Control of Phytophthora Root Rot of Avocado with *p*-Dimethylaminobenzenediazo Sodium Sulfonate (Dexon)

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ABSTRACT

Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate) controlled Phytophthora root rot of avocado seedlings in the glasshouse when repeated applications were made. Control or reduction of disease severity was also obtained by repeated applications to large avocado trees in field plots. In artificially infested soil, the relationship between dosage of the chemical and amount of inoculum of the fungus was evident in terms of effect on disease severity and growth of seedlings. Dexon was fungistatic; *Phytophthora cinnamomi* survived exposure to 500 ppm of Dexon for up to 20 days, depending on the medium in which the fungus was growing. Dexon acted as an antisporulant; 5 ppm of the chemical reduced formation of sporangia and chlamydospores by 90-100%.

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Many fungicidal chemicals can be used for seed or soil treatments at or before planting time. Relatively few soil fungicides combine effectiveness in preventing or controlling root diseases with low phytotoxicity so that they can be used in treating established plants. This paper concerns this latter aspect of application of soil fungicides, and is based on control in the field as well as in the glasshouse of Phytophthora root rot of a subtropical fruit tree, the avocado (*Persea americana* Mill.). The causal agent is *Phytophthora cinnamomi* Rands.

The fungicide used in this study was Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate) tested originally in our laboratory as Bayer 22-555. Data are presented also on the effectiveness of Dexon on vegetative growth, sporulation, and spore germination in *P. cinnamomi*. A preliminary report of a portion of the work covered in this paper has been presented (13).

Dexon is most effective against soil-borne pathogens in the genera *Phytophthora, Pythium*, and *Aphanomyces*, and relatively ineffective against *Fusarium, Rhizoctonia, Sclerotium*, and *Verticillium*, and against saprophytic fungi in the Ascomycetes and Fungi Imperfecti. A number of reports have appeared in recent years of the effectiveness of Dexon as a seed treatment or a soil drench for control of Phytophthora, Pythium, or Aphanomyces root rots and damping off of vegetables or ornamentals (3, 4, 5, 6, 7, 8, 9, 10).

MATERIALS AND METHODS.—Dexon was supplied by Chemagro Chemical Corp. in wettable (35 and 70%) and granular (5%) formulations. In soil infestation experiments, the isolate of *P. cinnamoni* used was SB 216-1, a single zoospore culture of an isolate from diseased avocado roots in California.

The naturally infested soil used in the glasshouse tests was a sandy loam (Fallbrook series) with a pH of 6.5-6.8. Mexican avocado seedlings (Topa Topa variety) were grown in U.C. mix (2) in 4-inch clay pots or in small peat pots, then transferred into the infested soil in 6-inch clay pots or in 1-gal cans. Solutions of Dexon were applied immediately, using sufficient solution to wet all of the soil in the container. Three concentrations were normally used, with treatments replicated at least five times. In some tests, granular Dexon was spread on the surface of the soil in the containers, then water was applied. Treatments were applied generally at weekly intervals for 12 weeks. Plants were then harvested, roots and tops weighed, percentages of healthy roots estimated, roots cultured for presence of *P. cinnamomi*, and the soil was tested for presence of the pathogen by the avocado fruit assay method (14, 16).

For the tests with artificially infested soil, *P. cinnamomi* mycelial mats were grown in 100 ml of potato-dextrose broth in 1-liter bottles placed in a horizontal position, following the method of Tsao & Garber (11). For X 1 inoculum, one-third of the mycelial mat from one bottle was chopped in a blender, mixed with ca. 50 g of sand, then mixed thoroughly with sufficient soil (U.C. mix) to fill five 4-inch pots; for the X 5 inoculum, mycelium from one and two-thirds bottles was used. *Persea indica* seedlings were planted in each pot, and water or the Dexon solutions was added immediately; the soil was treated once a week for 4 weeks. Plants were then harvested and assayed as noted above.

Avocado fruit were used as a trap for isolating *P. cinnamomi* from soil (14) and for a semiquantitative measure of the population of the pathogen in soil. Mature, firm avocado fruit (Fuerte variety) were placed in waxed paper cups containing the soil to be assayed, the fruit was pressed into the soil, and the soil was flooded with water. Spots developing on the fruit from infection by *P. cinnamomi* were counted as soon as evident (in 3-5 days at 24 C).

Fungitoxicity tests were made in the dark, using agar and the soil vial method (12). The effect of Dexon on formation of sporangia and chlamy-

dospores, and on germination of chlamydospores and zoospores, was studied in aqueous solution in the dark at 24 C.

Dexon was tested under field conditions in avocado groves in several areas in southern California. Concentrated solutions of the wettable material were applied with sprinkling cans to the soil surface around large (6- to 12-year-old) avocado trees, and the chemical was immediately diluted by applying water from low sprinklers set under each tree; or concentrated solutions were placed in an applicator attached to the irrigation line leading to the sprinklers. In both cases, 1 gal of solution was applied/ft^2 of soil surface. On some plots, granular Dexon was spread evenly under the trees at the rate of 3.0 lb. of 5% granular material/175 ft.^{2} of soil surface, and water was immediately applied at the rate of 1 gal/ ft^2. Treatments of 50 and 100 ppm Dexon in solution were usually applied at monthly intervals during the irrigation season (9 or 10 months). Results were evaluated by tree growth as determined by measurement of circumference of trunks, yield, disease progress (by evaluation of tree condition), and fungus population in soil and roots. Tree condition or disease severity was evaluated on a scale in which 0 = healthy, 1 = early stage of disease, 2 = moderate disease, 3 = advanced disease, 4 = very advanced disease, 5 = dead. Residue analyses were made on fruit by M. J. Kolbezen, University of California, Riverside, using the method of Anderson & Adams (1).

RESULTS.—*Glasshouse trials.*—In several experiments, weekly applications of 10 or 20 ppm Dexon beginning as soon as seedlings were planted in naturally infested soil controlled Phytophthora root rot effectively (Table 1). The 40-ppm treatment was somewhat phytotoxic. Even though 94% of the roots were healthy in the 20 ppm series, the pathogen was readily recovered from roots and soil in that treatment as well as in the other treatments. Repeated treatment with 40 ppm of Dexon greatly reduced the population of *P. cinnamomi* but did not eliminate the fungus.

In another glasshouse test, weekly applications of 10 ppm Dexon and weekly or biweekly applications of 20 ppm controlled the disease (Fig. 1); biweekly and monthly applications of 10 ppm and monthly applications of 20 ppm were ineffective. Weekly applications of 10 and 20 ppm (w/w) of the 5% granular formulation to the soil surface also controlled the disease in the glasshouse (Table 2).

Similar control was obtained by treating artificially infested soil with Dexon, with indications of a significant relation between dosage of chemical, inoculum dosage, and response in terms of disease prevention (Fig. 2). The relationship between dosage of inoculum and response, measured in growth of the *P. indica* seedlings, was evident, as was the relationship between amount of inoculum and recovery of the fungus, measured by numbers of spots per avocado fruit. With the X 1 amount of inoculum, the number of spots per fruit was reduced from 7.5 to 5.1 to 0.8 as the dosage of Dexon was increased from 0 to 10 ppm to 20 ppm.

Effect of Dexon on P. cinnamomi.—Dexon was fungistatic rather than fungicidal to mycelium of *P. cinnamomi*. When Dexon was incorporated in glucose potato agar or V-8 agar, 500 ppm retarded (by ca. 80%) but did not prevent growth. Exposure to 3,200 ppm of Dexon in soil for 24 hr in the standard laboratory vial test did not kill the fungus. In liquid culture (V-8 broth), dry weight of mycelium was reduced by the following percentages in 10, 50, 100, and 500 ppm Dexon: 84, 94, 96, and 96, respectively.

P. cinnamomi survived exposure to 1,000 ppm Dexon for 20 days, when the fungus was in small, diseased roots and survived exposure to 500 ppm for 20 days when discs from V-8 cultures of the fungus were used.

In three tests on the effect of Dexon upon germination of chlamydospores and zoospores, the percentage of reduction in germination of chlamydospores at 10, 50, 100, and 200 ppm, respectively, was 55, 76, 84, and 91. The slope of the dosage-response curve was thus very flat. Inhibition of germination of zoospores was usually not complete even at 200 ppm, although there was generally a reduction of ca. 50% at 5 to 10 ppm.

Dexon was most effective in inhibition of produc-

TABLE 1. Control of Phytophthora root rot of avocado seedlings with weekly Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate) drenches in the glasshouse[a]

Treatment	Increase in height	Weight of roots	Weight of tops	Healthy roots	Recovery of *Phytophthora cinnamomi*
	cm	*g*	*g*	%	
1. Untreated	3.0*[b]	3.5***	12.8***	0	+
2. Dexon, 10 ppm	7.0	23.4	20.4	75	+
3. Dexon, 20 ppm	10.5	26.8	30.2	94	+
4. Dexon, 40 ppm	8.4	12.4[c]	25.3[c]	58[c]	+

[a] Each figure is the mean of five plants, grown for 12 weeks in soil infested with *Phytophthora cinnamomi*.
[b] * = Significantly different from treatments two, three, and four at .05 level.
*** = Significantly different from treatments two and three at .001 level.
[c] Reduction in weight and in percentage of healthy roots probably is the result of phytotoxicity.

tion of sporangia and chlamydospores. In several experiments, production of sporangia in nonsterile soil extract (15) was reduced by 94 to 96% in 5 ppm Dexon. Similar results were obtained with formation of chlamydospores in V-8 broth, with 98-100% reduction at 5 to 10 ppm Dexon.

Field trials.—Control of Phytophthora root rot was obtained in several field plots. The initial field plot involved four trees treated with Dexon and four untreated in early stage of disease, all growing in infested soil. After 4 years of monthly treatments (8-10 treatments/year), three of the four Dexon-

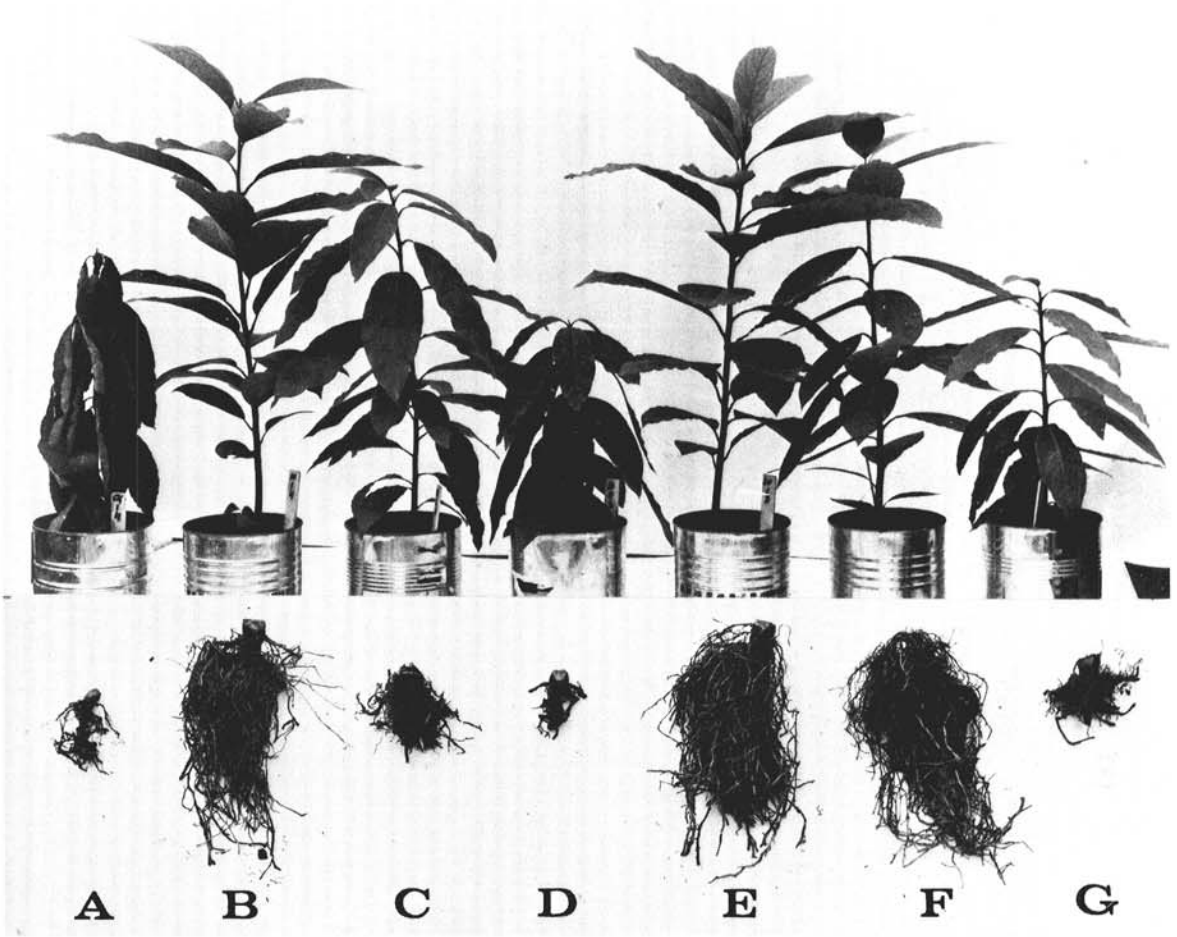

Fig. 1. Control of Phytophthora root rot of avocado seedlings with Dexon drenches. **A)** untreated. **B)** 10 ppm Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate) weekly. **C)** 10 ppm Dexon every 2 weeks. **D)** 10 ppm Dexon every 4 weeks. **E)** 20 ppm Dexon weekly. **F)** 20 ppm Dexon every 2 weeks. **G)** 20 ppm Dexon every 4 weeks.

TABLE 2. Control of Phytophthora root rot of avocado seedlings by weekly applications of granular Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate) applied to surface of soil in the glasshouse[a]

Treatment	Increase in height[a]	Weight of roots	Weight of tops	Healthy roots
	cm	*g*	*g*	%
Untreated	2.0	5.2	20.2	4.6
Dexon, 10 ppm	14.6	17.9	40	72
Dexon, 20 ppm	12.6	23.2	35	80
Dexon, 40 ppm	5.9[b]	21.5	31.4	76
Dexon, 80 ppm	2.3[b]	6.1[b]	21.2[b]	39[b]

[a] Each figure is the mean of five plants grown for 12 weeks in soil infested with *Phytophthora cinnamomi.*

[b] Reduction in growth, in weight of roots and tops, and in percentage of healthy roots probably is the result of phytotoxicity.

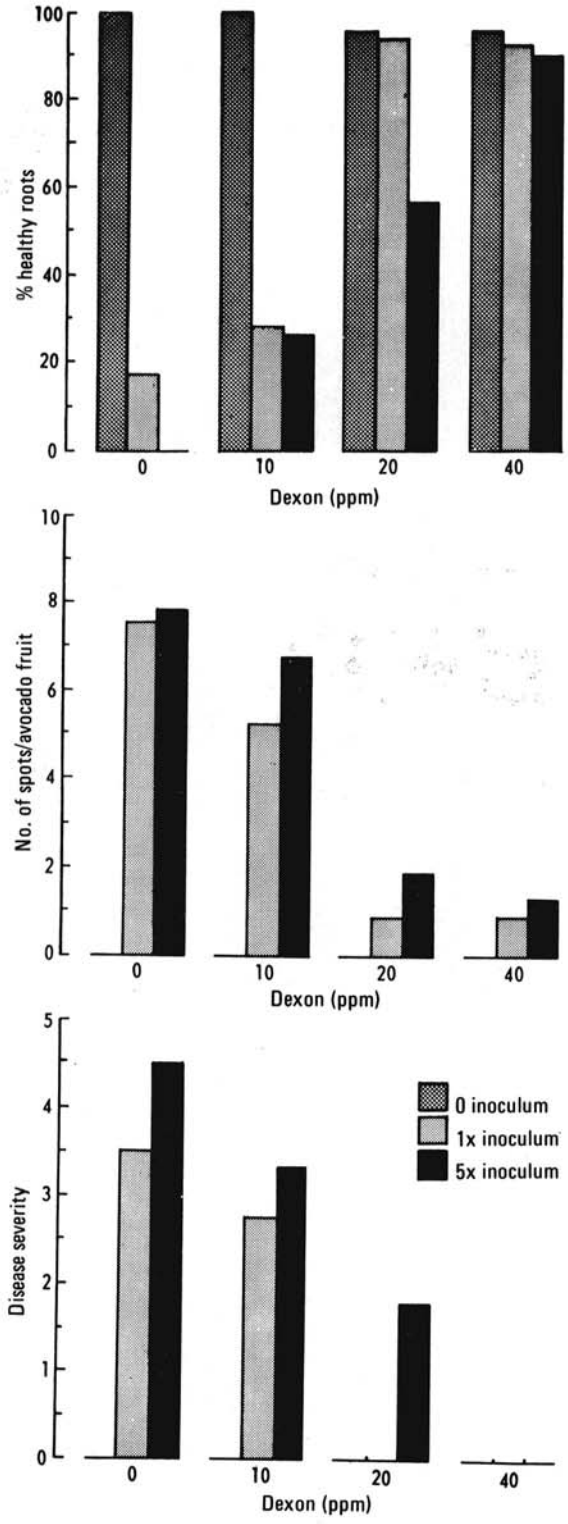

Fig. 2. Effect of Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate) drenches on Phytophthora root rot development in avocado seedlings and *Phytophthora* population in soil artificially infested with *Phytophthora cinnamomi* (see text for explanation of "disease severity" and × 1 and × 5 inoculum").

treated trees were still in good condition (Fig. 3), and one was in a more advanced stage of disease. Three of the four controls had either died or been removed when they reached an advanced stage of disease, and one was in very poor condition. This plot was continued in similar condition for 7 years with monthly applications of Dexon.

The largest, randomized plot involved 21 trees, with seven trees treated with 50 ppm Dexon (w/v) (70% wettable); seven, with 100 ppm Dexon; and seven, with water only. Most of the trees were in early stages of disease when treatment was begun. Monthly drenches of 100 ppm Dexon retarded the progress of the disease over a 4-year period, as measured by evaluation of the disease severity and by the increase in trunk circumference (Table 3). Treated trees on the average also produced more fruit each year than did untreated trees, but with the notably variable yield of avocado trees the differences were not quite significant.

On another plot, results were not so striking as on the first two plots, but some reduction in progress of Phytophthora root rot and increase in tree growth were obtained. On this plot, the untreated trees averaged 3.4, 4.3, and 4.7 cm increase in circumference 22, 32, and 45 months, respectively, after beginning treatment, whereas the trees treated with 100 ppm Dexon (w/v) (70% wettable) averaged 4.2, 5.7, and 6.8 cm increase, respectively, in the same time periods.

The first plot treated with granular Dexon showed striking response, with the five treated trees remaining healthy for 18 months, and the five untreated trees rapidly declining (Table 4). On two additional plots where granular Dexon was used, trees grew better than the untreated trees and had less severe root rot symptoms. On four other plots, no response was obtained, for reasons not yet determined.

No Dexon residues were found in fruit from treated trees, and the chemical was approved for use on avocados in 1964. Under recent revisions of residue regulations, the chemical is at present not available for use on bearing avocado trees in the United States.

DISCUSSION.—Dexon consistently controlled Phytophthora root rot in the glasshouse, but repeated applications were necessary. This is in contrast to the results of Mitchell & Hagedorn (6), who reported persistence of the chemical into the following season under their conditions. The latter authors used very heavy dosages of Dexon, which could account for some of the persistence; no information was presented on the pH of the soil used. Under California conditions, both in glasshouse and field trials, repeated applications are necessary for soils of pH 6.5-7.

The laboratory results indicate that Dexon is effective primarily because it inhibits spore production; it apparently acts as an antisporulant. If a supply of Dexon is maintained at a level of 5 to 20 ppm in the soil, *Phytophthora cinnamomi* will produce few if any sporangia or chlamydospores, and

mycelial growth will be retarded. Thus, new roots can emerge and root invasion by the pathogen will be reduced. The fungistatic nature of the chemical is also demonstrated by the fact that *P. cinnamoni* was readily isolated from soils treated with Dexon for 7 years.

Thus, with large avocado trees in the field it was possible to control, or at least to palliate, a root

Fig. 3. Control of Phytophthora root rot of avocado with Dexon (*p*-Dimenthylaminobenzenediazo sodium sulfonate) in field plot. Untreated tree in center of photo, with Dexon-treated trees on each side. All trees infected with *P. cinnamomi* and in early stages of disease at beginning of treatment.

TABLE 3. Control of Phytophthora root rot of 7-year-old avocado trees with Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate) in the field[a]

Treatment	Disease severity[b]			Mean increase in trunk circumference in			Mean lb. fruit/tree		
	At Beginning	2 yr later	4 yr later	2 yr	3 yr	4 yr	2nd yr	3rd yr	4th yr
Untreated	0.1	3.6	3.1	4.7cm	6.0	8.0	73	65	155
Dexon, 50 ppm	0.1	2.1	2.4	6.1	7.7	8.5	81	67	235
Dexon, 100 ppm	0.5	1.5	1.1	8.5	10.5	13.9[c]	131	121	297
Healthy trees									273

[a] Treatments applied monthly during irrigation season; seven replicates.
[b] Disease severity based on scale: 0 = healthy; 5 = dead.
[c] Statistically significant at .05 level.

TABLE 4. Control of Phytophthora root rot of 5-year-old avocado trees with granular Dexon (*p*-Dimethylaminobenzenediazo sodium sulfonate)[a]

Treatment	At beginning	Disease severity[b] after				
		0.5 yr	1 yr	1.5 yr	2 yr	3 yr
Untreated	0.1	2.3	3.1	3.0	4.1	4.3
Dexon granular	0	0.4	0.3	0.0	0.4	2.6

[a] Granular Dexon applied monthly during irrigation season; five replicates.
[b] Based on a scale where 0 = healthy; 5 = dead.

disease, induced by a species of *Phytophthora*, by continued application of a fungicide such as Dexon. Results were somewhat variable in the field, but responses were good on a sufficiently large number of plots, and under randomized and replicated conditions, as to indicate that such control is possible. The practicability of such treatment will depend on economic conditions, involving the expense of material plus application in relation to crop production. In a high-value per acre crop such as the avocado, under optimum conditions for disease control and good prices for fruit, usage may be feasible.

The lack of persistence of Dexon in the soil under conditions of these tests, as demonstrated in the glasshouse tests and by lack of control with 50 ppm dosages in the field, is one disadvantage of this type of chemical for continued use of tree crops. Sensitivity of Dexon to light is also a disadvantage, but we minimized this in our plots by applying the material under reduced light conditions, as under tree canopies, and by immediately watering the chemical into the soil by turning on sprinklers while the concentrated solution was being applied to the soil surface.

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