Effect of Protectant Fungicides on the Developmental Stages of *Phytophthora infestans* in Potato Foliage

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Supported in part by USDA-CSRS Grant #701-15-54.
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Accepted for publication 26 June 1980.

ABSTRACT

Bruck, R. I., Fry, W. E., Apple, A. E., and Mundt, C. C. 1981. Effect of protectant fungicides on the developmental stages of *Phytophthora infestans* in potato foliage. Phytopathology 71:164-166.

Two protectant fungicides, mancozeb and chlorothalonil, inhibited the development of in vitro and in vivo *Phytophthora infestans*. Germination of zoospores or sporangia was inhibited by low concentrations of either fungicide. Following fungicide application on infected leaves, lesion expansion and fungal sporulation were suppressed. The timing of fungicide application in relation to the stage of late blight development had a significant influence on fungicide efficacy. The most important effects of these fungicides were inhibition of spore germination and suppression of the viability of sporangia produced from treated foliage.

Potato late blight induced by *Phytophthora infestans* (Mont.) d By. is potentially the greatest threat to successful cultivation of potatoes in many areas of the world. Resistance alone has not effectively controlled blight (5). Therefore, control frequently is accomplished by the routine application of protectant fungicides, which must be applied before infection for effective control (7). Mistiming of sprays may have serious consequences. Mancozeb first applied when 0.5% of the potato foliage in small field plots had visible symptoms of late blight, did not slow the rate of epidemic development sufficiently to provide adequate control (6). Delaying the application of fentin hydroxide until late blight symptoms appeared resulted in reduced yield and increased tuber blight (8). Although the major epidemiological effect of protectant fungicides is to reduce the proportion of spores that successfully penetrate into host tissues, a protectant may affect the pathogen through other modes of action. For example, ethylenebisdithiocarbamate fungicides may reduce sporulation in addition to reducing infection efficiency of spores (11). These fungicides reduce the sporulation of *Cochliobolus sativus* on wheat (3) and *Venturia inequalis* on apple foliage (10). When applied to potato foliage, maneb reduced sporulation of *P. infestans* to $<10\%$ that on unsprayed leaves (7). Less is known about chlorothalonil, another broad-spectrum protectant fungicide. However, Lukens and Ou (9) observed that chlorothalonil suppressed appressorial development of and lesion formation caused by *Alternaria solani* on tomato.

Mancozeb and chlorothalonil are presently the major fungicides used for late blight control in the northeastern USA. In order to develop an accurate model of fungicide influence on late blight epidemics, the effects of these fungicides on spore germination, infection efficiency, sporulation, and resultant viability of *P. infestans* sporangia or zoospores need to be more precisely quantified than has been done previously. This paper reports those effects.

MATERIALS AND METHODS

Fungal and plant culture. *P. infestans* race 1,2,3,4 obtained from H. D. Thurston, Cornell University, was reisolated periodically from potato foliage and maintained on amended lima bean agar (ALBA) (1). Cultures were grown in the dark at 18 C. Inoculum was prepared by washing sporangia from ALBA in 9-cm-diameter plastic petri dishes with 20 ml of a 1% glucose solution. This sporangial suspension was mixed with an equal volume of ice and incubated at 1 C for 2 hr. The remaining ice was removed and the suspension equilibrated to 21–24 C. Zoospores were liberated within 2 hr.

Solanum tuberosum L. 'Norchip' plants were grown in the greenhouse (16–26 C) in clay pots (approximate volume, 1,040 cm^3), containing peat-vermiculite mixture (1:1, v/v) with 0.3 kg each of N, P, and K per cubic yard of mixture. For all experiments the plants were 28–38 days old, and at the 4- to 8-leaf stage.

Effects of mancozeb and chlorothalonil on spore germination in vitro. A suspension of either sporangia or zoospores was agitated for 20 sec on a Vortex "Genie" (Scientific Industries Inc., Bohemia, NY 11716) with an appropriate concentration of mancozeb (Manzate 200®, 80 WP) or chlorothalonil (Bravo®, 75 WP). This mixture was poured on a 9-cm-diameter petri dish containing 20 ml of solidified ALBA. The mixture was distributed evenly and the excess liquid was poured off. Plates were incubated at 23 C until at least 90% of the sporangia or zoospores not treated with the fungicides had germinated (usually within 24 hr). Germination of at least 200 sporangia or zoospores was determined. All treatments were repeated twice and each experiment was replicated at least twice.

Effects of mancozeb and chlorothalonil on spore germination in vivo. Potted plants were sprayed to runoff with the desired concentration of mancozeb or chlorothalonil and then allowed to dry for 1–2 hr. A suspension of zoospores or sporangia, 5,000 or 10,000/ml, respectively, was sprayed on the plant until run-off. The plants were placed in a 100% relative humidity (RH) chamber at 19 C for 72–96 hr. Terminal leaflets were excised from the plant and observed under a compound microscope for microscopic lesion development. Four 1-cm^2 fields per leaflet were observed, and averages were calculated for each treatment. Counts of lesions were done on at least four leaflets for each treatment, and all experiments were repeated at least twice.

Effects on sporulation and lesion development. Terminal leaflets of potted potato plants were inoculated by suspending a 10 μl drop of *P. infestans* (2,500 zoospores or 2,500–5,000 sporangia per milliliter on the abaxial surface of the leaflet at the junction of the midvein and a side vein. Inoculated plants were maintained at 100% RH and 19 C for 24 hr, and then at 50–85% RH at 16–26 C for 96 hr. Mancozeb or chlorothalonil at any of several concentrations was atomized to runoff onto both surfaces of leaves at 1, 3, or 5 days after inoculation. Five days after inoculation, terminal leaflets with well-developed lesions were placed in moist chambers at 18 C to induce sporulation. After incubation for either 24 or 48 hr, six leaf disks (7 mm in diameter) cut from the margin of the lesion where sporulation appeared to be the greatest were agitated in 1.5 ml of a 9.5% ethanol solution on a Vortex "Genie" for 20 sec to dislodge the sporangia from sporangiophores. When lesions were

0031-949X/81/02016403/$03.00/0

small and/or sporulating poorly, disks from several lesions were combined. At least two leaflets were sampled from each plant, with two plants per treatment. Each experiment was replicated at least twice.

Sporangia were counted in a haemacytometer and sporulation per unit area of lesion sampled was calculated. Sporulation for each treatment was expressed as a percentage of control (no fungicide). Analyses of variance (ANOVA) were performed on the arcsin transformed percentages.

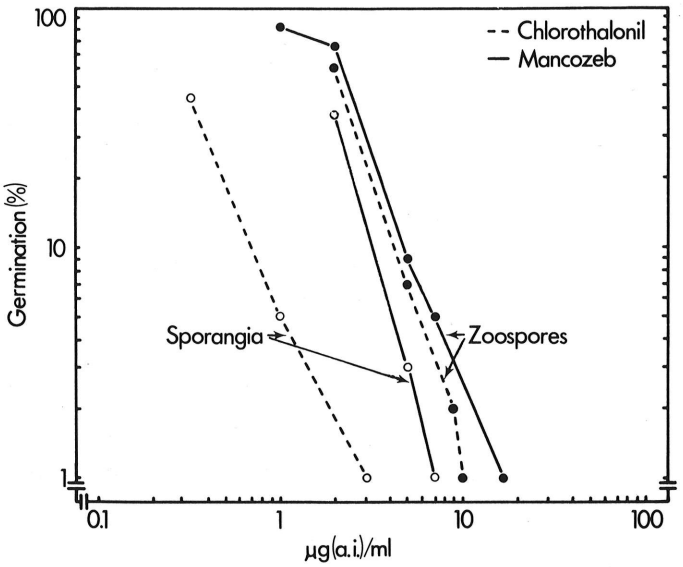

Fig. 1. Effect of chlorothalonil and mancozeb on direct in vitro germination of *Phytophthora infestans* sporangia and zoospores. Propagules were mixed with the indicated concentration of fungicide and then incubated on amended lima bean agar for 24 hr at 23 C. At least 90% of the sporangia or zoospores not treated with fungicide had germinated and germination is presented as percent of the control. Standard deviations of the mean were not normally distributed, even using the arcs in transformation. However, at germination percentages below 10%, standard deviations ranged from 0.0 to ± 2.3.

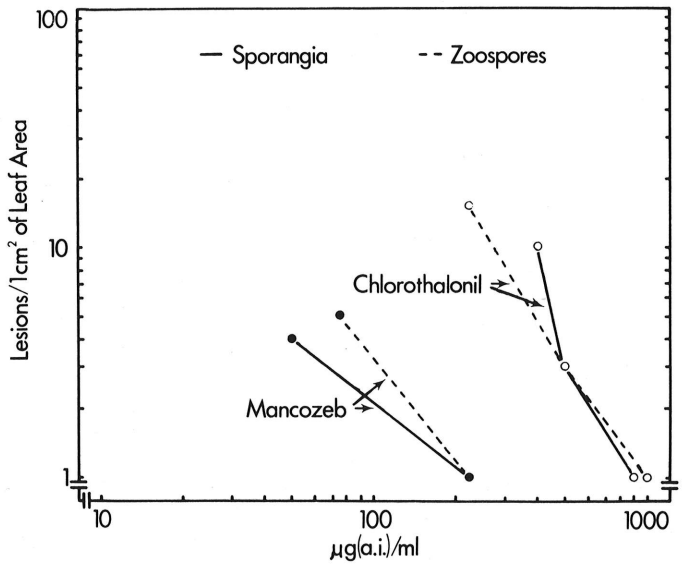

Fig. 2. The effect of chlorothalonil and mancozeb on successful development of lesions induced by *Phytophthora infestans* on potato foliage. Zoospores or sporangia were applied to foliage that had been sprayed (to run-off) with the indicated fungicide concentration. Leaves not treated with fungicide developed an average of 39.5 lesions per square centimeter of leaf area for zoospores or 36 lesions per square centimeter of leaf area for sporangia. Standard deviations of the mean for data points were not normally distributed and ranged from ± 0.2 at small numbers of lesions to ± 5.1 at larger numbers of lesions.

The effect of mancozeb and chlorothalonil on lesion expansion was investigated. The greatest width and length of individual selected lesions were measured over time on plants maintained at 24 C. Lesion area was calculated by the formula:

$$\text{Lesion area} = \pi/4 \text{ width} \times \text{length}.$$

Sporangia viability studies. Lesions bearing sporangia were agitated for 20 sec in 3 ml of an oxytetracycline dihydrate solution (35 μg/ml). The oxytetracycline dihydrate was used to suppress bacterial contamination and had a slightly inhibitory effect on

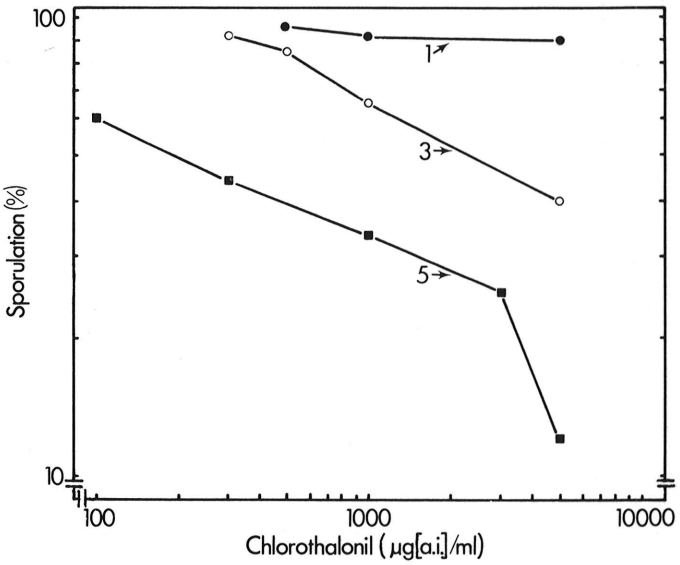

Fig. 3. Effect of chlorothalonil (applied 1, 3, or 5 days after inoculation) on sporulation of *Phytophthora infestans* from lesions in potato foliage. Samples from lesions 5 days after inoculation were placed in moist chambers for 24 or 48 hr to induce sporulation. Average sporulation from untreated lesions was 4.15×10^4 sporangia per square centimeter of leaf tissue sampled. Data are presented as percent of sporulation from control lesions. Standard deviations of the mean for data points varied from ± 0.8 to ± 11.2%.

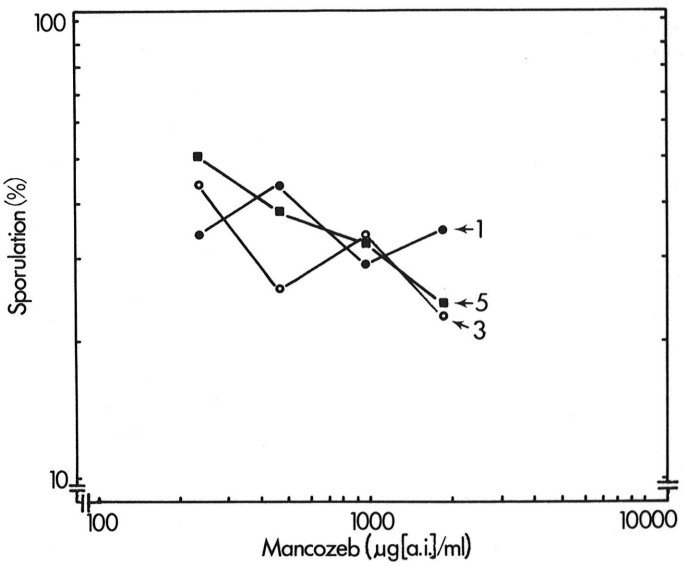

Fig. 4. Effect of mancozeb (applied 1, 3, or 5 days after inoculation) on sporulation of *Phytophthora infestans* from lesions in potato foliage. Samples from lesions 5 days after inoculation were placed in moist chambers for 48 hr to induce sporulation. Average sporulation from untreated lesions was 5.18×10^4 sporangia per square centimeter of leaf tissue sampled. Data are presented as percent of sporulation from control lesions. Standard deviations of the mean ranged from ± 1.3 to ± 15.0%.

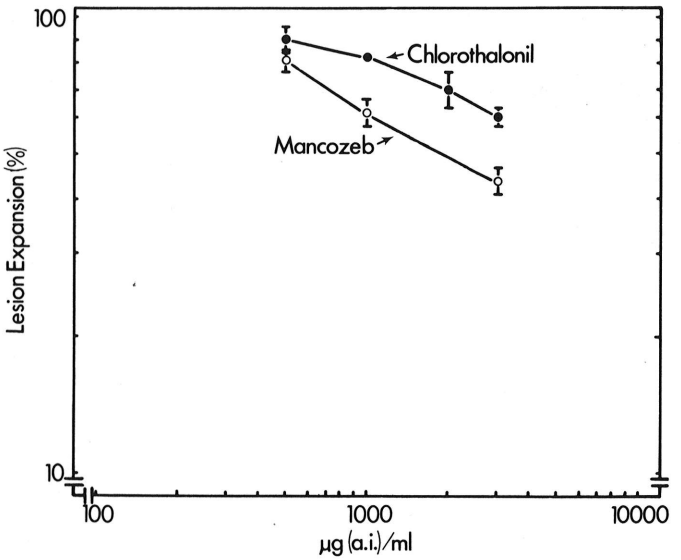

Fig. 5. Effect of chlorothalonil and mancozeb on the rate of lesion expansion of *Phytophthora infestans* in potato foliage. Lesions not treated with fungicide expanded at the rate of 3.56 cm^2/day. Data are presented as percent of control. Vertical lines indicate standard deviations of the mean.

sporangium germination (4). The sporangial suspension was distributed onto ALBA and the excess liquid was poured off. Germination was determined after 24 hr at 18 C. When possible, at least 200 sporangia per plate were counted to determine percent germination (viability).

RESULTS

Both mancozeb and chlorothalonil inhibited zoospore and sporangium germination at low concentrations in vitro (Fig. 1). Aqueous concentrations of both fungicides as low as 1 μg (a.i.) per milliliter suppressed spore germination. Zoospores were consistently more resistant to fungicide effects than were sporangia. Sporangium germination was suppressed to 1% of that of controls with 3 and 7 μg (a.i.)/ml for chlorothalonil and mancozeb, respectively. At equivalent dosages (μg/ml), chlorothalonil consistently suppressed spore germination more effectively than did mancozeb.

Penetration of *P. infestans* into potato leaves was greatly suppressed by fungicide sprays. In contrast to spore germination on ALBA, equivalent suppression of infection was effected by smaller amounts of mancozeb than of chlorothalonil (Fig. 2). In vivo, zoospores and sporangia were affected about equally by both fungicides. The average number of infection sites per square centimeter of leaf surface was reduced to ≤ 1 lesion at approximately 250 μg (a.i.) of mancozeb per milliliter and 950 μg (a.i.) of chlorothalonil per milliliter (Fig. 2). Successful infection sites expanded and eventually destroyed the leaf, regardless of fungicide dosage.

The sporulation from lesions treated with mancozeb or chlorothalonil was affected by fungicide dosage and the relative time of fungicide application (Figs. 3 and 4). Chlorothalonil applied at dosages up to 5,000 μg (a.i.) per milliliter 1 day after inoculation had little effect on sporulation. However, when applied 3 or 5 days after inoculation, chlorothalonil (5,000 μg [a.i.] per milliliter) reduced sporulation to 38 or 12% of control, respectively. Similarly, mancozeb (1,920 μg [a.i.] per milliliter) suppressed sporulation to 35, 22, or 24% of control when applied at 1, 3, or 5 days after inoculation, respectively (Fig. 4).

Mancozeb retarded lesion expansion more than did chlorothalonil; however, the difference was statistically insignificant ($P = 0.05$) (Fig. 5). Chlorothalonil reduced the rate of lesion expansion to about 64% that of the controls or to 2.17 cm^2/day when applied at 3,000 μg (a.i.) per milliliter 3 days after inoculation.

Sporangia washed from leaves sprayed with either mancozeb or chlorothalonil were affected by fungicide washed from the leaf surface, but the viability of sporangia produced on sprayed lesions was not noticeably different from that of sporangia from unsprayed lesions. Germination of sporangia washed from lesions sprayed with chlorothalonil (3,000 μg [a.i.] per milliliter) at 3 or 5 days after inoculation was suppressed to 33 and 25% of control, respectively. Germination of sporangia from lesions sprayed with mancozeb (2,200 μg [a.i.] per milliliter) at 3 days after inoculation was suppressed to 24% that of the controls. However, for both fungicides, the germination of sporangia from untreated lesions exposed to washings from sprayed, nonsporulating lesions was similarly reduced.

DISCUSSION

Chlorothalonil and mancozeb had their greatest effect on spore germination, but they also retarded lesion expansion and pathogen sporulation from treated lesions. Very low levels of mancozeb or chlorothalonil suppressed in vitro spore germination to less than 1% of controls. Much higher concentrations of the fungicides reduced the in vivo inoculum efficiency and sporulation. Lesion expansion was less affected by fungicide application.

These data confirm our expectation that mancozeb and chlorothalonil have small effects on *P. infestans* mycelium in leaf tissue relative to their large effects on inhibition of sporangia and zoospore germination. Thus, our data are consistent with the observation that weekly applications of mancozeb retarded established epidemics of potato late blight only after a delay of 8–10 days after applications were begun (6). In contrast, metalaxyl (Ridomil®), an acylalanine-based fungicide which did not affect germination of this isolate of *P. infestans* spores but which very markedly suppressed lesion expansion, sporulation, and sporangial viability (1), retarded an epidemic of potato late blight within 2 days (6). In experiments similar to those described here, metalaxyl (100 μg [a.i.] per milliliter) applied 3 days after inoculation retarded lesion expansion to 23% that of the controls (unsprayed infected leaves), suppressed sporulation to 8% that of the controls, and prevented sporangia from sprayed leaves from germinating (1).

These data are necessary in predicting the effect of mancozeb and chlorothalonil on pathogen development. When employed dynamically within a plant disease simulation (eg, Bruhn [2]) the effect of residues on epidemic development can be calculated and the need for fungicide treatment may be determined.

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