The Systemic Antifungal Activity of Ridomil against *Phytophthora infestans* on Tomato Plants

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ABSTRACT

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Ridomil® (DL-methyl N-[2,6-dimethylphenyl]-N-[2-methoxyacetyl] alaninate), a new systemic fungicide effective against plant pathogenic species of the Peronosporales, effectively controlled *Phytophthora infestans* on tomato plants. A single soil drench containing 0.25 mg a.i. applied to a 7-leaf plant in a 1 L pot was sufficient to protect plants against blight. Protection of potted plants treated by soil drench was observed within 1 hr from time of fungicide application at 25 C. The fungicide was slightly toxic to sporangia and zoospores in vitro. Penetration and initial establishment of *P. infestans* in leaves and fruits was not inhibited by the fungicide. Instead, minute, restricted, sterile lesions were produced, even if the compound was applied 2–3 days after inoculation. To achieve the most efficient control of blight, plants must be treated with the fungicide either before, or within the first 2 days after, inoculation.

Additional key words: Lycopersicon esculentum.

Ridomil® is a new systemic fungicide (CGA 48988, DL-methyl N-[2,6-dimethylphenyl]-N-[2-methoxyacetyl] alaninate) that recently has become available to experimenters investigating the control of plant pathogenic Phycomycetes. The fungicide was reported to be a more effective protectant than any other fungicide tested, and to have curative controlling effects against downy mildews (2,3,6). The mechanism of its action is not known.

The experiments reported here were undertaken to determine the influence of Ridomil on the various stages of pathogenesis of *Phytophthora infestans* d By. on tomato plants and to develop a better understanding of the nature of its antifungal activity. A preliminary report on this study was published (1).

MATERIALS AND METHODS

Plants and pathogen. One or more of the following four tomato (*Lycopersicon esculentum* Mill.) cultivars were used in each experiment: Rehovot 13, Hosen Ayalon, Orit, and Naáma. Plants were grown in the greenhouse (18–30 C) in 0.5, 1, 2, or 10 L plastic pots (one plant per pot) filled with about 0.4, 0.8, 1.7, and 9 kg, respectively, of an air-dried potting mixture (garden soil: compost: vermiculite; 3:1:1, v/v). Plants were watered daily with about 20% of the pot volume, and irrigated with Hoagland's solution once a week.

A local, unclassified race of *P. infestans*, collected from a tomato field in November 1977, was used in this study. The pathogen was grown on young tomato plants in the laboratory. Freshly produced sporangia collected from infected leaves were used for inoculation.

Fungal germination. A suspension of sporangia was mixed with Ridomil to a final concentration of 0, 5, 25, 50, 125, and 250 μg/ml, incubated at 2 C for 1 hr, and then at 15 C. Zoospore release was examined at 3 hr and zoospore germination at 20 hr.

Host inoculation and assessment of disease development. Whatman No. 1 filter paper disks 9 mm in diameter were saturated with about 0.2 ml of sporangial suspension of the fungus (about 5×10^4 sporangia per milliliter, unless otherwise stated) and placed in the center of the lower leaf surface of each leaflet, one disk per leaflet. Inoculation of fruits (attached or detached) was done either by spraying them with sporangial suspension or by inserting a filter paper disk saturated with sporangial suspension beneath the calyx. Inoculated plants were incubated in a dew chamber at 18 C for 20 hr in the dark, and then transferred to a 20 C cabinet (50–60% RH, illuminated at 12 hr/day with cool-white fluorescent light of about 150 μEinsteins $m^{-2}sec^{-1}$ [$\cong$ 4,100 lux]) for disease developmment. Usually, four replicate plants were used in each treatment.

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Disease production was evaluated by measuring lesion diameter (the largest was 4 cm, the leaflet width). To measure the effect of the fungicide on sporulation, infected leaves were detached from treated plants and placed in a moist chamber for 24 hr in the dark (20 C). Individual leaflets were transferred to a known volume of a fixative solution, and sporangia were counted with the aid of a cytometer (four counts per leaflet).

To study the movement of Ridomil from roots into leaves, the following modified inoculation technique was adopted: leaflets were excised from the experimental plants, placed adaxial surface upward on moist filter paper in plastic trays, and inoculated by the filter paper disk method, as above. Trays were covered with moist transparent plastic bags and incubated in a 20 C cabinet for 1 wk. Disease development was determined according to the diameter of the sporulating lesion.

Fungicide and fungicide application. Ridomil was applied to tomato plants either in inoculum suspensions, as a single soil drench, or in a single foliar spray.

Most tests were conducted with a wettable powder formulation containing 50% active ingredient (a.i.) but limited studies were conducted with a 25% (a.i.) formulation. Fungicide concentrations always were based on a.i. equivalents.

Unless otherwise stated, plants treated with the fungicide were kept in the greenhouse until inoculation. Most experiments were conducted with 6- to 7-leaf plants growing in 0.5- or 1-L pots. For some experiments plants were grown to flowering, or until fruit set in 2- or 10-L pots. All experiments were conducted at least twice with four or more replicates per treatment.

TABLE 1. The effect of various combinations of volume and concentration of Ridomil soil drenches on infection of leaves of tomato plants inoculated with *Phytophthora infestans*.

Ridomil concentration (μg a.i./ml)	Lesion diameters[a] (cm) at 7 days after inoculation[b] of plants in 0.5-L pots of soil treated with four different volumes of the indicated concentrations of the fungicide			
	5 ml	10 ml	15 ml	20 ml
0	3.7 ± 0.5	3.7 ± 0.5	3.7 ± 0.5	3.7 ± 0.5
5	3.5 ± 0	3.2 ± 0.6	2.0 ± 0.5	1.8 ± 0.3
25	1.3 ± 0.3	1.3 ± 0.3	1.0 ± 0	0.9 ± 0.1
50	0.8 ± 0.3	0.9 ± 0.1	0.8 ± 0.3	0.8 ± 0.3
250	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0	0.6 ± 0.4
500	0.8 ± 0.4	0.8 ± 0	0.5 ± 0	0.4 ± 0.2

[a]Means and standard deviations of means.
[b]Intact leaflets were inoculated with about 0.2 ml of sporangial suspension (about 5×10^4 sporangia per milliliter) per leaflet using the filter paper disk method.

RESULTS

The effect of Ridomil on fungal germination. Percentage of zoosporangia releasing zoospores (normally ranged between 50–80%) was unaffected by Ridomil up to 25 μg/ml. At >50 μg/ml, zoospore release was reduced by about 80%. Zoospore motility was normal at 5 μg/ml, but slowed down at 25 μg/ml. No motility was observed at > 50 μg/ml. At > 125 μg/ml, zoospores were mostly ruptured.

In water, all zoospores germinated and produced a germ tube of 20–50 μm in length. In the presence of 5 or 25 μg Ridomil per milliliter full germination occurred but germ tube was reduced to 10–15 μm. At 50 μg/ml, only 50% of the zoospores germinated. These germ tubes were 5–10 μm in length. At 100 μg/ml, zoospores did not germinate.

The effect of Ridomil on infection. *Inoculum suspensions.* Sporangial suspensions (5×10^4 sporangia per milliliter) were mixed with Ridomil to various final concentrations and used for inoculation. Infectivity was evaluated according to lesion diameter measured at various intervals after inoculation. The fungicide concentration needed to reduce lesion size on the 7th day by about 50% as against water control, was 50 μg/ml. A concentration of 250 μg/ml was needed to reduce lesion size by about 90% (Fig. 1). At 1,000 μg Ridomil per milliliter, no disease developed.

Soil drenches. A single amount of Ridomil, in various combinations of volume and concentration, was poured onto the soil surface of potted tomato plants (cv. Rehovot 13, four-leaf plants growing in 0.5-L pots). Plants were inoculated at 11 days after drenching using the filter paper disk method. An amount of 250–500 μg of Ridomil was found to protect plants against the blight (Table 1). Protection was recognized by the appearance of a small (4–8 mm in diameter), restricted lesion on the inoculated target (Fig. 2), which produced no sporangia upon transfer to a moist chamber. The fungicide also protected plants exposed to high inoculum doses of the pathogen (Table 2). However, larger lesions (6–10 mm), although restricted and sterile, were produced as a result of inoculation with a large number of sporangia.

The persistance of the systemic antifungal activity of Ridomil was investigated using tomato plants (cv. Orit) at the four-leaf

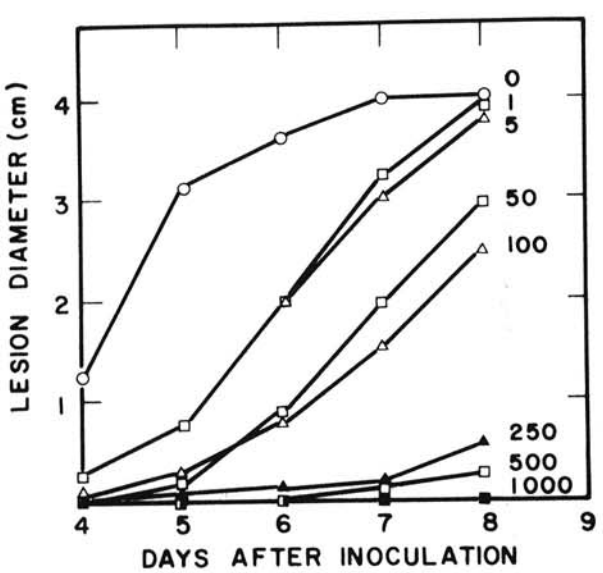

Fig. 1. Effects of Ridomil mixed in sporangial *Phytophthora infestans* inoculum on diameters of lesions caused on tomato leaves. Figures on curves indicate final concentration (μg/ml) of Ridomil. Standard deviations of means for lesion diameter (cm) ranges of 0.0–0.5, 0.6–1.0, 1.1–2.0, 2.1–3.0, and 3.1–4.0 were 0.1, 0.4, 0.6, 0.6, and 0.4, respectively.

Fig. 2. Effects of Ridomil, soil drench applied to potted plants, on the development of late blight of tomatoes. Right: inoculated, no Ridomil. Left: inoculated, 1 mg Ridomil per plant. Middle: noninoculated, 1 mg Ridomil per plant. Inoculation was at 11 days after Ridomil application. Pictures were taken at 5 days after inoculation.

stage. The soil containing the plants was drenched with either 0, 1, or 5 mg of the fungicide per 0.5-L pot and plants were transplanted, with original soil attached, to 10-L pots at 15 days after treatment. Inoculations were made at 15, 42, and 51 days after drenching when plants had reached the 7, 14, and 18-leaf stage, respectively. Lesion sizes in plants treated with 1 mg Ridomil and inoculated at 15, 42, and 51 days after treatment, was suppressed by about 86, 33, and 10%, respectively, in comparison with control inoculated plants. The lesion size of plants treated with 5 mg of the compound was suppressed by 87, 58, and 50% upon inoculation at 15, 42, and 51 days after fungicide application, respectively.

Foliar spray. Aboveground parts (leaves, petioles, and stems) of six-leaf plants (cv. Orit) were sprayed with Ridomil ($\sim$ 10 ml per plant). Intact leaves of sprayed and unsprayed plants were inoculated at various intervals after treatment. Plants developed about two leaves per week and set fruits at about 35 days after spraying. Foliar sprays at concentrations of 50, 250, 500, and 1,000 μg/ml were found to protect the plants from the disease for a period of 6, 14, 34, and $>$ 44 days, respectively. Fruits of plants sprayed with 500 or 1,000 μg/ml were not protected even though the leaves were protected from blight by the chemical.

The effect of Ridomil on lesion appearance and lesion expansion. Two experiments were conducted to study the effect of Ridomil on lesion appearance in already inoculated plants. In the first, four-leaf plants (cv. Hosen Ayalon) in 0.5-L pots, were inoculated with about 4×10^4 sporangia per milliliter and drenched with 0.5 mg Ridomil per pot at 0, 2, 3, 5, and 6 days after inoculation.

In the second, six-leaf plants growing in 1-L pots were inoculated with about 1×10^5 sporangia per milliliter and drenched with 1 mg Ridomil per pot at 0, 1, 2, 3-⅓, 4, and 6 days after inoculation. Lesions first appeared at about 60 hr after inoculation with the high-inoculum dose, and at about 90 hr with the low-inoculum dose. Final lesion measurements were taken on the 6th day after inoculation (Fig. 3). Ridomil prevented normal lesion production, even if applied at 2 (with the high-inoculum dose) or 3 (with the low-inoculum dose) days after inoculation. Ridomil had only a slight effect if applied to plants with lesions developed to 1 cm or more.

In another series of experiments, plants (cv. Orit, at the 7-leaf stage in 2-L pots), were treated with the fungicide 72 hr after inoculation when lesions $\sim$0.7 cm in diameter were observed. The compound was applied at 50, 250, or 500 μg/ml either as a soil drench (75 ml/plant) or as a foliar spray (10 ml/plant). Lesion expansion was followed at daily intervals. Expansion of lesions (as

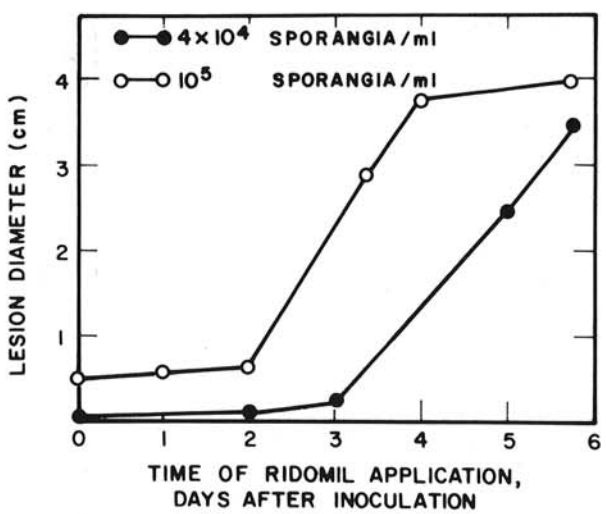

Fig. 3. The inhibitory effect of Ridomil on the development of lesions caused by *Phytophthora infestans* on tomato leaves at two inoculum dosages. Fungicide applied as soil drench (0.5, and 1.0 mg Ridomil per plant at low and high inoculum dose, respectively) at various intervals after inoculation. Data were recorded on the 6th day after inoculation. Standard deviations of means for lesion size (cm) of 0.0–0.5, 0.5–1.0, 2.1–3.0, and 3.1–4.0 were 0.2, 0.1, 0.6, and 0.1, respectively.

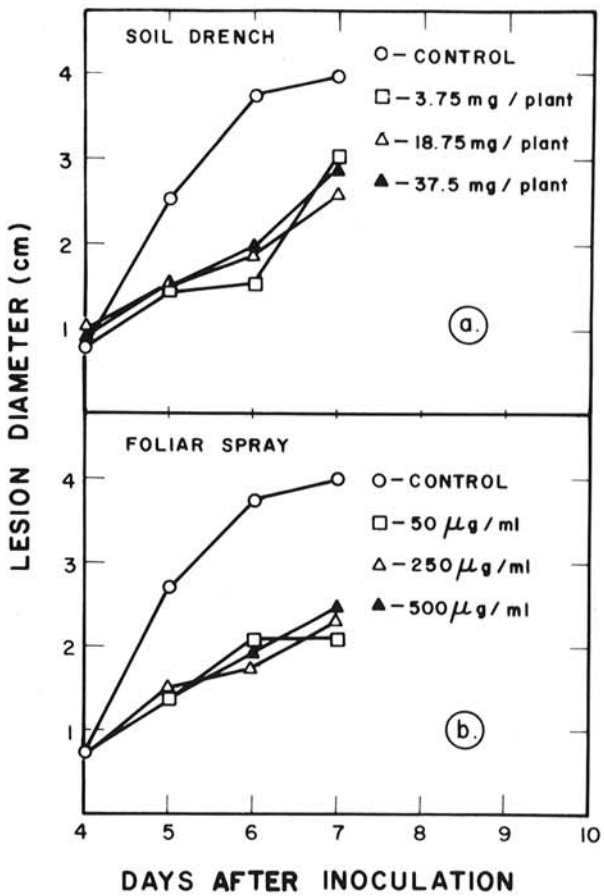

Fig. 4. The reduced expansion of lesions caused by *Phytophthora infestans* on tomato leaves as a result of Ridomil application 3 days after inoculation when lesions were $\sim$0.7 cm in diameter. Standard deviations of means for lesion size (cm) of 0.6–1.0, 1.1–2.0, 2.1–3.0, and 3.1–4.0 were, for soil drench: 0.2, 0.2, 0.4, and 0.1, respectively, and for foliar spray: 0, 0.3, 0.3, and 0.1, respectively.

TABLE 2. The effect of Ridomil soil drench on sizes of lesions caused by *Phytophthora infestans* on tomato plants inoculated with increasing doses of inoculum

Ridomil[a] (mg, a.i.)	Lesion diameters[b](cm) on leaves of tomato plants inoculated[c] with:								
	40,000 sporangia/ml			75,000 sporangia/ml			150,000 sporangia/ml		
	4 days	6 days	8 days	4 days	6 days	8 days	4 days	6 days	8 days
0	0.5±0.2	2.5±0.5	3.3±0.3	1.2±0.3	3.0±0	4.0±0	1.7±0.3	3.3±0.3	3.8±0.3
5	0.1±0.1	0.6±0.1	0.7±0.1	0.2±0.1	0.7±0.1	0.8±0.1	0.7±0.1	1.0±0	1.0±0.1
10	0.1±0	0.4±0.1	0.7±0.2	0.2±0.2	0.6±0.2	0.8±0.2	0.6±0.1	1.0±0.1	1.0±0

[a]Each rate of Ridomil applied to four single tomato plants each growing in a 1-L pot.
[b]Means and standard deviation of means.
[c]Inoculation was done as described in footnote "b" of Table 1, except the inocula were of three different concentrations.

measured on the 6th day after inoculation) was retarded by about 50% in Ridomil-treated plants, regardless of fungicide concentration or method of application (Fig. 4).

The effect of Ridomil on sporulation. Normally-infected 7-leaf plants (cv. Hosen Ayalon, in 1-L pots) were drenched or sprayed with Ridomil in order to examine its effect on production of sporangia by the pathogen. Plants were inoculated with the filter paper disk method and fungicide was applied on the 6th day after inoculation when lesions diameters were ~ 2.5 cm. Terminal leaflets were detached from the treated plants (16 leaflets, four per plant) at 4, 9 (in soil drench only), and 30 hr after Ridomil application and placed in moist chambers for sporulation (48 hr in darkness at 20 C).

The inhibitory effect of the fungicide increased by lengthening the interval between application and onset of the dew period (Fig. 5). With an interval of 4 hr, foliar sprays (Fig. 5b) seemed to be more suppressive to the production of sporangia than were soil drenches (Fig. 5a), especially with low doses of Ridomil.

In another experiment, infected 20-leaf plants (cv. Naáma, 80 cm high) growing in 10-L pots, were drenched with 10 mg Ridomil per pot on the 6th day after inoculation, when lesions reached ~ 2.5 cm in diameter. Leaflets were taken for sporulation at 24 hr after application. In this case, sporangial yield per leaflet was reduced by 56% compared to untreated plants.

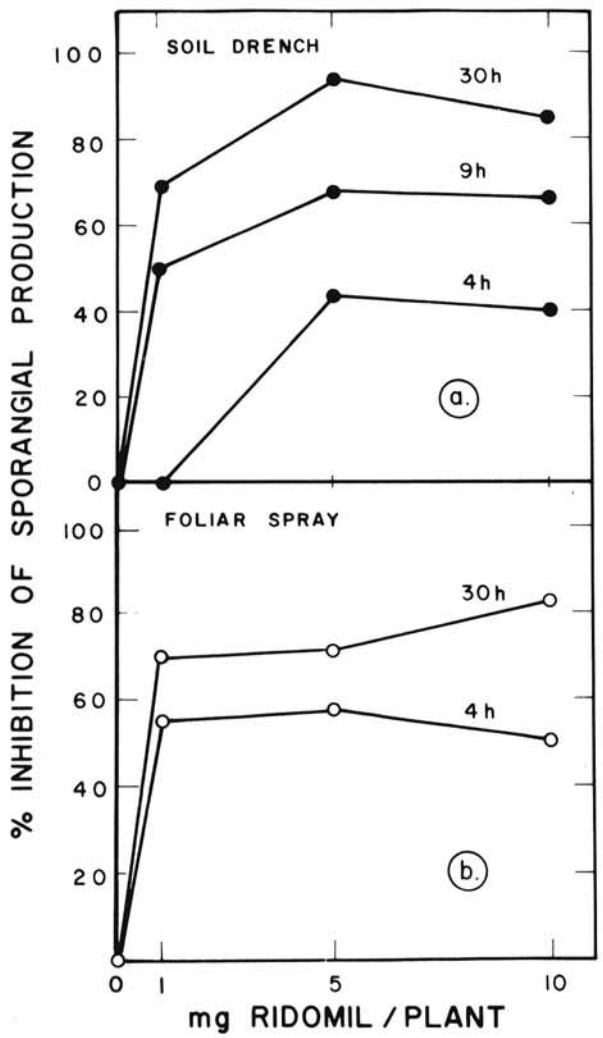

Fig. 5. The effect of Ridomil on production of sporangia by *Phytophthora infestans* on preformed normally-developed lesions. Leaflets were excised for sporulation at 4, 9 (for soil drench only), and 30 hr after fungicidal application. Average yield of sporangia per leaflet in control untreated plants was 96,500 ± 30,500. Standard deviations of means for grouped soil drench treatments were 27, 28, and 11% for 4, 9, and 30 hr, respectively. Standard deviations of means for grouped foliar spray treatments were 26 and 12% for 4 and 30 hr, respectively.

Movement of the systemic antifungal activity of Ridomil. A 100-ml aliquot of Ridomil suspensions of various concentrations was poured onto the soil surface of 7-leaf tomato plants growing in 2-L pots. Plants were kept at either 15, 20, or 25 C in the light. Terminal leaflets were detached at various periods after fungicide application and inoculated. Disease records were taken 1 wk after inoculation. The time required for leaves to be fully protected from the blight depended upon both temperature and fungicide dose. At 17.5 mg Ridomil per plant, full protection was attained within 1 and 2 hr at 25 and 20 C, respectively. At 15 C, only partial protection was observed at 4 hr. A reduced fungicide dose (8.75 mg/plant) resulted in poor protection after 1 hr, at 25 C.

To examine the translocation of Ridomil into tomato fruits, potted plants at the 18-leaf stage with 2 fruits (3–4 cm in diameter) on each were drenched with 17.5 mg Ridomil per plant and inoculated by spraying to runoff with sporangial suspension 24 hr after drenching. Ridomil-treated plants were fully protected against blight; small (1–2 mm diameter) sterile lesions developed on both leaves and fruits, while extensive blight of the leaves and brown dry rot of the fruits developed on the untreated plants.

In another experiment, the translocation of the fungicide from leaves sprayed with up to 10 mg/plant to nonsprayed leaves was examined. For this purpose, leaflets were sprayed on both adaxial and abaxial surfaces. Special care was taken to avoid any contact of the chemical with petioles, stems, or roots. Systemic translocation occurred neither upward from lower leaves, or downward from upper ones, nor from sprayed leaves into fruits.

DISCUSSION

Ridomil is an effective systemic fungicide against the late blight disease of tomato caused by *Phytophthora infestans*. A single application of this fungicide to young, potted tomato plants, either as soil drench or foliar spray, protected them from blight for at least 6 wk.

The fungicide has been reported to translocate acropetally in tomato plants (4), and basipetal translocation was observed in avocado (7). In our study, a rapid movement (within 1 hr) of the fungicide (or its derivatives) from roots to foliage was observed, but none from one leaf to another. The systemic protection achieved by spraying the aboveground parts of the plant seems to result from uptake through stem and petioles (4).

Ridomil was slightly toxic to sporangia and zoospores of *P. infestans,* but when mixed with fungal inoculum it did not prevent penetration into the leaf tissue, unless applied at high concentrations (Fig. 1). Staub et al (5) similarly observed that penetration and initiation of the first haustorium of *P. infestans* in tomato, and of *Plasmopara viticola* in grape, were the same on Ridomil-treated and untreated leaves.

The fungicide inhibited disease development even when applied only 24 hr before lesions otherwise would appear (Fig. 3), but exhibited only partial control of expansion of normal lesions (> 7 mm in diameter, Fig. 4a,b). This is unexplainable, especially considering the effective in vitro control of mycelial growth of *P. infestans* in culture (ED_{50} = 0.5 ppm; Schwinn, *personal communication*) and further investigation would be valuable. Also, the fact that Ridomil enabled the fungus to produce some sporangia on normal lesions, even when present at a high concentration (Fig. 5), would decrease the controlling effect of the fungicide if applied after lesion appearance.

It may be concluded, therefore, that Ridomil would be most effective if applied before inoculation or on the first 2 days after inoculation, before symptoms appear.

Its fungicidal action (2,3,6,7), together with a rapid uptake and prolonged period of effectiveness, makes Ridomil a promising compound for chemical control of plant pathogenic species of the Peronosporales.

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