# Correlation Between Immobilization of Zoospores by Fungicides and the Control of Phytophthora Root and Crown Rot of Transplanted Tomatoes

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ABSTRACT

A fungicide-screening technique based on the reduction in the duration of motility of *Phytophthora* spp. zoospores was developed. Zoospore suspensions were added to chemical suspensions in several concentrations in a 0.3-ml microbeaker. The time required for the cessation of zoospore motility in this milieu was determined. Two materials, Difolatan and zineb-maneb coordination product (Dithane M-45), previously untested in the field against *Phytophthora* root and crown rot of transplanted tomatoes as well as captan and copper sulfate, were effective in reducing motility time. As preplant root-dip treatments in the field, Difolatan and Dithane M-45 were more effective than the commercially used captan in retarding the development of foliage symptoms. The plots treated with Difolatan and Dithane M-45 yielded better than the control or captan-treated plots. Copper sulfate seemed to both hasten and increase symptom expression. Basic copper sulfate treatment reduced the yield of tomatoes to a level below the untreated plots. With the fungicides tested, both the poisoned food and greenhouse in vivo screening tests afforded better correlation with field studies than the zoospore immobilization method. Phytopathology 60:783-787.

Fungicide screening tests have been developed in which the targets are different stages in the life cycle of the fungus. Possible targets in fungi that spread and/or infect by means of zoospores are: the mycelium-producing sporangia; the sporangia; or the zoospores themselves. Several workers have used screening techniques against the mycelium of zoospore-forming fungi. Others have tested fungicides against sporangia. Goodwin et al. (2) tested the effects of several fungicidal materials against zoospores of *Pseudoperonospora humuli*. Ogawa et al. (6) tested the effect of several fungicides on the duration of motility of *P. humuli* zoospores. Field data were obtained for only one of the chemicals tested in their experiments.

An investigation to determine whether the effect of a chemical on duration of motility of zoospores was correlated with disease control was made using *Phytophthora* root and crown rot of transplanted tomatoes *Lycopersicon esculentum* Mill. Transplanted tomatoes are vulnerable to attack because their weakened and injured root systems can serve as sites of infection. In addition, secretions from damaged roots may attract zoospores. Roots and crowns of direct field-seeded plants are also attacked, though less so. The main problem in direct-seeded plants usually is damping-off. The symptoms of the disease as it occurs in the Sacramento-San Joaquin valleys of California were described by Citropoulos (1), Satour (7), and Satour & Butler (8). Citropoulos found *Phytophthora capsici* Leonian to be the cause of the disease, whereas Satour & Butler (8) showed that both *P. capsici* and *P. parasitica* Dast. can be involved in the disease in naturally infected plants.

MATERIALS AND METHODS.—*Phytophthora capsici* isolates 2 and 11 were used in laboratory and greenhouse experiments, and *P. parasitica* isolate 110 in greenhouse experiments. All were obtained from M. M. Satour, and the identification code used is the same as in his report (7). Used in some of the laboratory tests was a *Phytophthora citricola* Sawada isolated from hop by two of the authors (9).

Chemicals used in some of the tests or throughout the tests were: Botran 75W (75% 2,6-dichloro-4-nitroaniline); captan 50W (50% *N*-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide); copper sulfate (technical grade); Difolatan 80W (1,1,2,2, tetrachloroethylthiocyclohexene-1,2-dicarboximide); Dithane M-45 (80% coordination product of zinc ion and manganese ethylene bisdithiocarbamate); Dithane Z-78 (65% zinc ethylene bisdithiocarbamate); Fumazone (1,2-dibromo-3-chloropropane); benomyl (1-butylcarbamoyl-2-benzimidazole carbamic acid, methyl ester); Ortho Copper 53 (53% basic copper sulfate); Panogen (2.2% v/v methyl mercury dicyanate); Terrachlor (75% pentachloronitrobenzene); and Zerlate (ziram, 75% zinc dimethyl dithiocarbamate).

*Preparation of zoospores for laboratory and greenhouse studies.*—The *Phytophthora* spp. were grown on Difco lima-bean agar supplemented with 1% dextrose. Sporangia were induced by keeping small blocks of agar from 8- to 13-day-old cultures under sterile distilled water for 2-4 days at room temperature. The agar blocks were then transferred to fresh tap distilled water, chilled at 6 C for 15-30 min or longer, depending on the volume of water used, and then allowed to stand for about 15 min at room temperature before use. While not in use, the zoospore suspensions were kept at about 15 C to extend motility time.

*Laboratory tests.*—The effect of the chemicals on zoospore motility was tested in 0.3-ml microbeakers. The microbeakers were washed in hot alcoholic KOH and hot acidic alcohol, rinsed in running tap water for 2 days, rinsed in several changes of glass-distilled water, and then allowed to air-dry at room temperature. One-tenth ml of zoospore suspension was added to 0.1 ml of chemical suspension to final concentrations of 1, 10, 100, 200, and/or 1,000 ppm active of the chemical.

The longevity of motility was observed at room temperature (24 C) to a maximum of 30 min, at which time the experiment was terminated. The times were recorded of the initial inactivation (scored when the second immobile zoospore was observed), 50% inactivation, and 100% inactivation. The presence or absence of germination was recorded 24 hr after the zoospores and chemicals were mixed.

Poisoned food tests were conducted with several of the chemicals. The commercial chemicals were put into Difco lima-bean agar as distilled water suspensions just prior to pouring into petri dishes. Rate of radial growth was determined in 0.1, 1, 10, 100, and 1,000 ppm active of the chemicals.

*Greenhouse tests.*—Preliminary greenhouse experiments were conducted with the Improved Pearson tomato cultivar used in routine work at the Univ. of Calif. Dept. of Plant Pathology. The rest of the greenhouse studies and all the field studies were conducted with the VFN-8 variety, of commercial importance as a "hand-pick" cultivar. The VFN-8 seeds for greenhouse work were obtained from the Univ. of Calif. Vegetable Crops Dept.; the VFN-8 plants used in field tests were obtained from a commercial supplier.

The greenhouse-grown plants, 6-10 weeks old, were transplanted into moist U.C. Mix soil in plastic trays ("poly-trays", Burpee Seed Co.) after being dipped into suspensions of captan, Difolatan, basic copper sulfate, and Dithane M-45 of ½ lb. active/gal $H_2O$. Six plants were planted in each of three trays, for a total of 18 plants/chemical. Zoospore suspensions of cultures 11, 2, and 110 were poured between the plants in aliquots of 150-200 ml. Noninoculated chemically treated plants were also planted. The trays were watered every 3 to 4 days. A plant was rated as diseased when it fell over as a result of lesions at the base of its shoot.

*Field experiments.*—Preliminary field testing of several chemicals against the transplant problem had been conducted (T. Lyons, *unpublished data*) beginning in 1963. Basic copper sulfate, Panogen, Terrachlor, Dithane Z-78, Bordeaux mixture, captan, and Dexon were tested either in planting water or as dip treatments. Dip treatments were more effective and less expensive than planting-water treatments, which were discontinued after 1964. Dexon was eliminated from the field tests because of its phytotoxicity as a dip.

Field tests were made with Dithane M-45 and Difolatan, both having shown promise in laboratory tests; captan, which was promising in laboratory and preliminary field tests and now is used commercially, and basic copper sulfate, promising in laboratory studies but behaving anomalously in previous field trials. Zineb was replaced by maneb in field tests because of its superiority in laboratory tests. The trial was conducted on Tyler island, near Isleton, California, in the Sacramento-San Joaquin River delta, on a farm with a *Phytophthora* problem. There were eight roadways running north-south in the commercial field. A 5 × 5 latin square design was laid out between the second and seventh roadways. Each replication of a treatment was in paired rows 1.52 m apart, while the distance between rows having different treatments was 1.22 m. This set-up was used to facilitate hand harvesting. The five replications of each treatment occupied about 1/6 acre. The soil type within the test plot varied from Columbia loam, on the west end, to Egbert muck (peat) soil, on the east end. Prolonged wet weather during the spring delayed the planting until 27 April, about 2 weeks beyond the normal planting date. One-half lb. active of each chemical was mixed with 1 gal of water. The roots of VFN-8 were dipped into the suspension after trimming, which is the usual commercial practice. Only a handful of plants were dipped at a time. Treated areas at the base of the stem and roots were sent to the Agricultural Toxicology Laboratory on this campus to determine the amount of fungicide retained on these areas. Residues of 5,296 ppm captan and 8,318 ppm Difolatan were found per unit volume of root-shoot sample by electron-capture gas chromatography techniques (3, 4), and 2,500 ppm Dithane M-45 was found by a colorimetric method (5). Normal commercial procedures were followed after treatment. Planting was with a commercial planter, four rows at a time. The average distance between plants was about 0.53 m. Planting-water rate was 500 gal/acre, including 10 gal of 5-15-5 fertilizer and 8 oz of Lindane insecti-

TABLE 1. Time in min required for initial, 50%, and complete inactivation of the motility of the zoospores of *Phytophthora capsici* isolate 11 after treatment with chemical

| | Concn of chemicals | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1,000 ppm | | | 100 ppm | | | 10 ppm | | | 1 ppm | | | Control | | |
| Chemical | A[a] | B[a] | C[a] | A | B | C | A | B | C | A | B | C | A | B | C |
| Botran | 0.8 | 2.0 | 4.2 | 2.0 | 5.2 | +[b] | [c] | + | + | | + | + | + | + | + |
| Captan | 1.8 | 2.6 | 3.5 | 1.9 | 2.6 | 3.2 | 1.7 | 3.1 | 3.9 | 4.1 | 20.0 | + | + | + | + |
| $CuSO_4$ | 0.2 | 0.3 | 0.4 | 0.2 | 0.4 | 0.6 | 1.1 | 2.7 | 8.2 | + | + | + | + | + | + |
| Difolatan | 1.3 | 2.2 | 3.0 | 1.3 | 3.5 | 6.1 | 0.3 | 7.0 | 9.7 | 5.0 | 8.5 | 12.3 | + | + | + |
| Dithane M-45 | | | 0.3 | 0.6 | 0.8 | 1.0 | 0.9 | 1.2 | 2.1 | 7.6 | + | + | + | + | + |
| Dithane Z-78 | 1.0 | 1.3 | 1.5 | 1.3 | 1.5 | 1.8 | 2.1 | 2.7 | 4.9 | 6.0 | 27.0 | + | + | + | + |
| Panogen | | | 0.1 | | | 0.1 | | | 0.1 | 0.8 | 1.4 | 1.8 | + | + | + |
| PCNB | 2.2 | + | + | + | + | + | | + | + | | + | + | + | + | + |
| Ziram | | | 22.0 | | + | + | | + | + | | + | + | + | + | + |

[a] A = initial inactivation (time when second zoospore immobilized; B = time when an estimated 50% of zoospores immobilized; C = time when all zoospores immobilized.

[b] + = motility in excess of 30 min.

[c] = no data taken.

cide. The plantings were subirrigated three times during the growing season. Initial survival of the plants was checked 3 weeks after planting. Counts of plants with top symptoms were made 11, 13, 15, and 19 weeks (time of first harvest) after planting.

RESULTS.—*Laboratory studies.*—Table 1 shows the time required for immobilization of zoospores of *P. capsici* isolate 11 by the various concentrations of fungicides. The results are from one set of experiments of three replications/concentration of chemical. Results were similar for *P. capsici* isolate 2 and *P. citricola* isolate W-2. Not included in Table 1 are Fumazone and benomyl, which were ineffective even at 100 ppm. Panogen, the most effective chemical tested, was eliminated early in the testing because difficulty was anticipated in obtaining approval for use of organic mercury compounds. The time required for inactivation was generally very similar within the three replications of a given chemical during a given experiment, but it did vary greatly in some cases, especially with the lower concentrations of chemicals. Inactivation time with a single concentration of chemical also differed in different experiments. For example, complete immobilization of zoospores of isolate 11 by Dithane M-45 at 10 ppm took 2.5, 1.8, and 2.0 min in one experiment, and 4.5, 7.4, and 11.0 min in another experiment. Such differences were of similar magnitude for all the chemicals tested. In some experiments, the time of initial inactivation could not be determined, as some immobile zoospores were noticed immediately after the zoospore suspension and chemical suspension were mixed.

One ppm concentration of Difolatan, Dithane Z-78, Panogen, and captan are antigerminant to encysted zoospores of *P. capsici*, while even at 1,000 ppm, Botran or PCNB did not effect germination (Table 2). Mycelial growth rate of *P. capsici* was suppressed more with Dithane M-45 or captan at 0.1 to 10 ppm concentration, but at 100 ppm, mycelial growth was inhibited with all these compounds (Fig. 1).

*Greenhouse tests.*—Under greenhouse conditions, Difolatan was the most effective fungicide against both isolates of *P. capsici* tested (Fig. 2-A, B) and also against isolate 110 of *P. parasitica*. Dithane M-45, the second most effective chemical, was difficult to evaluate because of its phytotoxicity against both Improved Pearson and VFN-8 under greenhouse conditions. Phytotoxicity symptoms, beginning about 1 week after treatment, were a yellowing of the leaves beginning at the margin, and a stunting of growth. In 3 weeks, symptoms were manifested by about 75% of the VFN-8 plants treated with Dithane M-45. None of the other

TABLE 2. Qualitative readings of germination of encysted zoospores of *Phytophthora capsici* isolate 11 in various concentrations of chemicals[a]

| Chemical | 1,000 | 100 | 10 | 1 | Control |
|---|---|---|---|---|---|
| | *ppm* | *ppm* | *ppm* | *ppm* | |
| Botran | 5 | 5 | 5 | 5 | 5 |
| Captan | 0 | 0 | 0 | 0 | 5 |
| $CuSO_4$ | 0 | 0 | 0 | 5 | 5 |
| Difolatan | 0 | 0 | 0 | 0 | 5 |
| Dithane M-45 | 0 | 0 | 2 | 5 | 5 |
| Dithane Z-78 | 0 | 0 | 0 | 0 | 5 |
| Panogen | 0 | 0 | 0 | 0 | 5 |
| PCNB | 5 | 5 | 5 | 5 | 5 |
| Ziram | 2 | 5 | 5 | 5 | 5 |

[a] Ratings from 0, no germinated zoospores observed, to 5, most zoospores germinated.

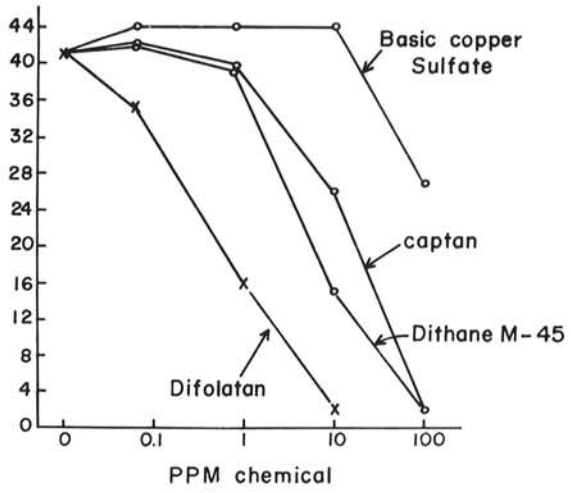


**Fig. 1.** Radial growth of *Phytophthora capsici* isolate 11, 180 hr after inoculation on variety of poisoned food medium.

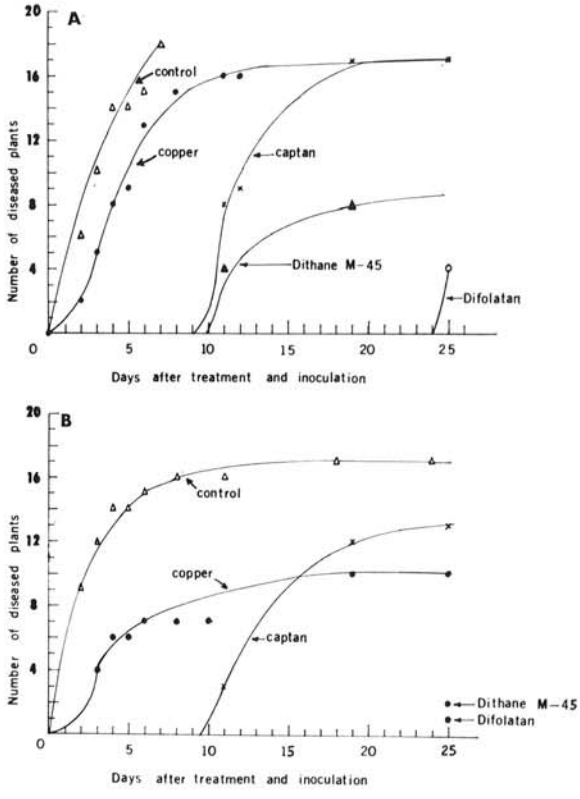


**Fig. 2.** Number of diseased tomato plants out of 18 plants dip-treated with fungicides and inoculated with *Phytophthora capsici* isolate II zoospores.

TABLE 3. Per cent of VFN-8 tomato plants dead 3 weeks after chemical dip treatment

| Chemical | % Dead, avg | % Range |
|---|---|---|
| Dithane M-45 | 4.7 | 3.0- 6.9 |
| Difolatan | 5.3 | 3.2- 9.6 |
| Captan | 7.3 | 4.7-10.1 |
| Basic $CuSO_4$ | 10.1 | 4.5-20.6 |
| Control | 6.2 | 1.6-10.4 |

chemicals tested were phytotoxic under the experimental conditions. The plants used in greenhouse tests were more succulent than field-transplanted plants. Because Fumazone might be used in irrigation water, it was tested under greenhouse conditions at 30 and 60 ppm. Neither concentrations, however, showed better control than no treatment against both isolates of *P. capsici* and isolate 110 of *P. parasitica*.

*Field experiments*.—Plants that had made little or no initial growth after transplanting died within 3 weeks. There was no significant difference in this regard between treatment and control plants (Table 3). Counts of plants expressing symptoms at different times after treatment and planting are given in Table 4. The sudden jump between 13 and 15 weeks in the number of plants with symptoms in the plots treated with Dithane M-45, Difolatan, and captan should be noted. At harvest, almost all plants had symptoms (no count was made), but with a difference in severity of symptoms. Qualitative ratings of plants based on size and *Phytophthora*-induced foliage symptoms before the first harvest clearly showed Difolatan and Dithane M-45 superior. No differences in rating were found between captan and the control, and copper sulfate was inferior in all five replications. The disease in the field was identified by D. H. Hall, Extension Plant Pathologist, UCD.

The treatments were harvested twice by commercial hand labor on 5 and 27 September 1967. Fifteen pickers were employed in the first pick, 20 in the second. The picking assignments were randomized, each worker picking the whole treatment within a replication. The tomatoes were picked into cannery boxes. The tons/acre (Table 5) were based on 44 lb. of tomatoes/box. The difference in yield between Dithane M-45 and the control was significant at the 1% level by Duncan's multiple-range test. Difolatan gave significantly better yields than the control at the 5% level, approaching the 1% level of significance. Dithane M-45 and Difolatan gave better yields than captan at the 5% level of significance. All treatments, including the control, gave yields significantly better at the 1% level than the plot treated with basic copper sulfate. No Difolatan, captan, or Dithane M-45 were detected in the harvested fruits by the methods referred to above.

TABLE 4. Incidence of top symptoms due to *Phytophthora* spp. on VFN-8 tomato plants after dip treatment with chemical, expressed in per cent

| Chemical | Weeks after treatment | | |
|---|---|---|---|
| | 11 | 13 | 15 |
| Dithane M-45 | 1.1 | 3.2 | 15.2 |
| Difolatan | 0.4 | 2.2 | 14.7 |
| Captan | 3.9 | 8.5 | 21.9 |
| Basic $CuSO_4$ | 21.4 | 23.8 | 31.1 |
| Control | 11.0 | 19.8 | 31.9 |

TABLE 5. Yields of tomato variety VFN-8 root-dip-treated before planting. Picked on 5 and 27 September 1967

| Chemical | Yield (tons/acre) | |
|---|---|---|
| | Mean | Range |
| Dithane M-45 | 36.6[a] | 31.6-41.6 |
| Difolatan | 35.5[b] | 32.0-39.0 |
| Captan | 32.1[c] | 26.0-38.6 |
| Basic $CuSO_4$ | 23.9[d] | 18.5-27.2 |
| Control | 31.1 | 29.0-32.6 |

[a] Significant difference when compared with control at the 1% level.
[b] Significant difference when compared with control at the 5% level.
[c] No significant difference when compared with control.
[d] All treatments, including control, significantly better at the 1% level than $CuSO_4$ treatments.

DISCUSSION.—Immobilization of zoospores correlated well with control of *Phytophthora* root rot in the field by Difolatan and Dithane M-45, neither previously tested in the field. Captan, in contrast, appeared effective in the laboratory and gave some control of top symptoms in the field, but gave yields that differed very little from control yields. Captan has been used for 2 years as a dip treatment in the area where the tests were made. Control of top symptoms with captan had been observed earlier in this area (T. Lyons, *unpublished data*), but he did not obtain yield data. As in previous tests (T. Lyons, *unpublished data*), although $CuSO_4$ immobilized zoospores at 10 ppm, it seemed to increase the susceptibility of transplants to *Phytophthora* spp. in the field. That this increase in susceptibility to *Phytophthora* was probably not due to a phytotoxic effect can be inferred from the non-phytotoxicity of the compound in greenhouse tests. In the greenhouse, basic $CuSO_4$ appeared to give some protection (Fig. 2-A, B). Tests with mycelial instead of zoospore inoculum yielded results similar to Fig. 2. The effect of copper sulfate is still to be worked out. Dithane Z-78 showed some effectiveness in the laboratory similar to previous results (T. Lyons, *unpublished data*) in the field. Both Terrachlor and Botran, previously found ineffective under field conditions, were ineffective in both zoospore immobilization and poisoned food tests. Dexon, an effective fungicide against other *Phytophthora* spp., was not used in the laboratory studies because of its phytotoxicity in preliminary field tests and its breakdown in light.

Although the zoospore-immobilization screening method provided two chemicals that subsequently proved effective under field conditions, the method is quite cumbersome at present. The two chemicals could just as easily have been selected by use of the poisoned food technique. Unfortunately, the compound found effective against zoospore motility and germination but ineffective in poisoned food tests, $CuSO_4$, was ineffective in the field. However, the possibility cannot be

excluded of finding an effective compound only by the immobilization technique.

In addition to the above, there are several other difficulties with the method. Two isolates of *P. capsici* isolated from tomato plants by M. M. Satour produced zoospores that came out of sporangia but did not swim, and therefore could not be used in the above tests. Citropoulos (1) reported similar behavior for several *P. capsici* that he isolated from tomato. Several *P. capsici* isolates produced too few sporangia to produce enough zoospores for a meaningful test. The duration of motility time of untreated zoospores from different cultures of the same isolate varied from several min to several hr. Thus, it was difficult in some cases to determine whether the zoospores stopped naturally or were stopped by the chemical. An attempt to prolong the motility of the untreated zoospores by conducting the experiments in a 15-C walk-in type environmental chamber was abandoned because of the increased motility time of the chemically treated zoospores. Another difficulty with the method is the repeatability of the results, which were fairly close in some cases but not in others.

The sudden increase in symptoms in plots treated with Difolatan and Dithane M-45 could be due either to the growing away of roots from the chemically protected area or to degradation of the chemicals. Although protection decreased with time, the protection given the plant during initial growth was what was desired in terms of crop production.

Besides reducing the quantity of fruit, the *Phytophthoras* affect fruit quality. The fruits on severely affected plants are often smaller, and fruits on plants with severely affected foliage are more prone to sunburn. In commercial picking, most of the control plot and copper sulfate plots would have been skipped in favor of the more healthy-appearing fruits in plots treated with the effective chemicals, because such fruits are easier to pick.

Although Dithane M-45 was phytotoxic in the greenhouse, no phytotoxicity symptoms were noticed in the field.

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