis was propagated on cucumber (cv. Dalila) plants as described elsewhere (4). Sporangia were washed off with cold (4°C) glass distilled water and their concentration adjusted to 1,200 sporangia/ml (unless stated otherwise). Cucumber and melon plants were inoculated with sporangial suspension as described for *P. infestans.*

Peronospora tabacina was propagated on tobacco plants. Inoculum was prepared as described for *P. cubensis* and adjusted to 10^4 conidia/ml before inoculation.

After inoculation, plants were kept in a dew chamber at 18°C in the dark for 20 h to ensure infection. Plants were then transferred to a 20°C growth chambers (60% relative humidity), with a 12-h light-dark cycle for symptom development (4,8).

Disease assessment. Late blight development was estimated as proportion of leaf area blighted according to the scale described before (8). Downy mildew symptoms were rated according to the scale described previously (4). Percentage of disease control was calculated as the ratios of diseased leaf areas determined for treated and nontreated plants.

Spore germination, mycelial growth, and sporulation. The effect of DMM on zoospore release, cyst germination and mycelial growth in vitro was tested with *P. infestans* only.

Sporangial suspensions were mixed with DMM of various concentrations and 20-µl droplets were transferred to depression glass slides. Slides were incubated in moist petri dishes at 15°C in the dark for 20 h. Some experiments conducted with motile zoospores produced by incubating sporangial suspension at 15°C for 2 to 3 h before DMM was applied.

Mycelial growth in vitro was tested in liquid rye-extract broth (60 g of rye, 20 g of sucrose, 2 g of yeast extract per liter of water) as described previously (8). Mycelial dry weight was determined after 10 days incubation at 18°C in the dark. Sporulation was examined in vivo with either intact plants or leaf disks floated on DMM suspensions in 24-well titer plates. Blight or downy mildew lesions were allowed to establish in the absence of DMM, and the fungicide was either sprayed onto the leaves or added to the leaf disks in the titer plates. Fungal sporulation was examined 20 to 44 h later with the aid of a UV microscope (6,7) or using a hemacytometer.

Some experiments were done with DMM (technical grade) mixed with lanolin (BDH, UK). Lanolin (0.75 g) and DMM (0.25 g) were mixed and gently applied to approximately 1-cm segments of hypocotyls, stems, or petioles.

Data analysis. Experiments were repeated at least once with three to six replicate plants per treatment. Results of a representative experiment are reported. Data were analyzed for significant differences using ANOVA program. ED_{90} values were calculated after log-logit transformation of dose-response data.

RESULTS

Effects on spore germination. DMM had no effect on zoospore discharge from sporangia of *P. infestans* at concentrations as high as 32 µg/ml. However, cystospore formation from zoospores was inhibited by 90% at 0.06 µg/ml. After motile zoospores were exposed to the fungicide for 20 h, the percentage of cells producing a germ tube was 100, 2.4, 1.4, and 0 at DMM concentrations of 0, 0.015, 0.03, and 0.06 µg/ml, respectively. A comparative experiment conducted with mancozeb revealed that germination occurred at a concentration range of 0.01 to 5 µg/ml. At the highest concentration, mancozeb prevented discharge of zoospores from sporangia.

Effect on mycelial growth in vitro. Two isolates of *P. infestans* were cultured in rye seed liquid medium amended with DMM dissolved in ethanol and mycelial dry weight was determined after 10 days of incubation at 18°C in the dark. Ethanol alone (final concentration 0.6%) had no effect on fungal growth or biomass. DMM at concentrations of up to 0.075 µg/ml had no significant effect on dry weight accumulation of either isolate S49 (metalaxyl-sensitive) or isolate MR1 (metalaxyl-resistant). Both isolates were inhibited by 90% with DMM of 0.3 µg/ml. MIC (minimal inhibitory concentration) values were 0.6 and 1.25 µg/ml for isolates MR1 and S49, respectively (Fig. 1).

Late blight in potato and tomato. Adaxial surfaces of potato plants were treated with various concentrations of DMM and 2

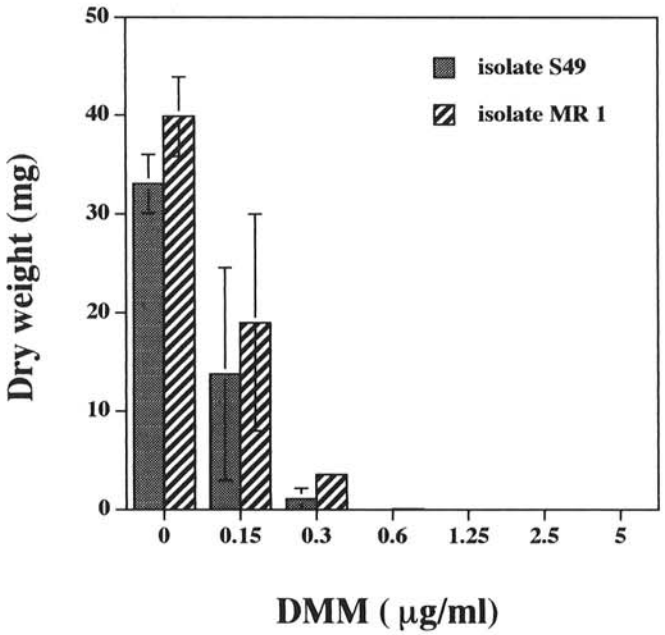

Fig. 1. The effect of dimethomorph (DMM) on mycelial growth of *Phytophthora infestans* in liquid medium. Means ± standard error (SE); where no SE bars are given, replicate were identical.

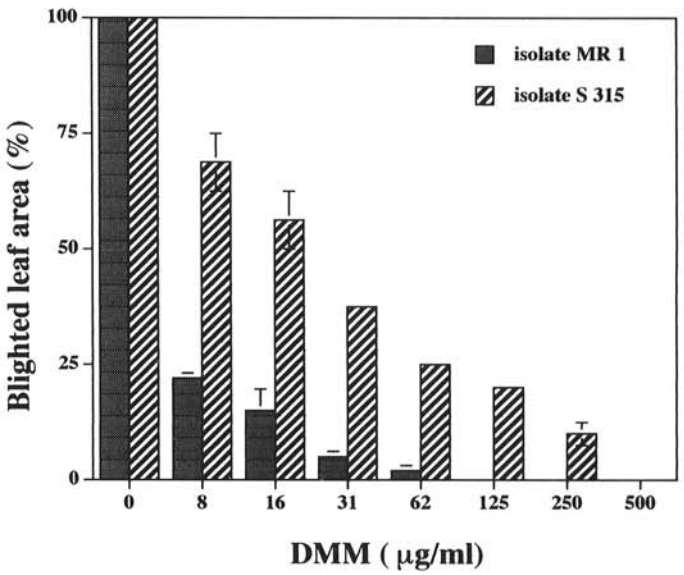

Fig. 2. Efficacy of dimethomorph (DMM) applied prophylactically to potato plants on late blight development induced by two isolates of *Phytophthora infestans*. Means ± standard error (SE); where no SE bars are given, replicate were identical.

days later were inoculated with sporangia (2,500 per ml) of *P. infestans*. Disease assessment 8 days after inoculation showed total control of disease for plants treated with 125 and 500 µg/ml of DMM for isolates MR1 and MS-315, respectively (Fig. 2). Concentrations required for 90% inhibition of the disease were 18.4 and 294 µg/ml for isolates MR1 and MS-315, respectively. A similar experiment conducted with tomato plants and isolate MR1 revealed ED_{90} value of 49 µg/ml and MIC of 250 µg/ml.

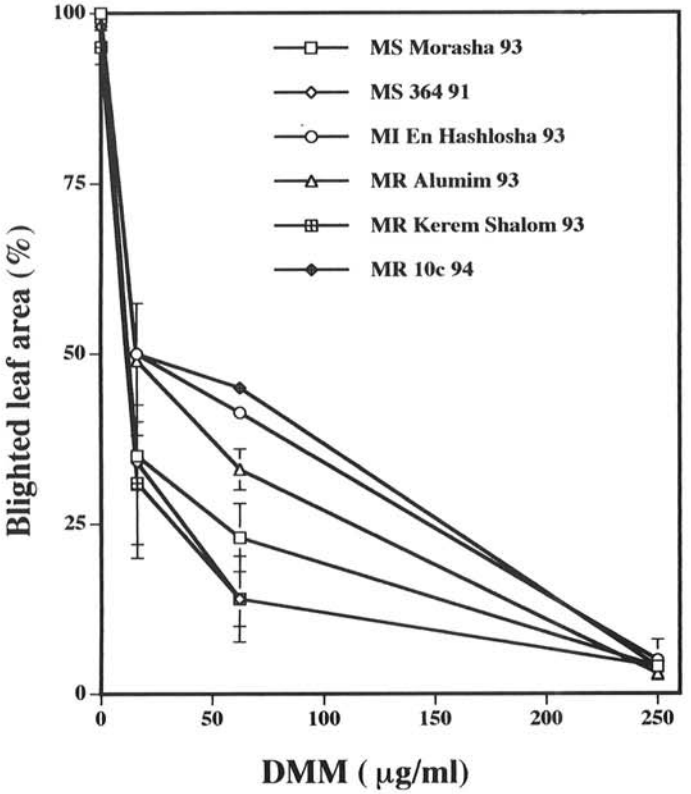

Fig. 3. Dimethomorph (DMM) sensitivity distribution in *Phytophthora infestans* on potato. Plants were sprayed with DMM, inoculated after 1 day with sporangial suspension (2,500 sporangia/ml) of *P. infestans* and evaluated for disease development a week later. MS = metalaxyl-sensitive; MI = metalaxyl-intermediate; MR = metalaxyl-resistant. Means ± standard error (SE); where no SE bars are given, replicate were identical.

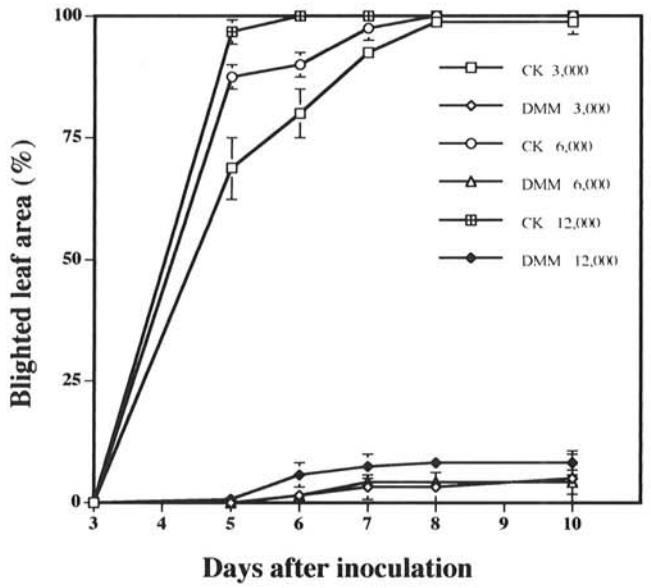

Fig. 4. Progress with time of late blight in control (CK) and DMM-treated (250 µg/ml) potato plants inoculated with three sporangial concentrations (3, 6, and 12 × 10^3 sporangia/ml) of *Phytophthora infestans* isolate MR1. Means ± standard error (SE); where no SE bars are given, replicate were identical.

Phytophthora infestans populations showed a relatively broad sensitivity distribution to DMM. Potato plants were inoculated with each of six other field isolates of the fungus 1 day after being sprayed with DMM (0, 16, 62, 250 µg/ml). Disease records taken 7 days later are presented in Figure 3. The following ED_{90} values were calculated for these isolates: 62 (isolate MS-364, A_2, Israel, 1991); 63 (MR-Kerem Shalom, A_1, Israel, 1993); 83 (MS-Morasha, A_1, Israel, 1993); 133 (MR-Alumim, A_1, Israel, 1991); 179 (MI-En Hashlosha, A_2, Israel, 1993) and 193 µg/ml (MR-10C, A_2, Switzerland, 1994).

DMM was also effective against late blight in potato when higher inoculum doses were used. For example, 5 days after inoculation with 3,000, 6,000, and 12,000 sporangia/ml (isolate MR1), disease levels (± SE) were 69 ± 5, 87 ± 1, and 97 ± 3%, respectively, with control plants, compared with 0, 0, and 1 ± 1% in plants sprayed with DMM of 250 ppm; 10 days after inoculation corresponding figures were 98 ± 2, 100 ± 0, and 100 ± 0%, compared with 4 ± 6, 3 ± 2, and 8 ± 3% on treated plants (Fig. 4).

Downy mildew in melons. Melon plants (cv. Ein-Dor) were used to assess the sensitivity of MR vs. MS isolates of *P. cubensis* to DMM. Plants were sprayed with various concentrations (4 to 250 µg/ml) of DMM on adaxial leaf surfaces and inoculated on the same surfaces with 1,200 sporangia/ml of either the MR or the MS isolate. MIC values, a week later, were 62 and 250 µg/ml for the MR and the MS isolate, respectively (Fig. 5). Corresponding ED_{90} values were 12.6 and 21.8 µg/ml, respectively.

Translaminar activity against late blight and downy mildews. DMM was applied to adaxial leaf surfaces of potato, cucumber, and tobacco, and plants were inoculated on either the adaxial or abaxial leaf surfaces with their respective pathogen.

Results for potato plants inoculated with the MR1 isolate of *P. infestans* and assessed 7 days after inoculation are given in Figure 6. When DMM and the fungus were applied to the adaxial leaf surfaces, ED_{90} and MIC values were 30 and 250 µg/ml, respectively. However, when DMM and the fungus were applied to opposite surfaces ED_{90} and MIC values were 120 and >500 µg/ml, respectively, suggesting that DMM was quite efficiently transported across the leaf blade.

Results for cucumbers are given in Figure 7. Cucumber plants (two-leaf stage, cv. Dalila) were sprayed with DMM (4 to 250

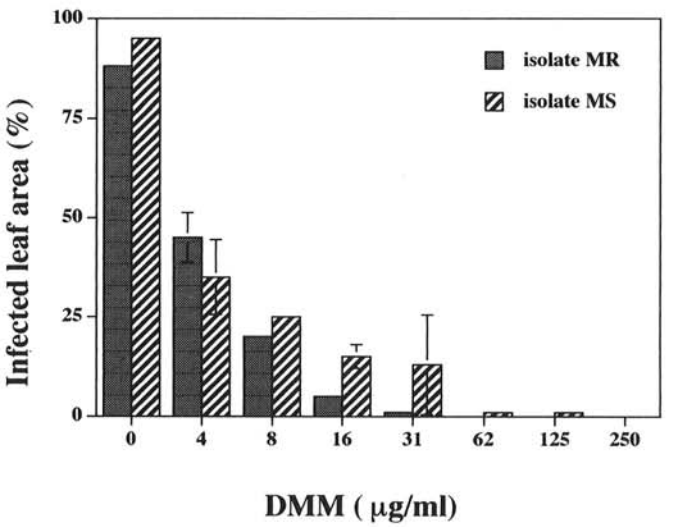

Fig. 5. Efficacy of dimethomorph (DMM) applied prophylactically to melon plants on downy mildew development induced by two isolates of *Pseudoperonospora cubensis*. Means ± standard error (SE); where no SE bars are given, replicate were identical.

μg/ml) on adaxial leaf surfaces and inoculated 2 days later with sporangial suspension of *P. cubensis* (MR isolate, 1,200 sporangia/ml) on either the adaxial (treated) or the abaxial (untreated) leaf surfaces. Disease records taken a week later revealed that DMM at 31 and 62 μg/ml was sufficient for complete control of the disease when inoculation was done on the adaxial or abaxial surfaces, respectively (Fig. 7). Corresponding ED_{90} values were 10.6 and 23.1 μg/ml, respectively.

With tobacco challenged with *Peronospora tabacina*, the ED_{90} value of DMM applied to adaxial leaf surface was 145 μg/ml and to abaxial surfaces 377 μg/ml. In both cases MIC was above 500 μg/ml (data not shown).

The ratio between control efficacy (90%) of DMM applied to adaxial versus abaxial surfaces was smallest for cucumber (1:2.2), medium for tobacco (1:2.6), and largest for potato (1:4). However, *P. infestans* seems to be more sensitive than *P. tabacina* to DMM, and *P. cubensis* most sensitive.

Residual activity against late blight in potato. Residual activity was determined by measuring disease symptoms in potato plants inoculated with *P. infestans* at various time intervals after application of DMM at various concentrations. Plants were strongly and significantly ($P < 0.05$) protected against late blight when challenged 6 h to 9 days after DMM application. ED_{90} values, however, increased (except for 6 h) with lengthening the interval period between spray and inoculation, suggesting some decay or dilution of the fungicide on leaf surfaces. Thus, ED_{90} values for DMM applied 6 h, 1 day, 3 days, 7 days, and 9 days before challenge inoculation were 197, 70, 80, 216, and 276 μg/ml, respectively. The lower efficacy of DMM in plants challenged 6 h after spray, compared with plants challenged 1 day or 3 days after spray, may indicate that at 6 h the compound has not yet reached its optimum diffusion in the leaf cuticle.

Curative activity against late blight and downy mildew. DMM applied to potato plants 1 day after inoculation (isolate MR1) did not significantly reduce blight symptoms development compared with nontreated plants. Thus, plants sprayed with 0, 250, 500, and 1000 μg/ml of DMM were all blighted up to 75% at 4 days postinoculation. However, when blighted leaflets were detached from such plants and placed in a moist chamber in the dark for 20 h (18°C) a significant reduction in sporangial yield was observed in treated leaves: sporangial yields were 20 ± 1, 2.8 ± 0.2, 0.7 ± 0.1, and 0.4 ± 0.1 × 10^3 sporangia/leaflet for plants treated with DMM of 0, 250, 500, and 1,000 μg/ml, respectively. Similar experiments conducted with the 15DC formulation (15% a.i.) of DMM showed that it was more effective than the 25WP formulation in curative applications. Thus, spray application of the 15DC formulation to potato plants at 250, 500, and 1,000 μg/ml, 1 day postinoculation, resulted (1 week later) in 13 ± 0, 62 ± 4, and 89 ± 4% inhibition of blight symptoms development, respectively, with a corresponding reduction in sporangial yields of 59 ± 2, 84 ± 3, and 98 ± 1%.

When cucumber plants were treated with 25WP DMM 1 day after inoculation with *P. cubensis* (MR isolate), a mild but significant reduction in disease development was observed. Plants treated with 250, 500, and 1,000 μg/ml were protected to an extent of 33 ± 14, 43 ± 0, and 62 ± 13%, respectively. Treated plants also brought about reduced sporulation of the fungus when placed for 24 h in a moist chamber (darkness, 18°C). The number of sporangiophores bearing sporangia emerging from stomata of the abaxial leaf surface was counted with the aid of a UV epifluorescent microscope after calcofluor staining (7): 54 ± 20, 20 ± 14, 15 ± 8, and 7 ± 9 sporangiophores per 1.88 mm^2 of leaf surface in plants treated with DMM of 0, 250, 500, and 1,000 μg/ml, respectively. The 15DC formulation had no activity at 500 μg /ml, applied as a postinfectional spray, against mildew symptom development, but reduced sporulation of *P. cubensis* by 67 ± 19%.

Effect on sporulation. Potato and cucumber plants were sprayed with DMM (25WP or 15DC) at 7 days after inoculation when blight or mildew lesions occupied about two-thirds of the leaflet (leaf) area. They were then incubated for 2 days in Perspex moist boxes in growth chambers at 18 to 20°C to induce fungal sporulation. Epifluorescent microscopical examination of Calcofluor-stained leaf tissue revealed a significant reduction in the number of fungal sporangiophores emerging from stomata of DMM-treated potato or cucumber leaves. In fungicide-free potato leaflets and cucumber leaves the mean numbers of sporangiophores per 1.88 mm^2 were 183 ± 90 and 133 ± 15, respectively. DMM reduced these figures significantly (Table 1). DMM was more effective against *P. cubensis* (55 to 100% inhibition) than

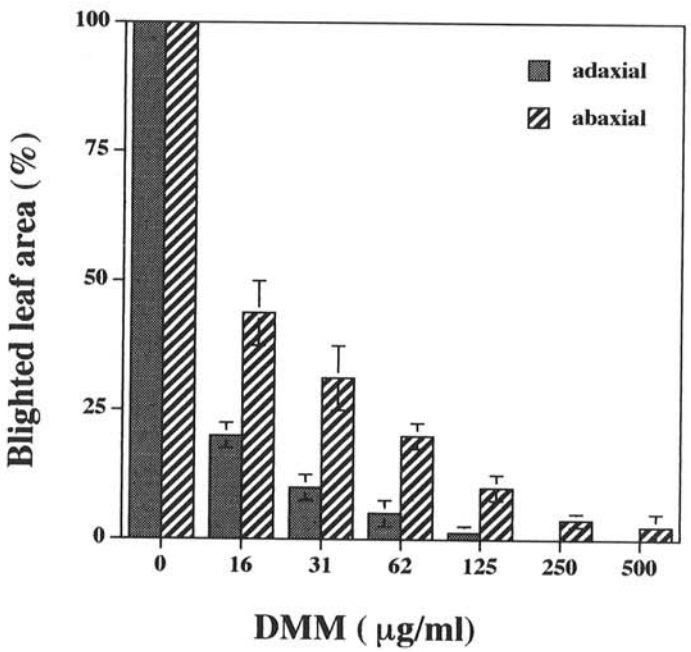

Fig. 6. Translaminar activity of dimethomorph (DMM) against *Phytophthora infestans* in potato plants. Plants were sprayed on adaxial (upper) leaf surfaces and fungal inoculation was made on either the adaxial or the abaxial (lower) leaf surfaces. Means ± standard error (SE); where no SE bars are given, replicate were identical.

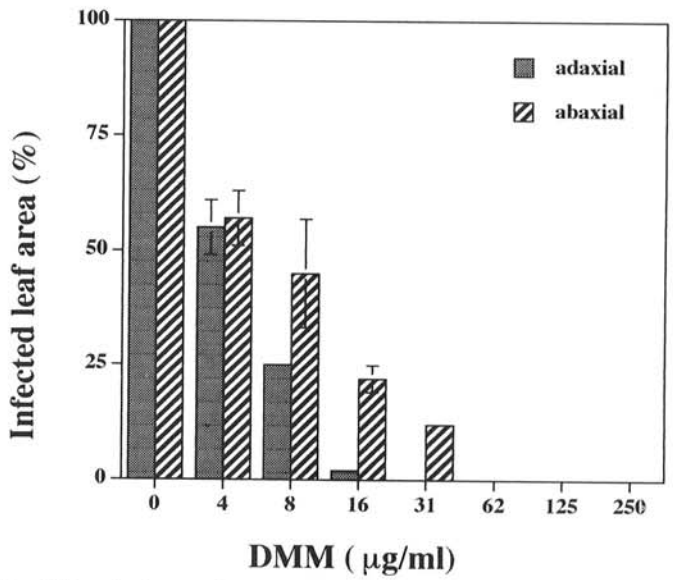

Fig. 7. Translaminar activity of dimethomorph (DMM) against *Pseudoperonospora cubensis* in cucumber plants. Plants were sprayed on adaxial (upper) leaf surfaces and fungal inoculation was made on either the adaxial or the abaxial (lower) leaf surfaces. Means ± standard error (SE); where no SE bars are given, replicate were identical.

against *P. infestans* (23 to 81% inhibition). The WP formulation was slightly, but significantly, more effective than the DC formulation in potato (except in 2,000 µg/ml) but not in cucumber (Table 1). UV microscopic examination of basic aniline blue-stained cucumber leaf tissue (6) showed an enhanced callose-encasement of haustoria in DMM-treated leaves relative to non-treated leaves (Fig. 8). This may be related to the reduced sporulation associated with DMM application because such encasement was reported to be associated with genetic resistance of melon to downy mildew (6).

The eradication of *P. infestans* by DMM was also evaluated with tomato leaf disks. Twelve-millimeter disks were floated on 1 ml of water each in titer plates and each inoculated with a 10-µl droplet of water containing 20 sporangia of *P. infestans* (MR1). Three days later when mean lesion diameter was 6 ± 1 mm with sporangia not yet produced, the water was withdrawn from wells and 1.0 ml of DMM (15DC) of various concentrations added. Lesion sizes and sporangial yields as measured 2 days later were 12 ± 0, 9 ± 1, 8 ± 1, 7 ± 1, and 6 ± 1 mm with corresponding sporangial counts of 21 ± 7, 6 ± 5, 3 ± 2, 3 ± 1, and 0.0 × 10^3 sporangia/disk in disks treated with DMM of 0, 0.08, 0.31, 1.25, and 5 ppm, respectively

Systemic translocation in detached leaves. DMM supplied to detached tomato leaves via the cut end of the petiole strongly protected against *P. infestans* (MR1). Compound leaves (7 leaflets) were dipped in 3 ml of DMM of various concentrations for 1 day until all the suspension was taken up, placed on moist filter paper, and inoculated with droplets containing 20 sporangia/droplet, one droplet/leaflet. Mean lesion diameters recorded 4 days after inoculation were 21 ± 5, 6 ± 8, 2 ± 4, 0.5 ± 0.5, and 0.0 mm for leaves supplied with 0, 3, 6.1, 12.5, and 25 µg/ml of DMM, respectively. Thus, 6 µg/ml of DMM (a total of 18 µg/leaf) was sufficient to reduce lesion diameter by about 90%, indicating on efficient xylem transport of the fungicide in tomato leaves.

Systemic translocation from leaves of intact plants. When applied to a single leaf of a tomato plant, DMM failed to protect other leaves from late blight disease. Eight-leaf tomato plants were treated on a single leaf (number 2 through 8) with 50 droplets of DMM (2,000 µg/ml) and 2 days later challenged inoculated with sporangia of *P. infestans* on all leaves. A week later all leaves of the plant, except the treated leaf, were severely blighted, suggesting that DMM failed to translocate from one leaf to another in either acropetal or basipetal direction. Results were similar in cucumber plants. Two-leaf plants were sprayed with DMM on either leaf 1 (from stem base) or leaf 2 and challenged with *P. cubensis* (1,000 sporangia/ml) 2 days later. After 1 week, disease severity was recorded in leaves 1 and 2. Treated leaves were fully protected against the mildew at all concentration (500 to 2,000 µg/ml) used, but the untreated leaves were as mildewed as those in inoculated control plants.

TABLE 1. Eradicative activity of two formulations of dimethomorph (DMM) (25% wettable powder [WP] and 15% dispersive concentrate [DC]) against *Phytophthora infestans* in potato and *Pseudoperonospora cubensis* in cucumber[a]

	Percent inhibition of sporulation (± SE)[c]			
	P. infestans[d]		*P. cubensis*[e]	
DMM[b]	25 WP	15 DC	25 WP	15 DC
125	40 ± 2	23 ± 1	55 ± 41	87 ± 18
500	52 ± 7	35 ± 4	94 ± 6	100
2,000	81 ± 14	71 ± 8	98 ± 4	100

[a] Plants were sprayed with DMM 7 days after inoculation when lesions covered about two-thirds of the leaflet (leaf) area.
[b] Concentration is ppm a.i.
[c] Relative to sporulation of nontreated plants.
[d] Randomly sampled leaflets.
[e] Leaf 2 from stem base in two-leaf plants.

When locally applied onto the vein junction of a single leaf of cucumber or a single leaflet of tomato DMM protected against *P. cubensis* and *P. infestans*, respectively. A single 20-µl droplet of DMM (2,000 µg/ml) reduced disease severity, compared with an untreated-inoculated leaf, by 76% in both pathosystems, suggesting that DMM may be taken up and translocated within a leaf (or leaflet). In both systems, however, adjacent leaflets or leaves were unprotected.

Systemic translocation from roots. Five-leaf tomato plants growing in 100-ml pots were drenched with 1.0 ml of DMM suspension containing 0.1 to 2 mg a.i. and inoculated with *P. infestans* (MR1, 3,000 sporangia/ml) 2 days later. Four days after inoculation, control-challenged plants were fully blighted. In contrast, the treated plants (all concentrations) were totally free of blight symptoms. The results indicate that tomato roots may take DMM and translocate it to the foliage. This, however, was not the case with cucumbers drenched with DMM and inoculated with *P. cubensis*. In an experiment similar to the one above, we observed that all plants treated by a soil drench were not protected, suggesting no uptake of DMM by cucumber roots.

Systemic translocation from the stem surface. DMM paste in lanolin (25% a.i.) was gently applied, with the aid of a wooden stick, to a petiole, hypocotyl, or a stem of a plant. About 20 mg of paste was applied to a petiole, and 50 mg to a hypocotyl or stem. Two days after application, plants were challenge inoculated with their respective pathogen. We observed that DMM-lanolin paste applied to a petiole of tomato or cucumber fully protected the leaf blade attached to this petiole against *P. infestans* or *P. cubensis*, respectively, but not the leaves above or below the treated leaf. DMM-lanolin paste applied to the first internode of the stem of five-leaf tomato plants fully protected all leaves against late blight except the cotyledons. A similar experiment conducted with cucumber plants with two leaves inoculated with *P. cubensis* gave similar results. However, in both tomato and cucumber, DMM-lanolin paste failed to protect the true leaves against disease if applied to the hypocotyl. Microscopical observations made with longitudinal sections taken from the hypocotyl-stem region of tomato plants revealed that the abaxial xylem (facing the epidermis) of the hypocotyl grows into the cotyledons but not into the stem. Rather, xylem strands coming from the root cylinder grow into the stem. Thus, the lack of protection results from a disconnection between the abaxial xylem strands of the hypocotyl and the stem.

Tenacity. DMM was found to efficiently resist washing from potato foliage. Plants were sprayed with a 500 µg/ml suspension of DMM on adaxial leaf surfaces and washed with excessive water at various time intervals after spray (1 to 48 h). At 50 h after spraying, plants were challenged with *P. infestans* (MR1, 2,500 sporangia/ml). A week later all treated plants were free of disease symptoms whereas untreated plants showed severe blight symptoms (82%).

DISCUSSION

DMM is a cinnamic acid–derivative fungicide active as foliar spray or seed dressing against fungal plant pathogens of the oomycetes (excluding *Pythium*) under greenhouse or field conditions (1,11,14,16). In this paper we report on the activity of DMM in controlling late blight in potato and tomato as well as downy mildews in cucurbits and tobacco. Special emphasis was given to the curative and eradicative activities of DMM.

DMM had an excellent prophylactic activity against late blight in potato and tomato and downy mildew in cucumber and melon. Its activity against blue mold of tobacco was somewhat lower than that against the former diseases, probably because *P. infestans* and *P. cubensis* produce zoospores and *P. tabacina* does not. The primary mode of action of DMM is not yet known but interference with cell wall assembly was implicated (2,10,15).

Judging from our results we assume that DMM acts on cell-wall biosynthesis because it had no effect on zoospore discharge but prevented zoospores from producing cell walls and consequently a germ tube. It also prevented direct sporangial germination and mycelial growth in vitro, probably for the same reason. Our data show that DMM has translaminar activity, local systemic translocation, and a strong resistance to washing. The ability of DMM to stop fungal colonization (symptoms) when applied 1 day postinfection was dependent on formulation and pathosystem: the 15DC formulation was more effective than the 25WP formulation in the potato–late blight system whereas the opposite was true in cucumber-downy mildew. Nevertheless, in both pathosystems fungal sporulation was strongly inhibited. Inhibition of sporangial formation was also observed when DMM was applied 7 days postinoculation to normally developed lesions just before sporangiophores were induced, by high humidity, to emerge from stomata. In both pathosystems spore production was more sensitive than symptom development to postinfectional application of DMM, suggesting that sporangial wall synthesis is more sensitive to DMM than is mycelial wall synthesis in planta. In cucumbers we observed that the haustoria of *P. cubensis* were heavily encased with callose as a result of the DMM treatment. Such encasement is characteristic of muskmelon genotypes resistant to downy mildew (6).

DMM translocated readily in the xylem but not in the phloem. We reached this conclusion from leaf petiole uptake studies and lanolin-paste application. In the former case excellent protection of detached leaves was observed. In the second case protection was evident only in leaves or leaflets distal to the point of application (above) but not at the proximal position to that point (below). The lack of phloem mobility is also evident from the fact that DMM applied to one leaf did not protect any other leaf from

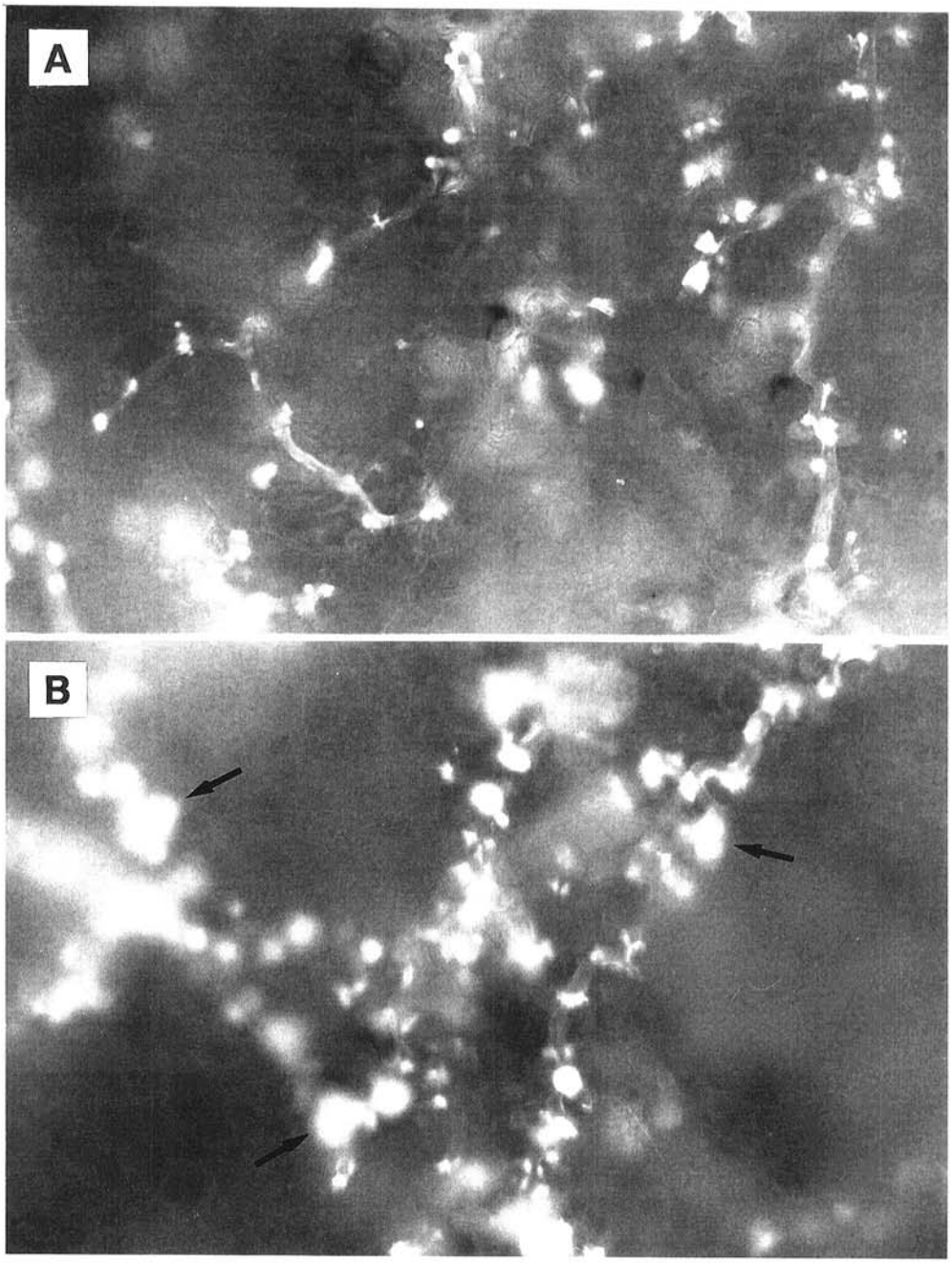

Fig. 8. UV micrographs of basic aniline blue-stained cucumber leaf disks infected with *Pseudoperonospora cubensis*. **A,** infected, not treated. **B,** treated with 125 ppm dimethomorph (DMM) (15DC [dispersive concentrate]) 7 days after inoculation. Note the heavily fluorescing callose encasements of haustoria (arrows). 270×. Figure magnification calculated for full page width. Actual magnification of prints as they are is 400×.

disease.

The systemic translocation of DMM from roots to foliage subsequent to soil drench was different for tomato and cucumber. While in tomato a soil drench effectively controlled *P. infestans*, in cucumber no control of *P. cubensis* was observed. Since both tomato and cucumber allowed for a systemic movement of DMM from stem to foliage, we concluded that roots of cucumber are probably impermeable to the fungicide.

Most importantly, DMM controlled late blight and cucurbit downy mildew whether produced by phenylamide-sensitive or -resistant fungal field isolates, thus making it a possible candidate to replace phenylamide fungicides in growing areas where they are ineffective due to the appearance of resistant subpopulations (5,12,13). Although DMM-resistant mutants could not be produced in the laboratory (3), DMM application in the field should be done with care to avoid possible build up of resistant strains.

DMM is an effective prophylactic, curative, and eradicant fungicide active against oomycete fungal pathogens regardless of their sensitivity to phenylamide fungicides. Its activity relates to cell wall biosynthesis. It has limited systemic movement in the plant but quite prolonged residual activity.

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