Biological Effect of Panogen PX in Soil on Common Root Rot and Growth Response of Wheat Seedlings

S. H. F. Chinn

Plant Pathologist, Research Station, Research Branch, Canada Agriculture, University Campus, Saskatoon, Saskatchewan.

Contribution No. 396.

Accepted for publication 26 August 1970.

ABSTRACT

Addition of Panogen PX at the rate of 5 ppm of the active chemical, methylmercury dicyandiamide, to soil had little direct effect on conidia of *Cochliobolus sativus,* the causal pathogen of common root rot of wheat. The treatment nevertheless reduced common root rot of wheat seedlings and increased seedling growth. The average decrease in root rot in four tests using different soils was 26.1%, and average increase in dry seedling weight was 30.6%. Some microorganisms, including *Penicillium* spp., increased profusely in treated soil. Several hypotheses concerning the nature of the mechanisms responsible for reduction in disease are proposed.

Increased growth response of wheat seedlings was attributed partially to control of common root rot. Other possible causes of increase in seedling weights are discussed.

Addition of Panogen PX at 1 part per million (ppm) had little influence on common root rot and growth of seedlings. At 25 ppm, the treatment reduced the level of disease but was phytotoxic. Phytopathology 61:98-101.

Additional key words: soil microbiology, fungicide, growth of wheat.

Relatively high application rates and costs are limiting factors in the use of fungicides for control of soilborne plant diseases, especially those concerned with low-priced crops such as wheat. Chinn & Ledingham (3), in a study of 16 granular chemicals applied to soil, noted that Diamond Alkali DAC (75% 3,3,4,4-tetrachlorotetrahydrothiophene-1,1-dioxide) and Stauffer N521 (90% 3,5-dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione) were the two most effective chemicals for eradicating conidia of *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur (imperfect stage: *Helminthosporium sativum* Pam., King, & Bakke), main causal pathogen of common root rot of wheat. The lowest completely effective or lethal rate for the two fungicides was 50 ppm, which seems impractical for control of common root rot in commercial grain fields.

Recent studies showed that Panogen PX [0.9% methylmercury dicyandiamide (MMDD)], at the rate of 25 ppm of the active ingredient, was completely effective, and at 5 ppm, slightly effective, for the eradication of conidia of *C. sativus* in light or medium soils. The effectiveness was somewhat less in heavier soils. Nevertheless, treatment at the lower rate reduced common root rot and increased seedling growth in all soil types. An investigation was conducted to confirm these responses and to explore their nature. The results of this study are reported here.

Materials and Methods.—Four soil types, ranging from a sandy soil from Grasswood, light loam from Warman, medium loam from Saskatoon, to heavy clay loam from Floral, were used. The soils were sieved to remove large particles and debris, then air-dried slightly to facilitate mixing. Panogen PX formulation (Morton Chemical of Canada) was used, and references to rate herein indicate the active ingredient only. MMDD, at the rate of 1, 5, or 25 ppm, was mixed thoroughly with the four soils on a dry wt basis. Water was added to bring soil to a moisture content about opt for seeding. The following tests were made with aliquots of soil from all treatments.

The fungicidal effect of MMDD on conidia of *C. sativus* in soil was determined by testing the effect of the various soil treatments on the fungus by the laboratory fungicide test of Chinn & Ledingham (3). Essentially, the test consisted of burying straw cultures of *C. sativus,* isolate H28, in 0.25-gal crocks containing aliquots of treated soil. After 4 days, the straws were removed and the conidia tested for viability. Two slides were prepared for each determination, and percentage germination was based on the examination of 50 conidia/slide by each of two persons.

The influence of the various treatments on viability of *C. sativus* conidia over a 4-week period was determined initially (2 hr after treatment) and at 1, 2, and 4 weeks. Tests were made by a procedure based on the fungicide test (3). Four straw cultures of isolate H28 were buried in each of the variously-treated soils in 0.25-gal crocks. One straw culture was removed from each crock at the indicated time, and conidia were tested for germinability as in the previous test.

The effect of MMDD on changes in total (bacteria and actinomycetes) and fungal populations in soil was determined initially (2 hr after treatment) and at 1, 2, and 4 weeks. The prevalence of *Penicillium* spp. on the fungal plates was also recorded. Estimations of populations were made by the dilution plate technique. Soil extract medium (4) was used for total, and Martin's medium (4) for fungal counts.

The effectiveness of various treatments on control of common root rot was determined by rating the amount of disease on Thatcher wheat seedlings grown in a greenhouse. Four replicates of 25 seeds/0.25-gal crock were sown and, after 4 weeks, the seedlings were harvested and assessed for disease according to the method of Ledingham et al. (5).

Isolations were made from subcrown internodes to determine what fungi were present in the tissues. Fifty subcrown internodes, with small sections of the crown attached, were randomly selected from seedlings grown in each fungicide treatment in each of the different soils. They were washed in running water for 3 to 4 hr; treated with mercuric chloride solution (1:1,000) for 1 min, then with 2% sodium sulfide to precipitate the mercury; rinsed 4 times in sterile distilled water; plated on acidified potato-dextrose agar; and examined after a 5-day incubation at room temp for the occurrence of *C. sativus* and *Penicillium* spp. All seedlings, including those that have their subcrown internodes excised, were counted and then oven-dried at 80 C for 48 hr and weighed.

RESULTS.—MMDD was not fungicidal at 1 ppm to conidia of *C. sativum* in the soils. Results show that the average percentage germination of conidia from the treated soils was 98% as compared to 97% in the untreated soils. It was slightly effective at 5 ppm in the two lighter soils (76 and 84%), but ineffective in the two heavier soils (95 and 100%). It was completely effective at 25 ppm in the two lighter loam, highly effective in the medium loam, and slightly effective in the heavy clay loam soils.

Viability of conidia of *C. sativus* remained high-to-practically-unchanged over the 4-week period in all four soils that were untreated or treated with 1 ppm MMDD. Averages of percentage germination of conidia from untreated soils at 0, 1, 2, and 4 weeks were 94, 96, 90, and 95%, respectively; whereas, those from 1-ppm-treated soils were 90, 93, 91, and 91%, respectively. Averages of percentage germination of conidia from 5-ppm-treated soils were hardly affected initially (90%), but decreased slightly at 1 week (79%) and moderately at 2 (61%) and 4 weeks (53%). At 25 ppm, percentage germination dropped to zero from the beginning in the three lighter soils. Some conidia from the heavy clay loam soil germinated initially, but germination also dropped to zero at 1 week. All treatments in general appear to influence viability slightly less in heavier than in lighter soils.

MMDD influenced quite similarly the total and fungal flora of all four soils; hence, results are presented for only two; namely, the sandy and medium loam soils. In untreated and 1-ppm-treated soils, total plate counts of bacteria and actinomycetes varied slightly during the 4-week period. With 5-ppm treatment, the counts also were quite static until the 3rd week, when they started to increase. Increases of 6- to 8-fold were noted by 4 weeks. The 25-ppm-treated counts were depressed at the beginning but returned to about their original numbers at 4 weeks. Changes in fungal counts during the 4-week period are shown in Table 1. A moderate decline occurred in the sandy soil with the untreated and 1-ppm treatment by 1 week, but remained constant thereafter to 4 weeks. Slight fluctuations occurred in medium loam soil throughout the experiment. With 5-ppm and 25-ppm treatments, fungal counts were low or depressed at the beginning. Marked increases occurred in some of these treatments by 1 week, and in all by 2 and 4 weeks.

Colony types from untreated and 1-ppm-treated soil were quite similar in kind and in number at all determinations. *Penicillium* spp., however, were the predominant fungi at 1 week, and almost the exclusive ones at 4 weeks in the 5- and 25-ppm-treated soils.

TABLE 1. Plate count of fungal population occurring at various periods in Grasswood sandy and Saskatoon medium loam soils treated with methylmercury dicyandiamide (MMDD)

Soil	Concn MMDD	Fungal count/g soil (10^3)			
		0[a]	1	2	4
	ppm	*weeks*			
Grasswood sandy	0	120	88	76	75
	1	140	73	72	82
	5	86	180	3,200	3,000
	25	4	1	70	1,950
Saskatoon medium loam	0	130	95	140	200
	1	110	100	100	170
	5	45	350	650	1,200
	25	24	710	2,700	4,000

[a] Test made 2 hr after treatment.

Disease ratings of seedlings from untreated soils ranged from 14.6 to 39 with an average of 29.3% (Table 2). Treatment with MMDD at 1 ppm reduced average disease ratings slightly; it caused little or no change in disease ratings of seedlings from two soils, moderate reduction from the third, and some increase from the fourth. At 5 ppm, the treatment reduced disease ratings considerably in all four soils, the average reduction being 26.1% (29.3-3.2). At 25 ppm, the treatment was highly inhibitory to plant growth in the three lightest soils; consequently, no disease ratings were taken. It reduced disease ratings considerably in the heavy clay loam soil.

Rates of MMDD treatment influenced in a conspicuous pattern the frequency of *C. sativus* and *Penicillium* spp. occurring on subcrown internodes of wheat seedlings harvested from all four soils (Table 3). *Cochliobolus sativus* occurred on an average of 57 and 39% of the subcrown internodes from seedlings grown in untreated and in 1-ppm-treated soils, respectively, while *Penicillium* spp. were virtually absent. On the other hand, *C. sativus* occurred on few or none and *Penicillium* spp. on an average of 52 and 57%, respectively, of the subcrown internodes from seedlings grown in 5- and 25-ppm-treated soils.

With minor exceptions, the influence of each rate of MMDD on seed germination and growth response of seedlings was quite similar in the four soils. Treatment at 1 ppm increased average germination slightly, although germination actually decreased slightly in one

TABLE 2. Disease ratings of wheat seedlings[a] grown in soils treated with methylmercury dicyandiamide (MMDD)

Soil	Concn MMDD (ppm)			
	0	1	5	25
Grasswood sandy	39.0	18.8	1.9	
Warman light loam	14.6	13.4	3.6	
Saskatoon medium loam	26.6	23.6	0.2	
Floral heavy clay loam	37.0	48.0	7.3	5.3
Average of four soils	29.3	25.9	3.2	

[a] Seedlings from 25 seeds/crock replicated 4 times.

TABLE 3. Average frequency of occurrence of *Cochliobolus sativus* and *Penicillium* spp. on subcrown internodes[a] of wheat seedlings grown in four soils treated with methylmercury dicyandiamide (MMDD)

	% Subcrown internodes invaded by	
Concn MMDD in soil (ppm)	*Cochliobolus sativus*	*Penicillium* spp.
0	57	1
1	39	1
5	6	52
25	0	57

[a] Fifty subcrown internodes from each treatment of the four soils were plated and examined.

and remained unchanged in another soil. At 5 and 25 ppm, the average increases were 12.4 and 11.2%, respectively. The dry wt of seedlings was highest in the 5-ppm-treated soil, the average per seedling being 0.065 g as compared to 0.056 in both the 1-ppm-treated and untreated soil, and 0.039 g in the 25-ppm-treated soil. With the latter soil, phytotoxicity depressed growth wt considerably.

DISCUSSION.—MMDD, when added at 1 ppm to soil, was sublethal to conidia of *C. sativus,* since the treatment caused no reduction in viability of the fungus within 4 days and only minor reduction even at the end of 4 weeks. As expected, wheat seedlings grown in 1-ppm-treated soil were nearly as heavily diseased and subcrown internodes as frequently invaded by *C. sativus* as in untreated soil. Soil treatment at 25 ppm was fungicidal, and this was reflected by low viability of conidia and, in the heavy clay loam soil, low disease rating of seedlings (phytotoxicity affected seedling growth in other soils).

The important consideration in this report, however, is concerned with the influence of soil treatment with 5 ppm MMDD on viability of *C. sativus* and concomitantly disease ratings of wheat seedlings. MMDD at 5 ppm must also be considered, as at 1 ppm, as being sublethal to *C. sativus,* as the treatment caused only slight reduction in viability of the fungus within 4 days. Moderate reduction, however, occurred by the end of the 4-week trial. This effect on the conidia at this rate of treatment may be attributed to indirect effects of the fungicide such as, for example, an alteration of the soil environment to one that was partly inimicable to the conidia. The pathogen, however, was not eradicated; a high percentage of conidia were viable in treated soil even at 4 weeks. Nevertheless, a highly significant reduction occurred in the level of disease in plants grown in the soil (Table 2). These seemingly anomalous results may be due to one or more of the factors discussed below.

Antibiotics in sufficient quantity to inhibit germination and subsequent infection of plants by conidia of *C. sativus* may have been produced in the 5-ppm-treated soil. Apparently, *Penicillium* spp. tolerated and, in fact, developed profusely in soil treated with MMDD at the rates of 5 and 25 ppm. This finding is in general agreement with the work of Booer concerning the tolerance of this group of fungi to mercurial compounds in soil (1), and with Munnecke & Moore (6) who reported that the population of *Penicillium* spp. was ca. inversely proportional to the concn of MMDD in soil.

Furthermore, the tolerance and growth of the total microbial population in MMDD-treated soil followed a trend quite similar to that of the fungal population. Work, however, was too limited to ascertain the type of bacteria and actinomycetes that survived the treatment and those that eventually populated the soil. Possibly, the bacterial flora was somewhat similar to that described by Spanis et al. (8), who demonstrated prolific growth of certain tolerant bacterial species in soils treated with Panodrench 4 [0.6% cyano (methylmercuri) guanidine]. In the present study, the suppression of soil flora was less in the heavy than in the sandy soil. Probably, MMDD was inactivated to a greater extent by adsorption on the clay particles in the former soil.

The increase in certain microorganisms may have been paralleled by a concomitant increase in antibiotic production. Wright (11, 12) has demonstrated, and Brian (2) has reviewed, various work on the production of antibiotics in soil following introduction of seed pieces or other organic matter. Possibly, the environment described here was even more conducive to the production of these metabolites, some of which may be active against *C. sativus.*

A cross-protection mechanism operating within a plant may also be involved. A large percentage of seedlings grown in soil treated with 5- or 25-ppm MMDD was healthy as indicated by low disease ratings (Table 2), and substantiated by low incidence of *C. sativus* on subcrown internodes (Table 3). Nevertheless, *Penicillium* spp. were isolated frequently from this source. Possibly, symptomless invasion of healthy plants by *Penicillium* spp. interfered with subsequent invasion by *C. sativus.*

MMDD added at a low rate to soil may act as a fungistatic agent, or it may augment naturally occurring fungistatic substances, thus minimizing spore germination and infection. Finally, the possibility also exists that conidia can be mildly injured when exposed in 5-ppm-treated soil. Such injury may contribute to their slow but gradual eradication from soil and their inability to invade plant tissues.

The growth response of the seedlings is noteworthy. Whether the 30% increase in seedling wt obtained in the 5-ppm-treated soil was due entirely or partly to control of common root rot is now being studied. Indication is that disease control is only part of the answer. Soil treatments with other chemicals have been reported to give spectacular yield increases in grapes (7), strawberries, cauliflower, and tobacco (10). Growth response may also be due to formation in soil of metabolites that are not generally considered as growth substances. Tumarryan et al. (9) showed that treatment of soil with streptomycin, penicillin, or culture filtrates of some bacteria increased fruit yield of tomato by as much as 40%. Conceivably, these substances may have been formed in soil treated with MMDD, and may have promoted seedling growth via a hormonal effect.

LITERATURE CITED

1. BOOER, J. R. 1944. The behavior of mercury compounds in soil. Ann. Appl. Biol. 31:340-359.
2. BRIAN, P. W. 1957. The ecological significance of antibiotic production, p. 168-188. *In* E. O. Williams & C. E. Spicer [ed.] Microbial ecology. 7th Symp. Soc. 93n. Microbiol. Cambridge, Univ. Press, London.
3. CHINN, S. H. F., & R. J. LEDINGHAM. 1962. A laboratory method for testing the fungicidal effect of chemicals on fungal spores in soil. Phytopathology 52:1041-1044.
4. JOHNSON, L. J., E. A. CURL, J. H. BOND, & H. A. FRIBOURG. 1959. Methods for studying soil microflora-plant disease relationship. Burgess Publishing Co., Minneapolis, Minn.
5. LEDINGHAM, R. J., B. J. SALLANS, & A. WENHARDT. 1960. Influence of cultural practices on incidence of common root rot in wheat in Saskatchewan. Can. J. Plant Sci. 40:310-316.
6. MUNNECKE, D. E., & B. J. MOORE. 1967. Fungicidal activity in soil in relation to time, concentration, and Penicillium population. Phytopathology 57:823 (Abstr.).
7. PLANCHON, J. E. 1875. La defense contre le Phylloxera. Ann. Agron. 1:74-97.
8. SPANIS, W. C., D. E. MUNNECKE, & R. A. SOLVERG. 1962. Biological breakdown of two organic mercurial fungicides. Phytopathology 52:455-462.
9. TUMARRYAN, V. G., E. J. AFRIKIJAN, & R. A. BOBIKYAN. 1964. Use of certain antibiotics and microbe antagonists in vegetable growing. Rev. Appl. Mycol. 43:289 (Abstr.).
10. WILHELM, S. 1965. Pythium ultimum and the soil fumigation growth response. Phytopathology 55: 1016-1020.
11. WRIGHT, J. M. 1956. The production of antibiotics in soil III. Production of gliotoxin in wheatstraw buried in soil. Ann. Appl. Biol. 44:461-466.
12. WRIGHT, J. M. 1956. The production of antibiotics in coats of seeds sown in soil. Ann. Appl. Biol. 44:561-566.