Residual Dexon and the Persistent Effect of Soil Treatments for Control of Pea Root Rot Caused by Aphanomyces euteiches

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ABSTRACT

Plots in five Wisconsin locations were treated with Dexon [sodium-*p*-(dimethylamino)benzenediazosulfonate] at 30 and 15 lb. actual/acre in October 1968 and at 15 and 7.5 lb. actual/acre in May 1969. They were planted and harvested in 1969 in a manner simulating commercial pea production. Adjusted yields showed increases attributable to treatment ranging up to 550%. Yields of plots treated at 15 and 30 lb./acre in October 1968 averaged 252 and 364%, respectively, over those for control plots at three sites. The spring preplant treatments of 15 and 7.5 lb./acre increased the 1969 yield by 385 and 184%, respectively, at Arlington where root rot was most severe. In 1970, the increases due to the 15- and 30-lb./acre treatments in 1968 were 33 and 84%, respectively. The persistence of the effect of Dexon treatment for pea root rot control apparently involved both reduced pathogen populations and persistence of relatively low levels of Dexon in the treated plots. Data for residual Dexon in soil indicated a loss of 50, 70, 80, and 90% after 1, 7, 21, and 90 days, respectively. Approximately 1 μg of residual Dexon/g of soil is required to inhibit zoospore formation. This level is exceeded in treated plots which continue to show repression of pea root rot. A treatment of 30 lb./acre which would be required to provide this level of residual Dexon for more than one season would not be economically feasible. Phytopathology 61:978-983.

Additional key words: fungicide residues.

The levels of fungicides residual in soil and their persistence in relation to their effectiveness in disease control are of considerable interest in determining applications required and in minimizing use of excessive dosages.

The effect of soil treatment with the chemical Dexon [sodium-*p*-(dimethylamino)benzenediazosulfonate] on the activity of root pathogens such as *Pythium* and *Aphanomyces* appears to persist for a relatively long period. Whether this reflects the persistence of active residual chemical or merely the period required for the pathogens to return to their original level of activity has not been established. Greenhouse tests by Alconero & Hagedorn (1) showed that a high dosage of Dexon (100 and 200 μg/g of soil) completely suppressed *Pythium* for 3 months. They showed by chemical analysis that residual Dexon 6 months after the treatment at 100 μg/g assayed from 1.4-1.8 μg/g. After the same period, Dexon levels for the treatment at 200 μg/g ranged from 4.1-7.2 μg/g.

Kahn & Baker (4) used two bioassay techniques that yielded equivocal results in short-term tests. Selective isolation of the fungi by means of antibiotic-treated potato discs indicated that there was a distinct decrease in the effect of the Dexon treatment over a period of 28 days. There was no apparent commensurate decrease in activity when the effect of treatment was assayed in terms of control of cucumber damping-off. Alconero & Hagedorn (1) suggested that *Pythium* spp. may differ in their sensitivity to Dexon, but the possibility exists that the two bioassays (3) measured populations of different species.

Previous work (4) showed that, in pot tests, the effect of Dexon treatment on activity of *A. euteiches* persisted indefinitely and constituted selective sterilization. This occurred only at a high dose of 100 μg Dexon/g of soil. Persistence for at least 2 years of an effect of treatment with Dexon at the rate of 60 and 30 lb./acre on pea root rot under field conditions was reported by Mitchell & Hagedorn (5, 6). The information reported herein has been accumulated in connection with field plot evaluation of the effectiveness of Dexon in the control of common root rot of pea. The effectiveness of specific soil treatments in terms of yield are related to the residue of Dexon present in the soil used. The relationship between the rate of degradation of Dexon in soil and its effectiveness in disease control is demonstrated.

MATERIALS AND METHODS.—Field plots were treated with Dexon in October 1966 and 1968 and in May 1969. Application was made in October 1966 to 20- × 50-ft plots at the rate of 60 and 30 lb. Dexon/acre (all rates given are on active chemical basis) by means of a hydraulic back-pack sprayer. Mixing the chemical with the soil was done initially with a hand rake; then, after all plots were treated, with a tractor-mounted disk. Perfection peas were grown in these plots in 1967, 1968, and 1969. All treatments were replicated 3 times. Samples for residue analysis were taken from eight locations in each of the plots and mixed thoroughly; then, three samples were drawn for assay. The residue values reported are average values for the three samples from each replicate plot.

The second series of plots were treated in October 1968 and May 1969. Dexon was applied to these 200- × 20-ft plots by means of a spray boom mounted immediately ahead of a tractor-mounted disk. Thus, the soil was mixed immediately after the chemical was applied. A final disking was made immediately after all treatments were applied to insure uniform incorpo-

ration to a depth of ca. 6 inches. Rates of active chemical applied were 30 and 15 lb./acre in the plots treated in October 1968, and 15 and 7.5 lb./acre in those treated in May 1969. Samples of soil from these plots were collected every 25 ft along two arbitrary lines 15 ft apart, and handled as noted above for the samples from the 1966 plots. All samples were stored at room temperature between sampling and assay.

A more precise study of the rate of disappearance of Dexon from soil was made in the laboratory by mixing a known amount of Dexon in dry sand with a weighed amount of sieved silt loam soil for 30 min on a roller mill in the dark to give a final concentration approximating 30 lb. Dexon/acre. Residual Dexon was determined immediately and at intervals over a 3-month period. The treatments were stored individually in double plastic bags and were kept at room temperature in the dark unless otherwise noted.

Analysis of soil for Dexon was made by a modification of the method suggested by C. A. Anderson, Chemagro Corp. (*personal communication*). Each sample of soil was thoroughly mixed, and 25 g was added to 700 ml of 2% sodium sulfite for extraction. The dry wt of a comparable sample of soil was used as the basis for computing residue in the soil. The suspension was mixed in a Waring Blendor at low speed for 3 min, then centrifuged for 10 min. The supernatant solution was filtered through Whatman No. 40 paper on a Büchner funnel and brought to 700 ml with distilled water. The extract was then transferred to a 3-liter beaker, and 15 ml of 4 N resorcinol and 20 ml of 4 N KOH were added. The beaker was placed on a magnetic stirrer with the liquid level 6 inches below a 150-w flood lamp for 30 min and the mixture vigorously agitated during irradiation. The irradiated solution was transferred to a 2-liter separatory funnel, and 30 ml 4 N HCl and 100 ml benzene were added. The contents of the funnel were shaken thoroughly for 2 min and the phases allowed to separate. The upper phase was centrifuged to break the emulsion and separate soil particles, and the benzene phase after transfer to a 125-ml Erlenmeyer flask was treated with anhydrous Na_2SO_4 to remove residual water. The absorbance at 450 mμ was proportional to the Dexon present. The sensitivity of the procedure permitted detection of Dexon to levels less than 0.1 μg/g soil. In the absence of soil, the assay resulted in values directly proportional to the amount of Dexon present.

Assay of soil samples with no history of contact with Dexon consistently gave zero values for Dexon. When Dexon was added to such samples, recovery was never complete. While there were undoubtable losses associated with the assay procedure, the rapid inactivation of Dexon in contact with soil made the evaluation of Dexon actually recovered difficult. As a consequence, all values are reported as determined. Comparative values are valid, as they were obtained by standard assay procedures. Crop yields were determined by harvesting entire plots and separating peas in a viner. Yields of peas were corrected to the equivalent yield for a tenderometer (TDR) value of 100 by the method of Hagedorn et al. (2).

RESULTS.—Residues of Dexon in 1969 in the soils of treated plots are shown in Table 1. At the Sun Prairie location in the plots treated in the fall of 1966, there was a definite decrease in Dexon levels between May and July during the period the crop was growing and ripening. Levels in the treated plots approached those of the control. Effects of the Dexon levels on yield were still apparent, as peas in treated plots matured later and had lower TDR readings. In addition, yields for treated plots, after adjustments based on equal TDR values, were more than double those for the control.

Dexon levels at planting and after harvesting in plots at six locations treated in October 1968 and May 1969 on a semicommercial scale showed a greater range of values.

The effectiveness of residual Dexon in controlling disease during a season in which conditions were conducive for root rot development is indicated by the data in Table 2. Unfortunately, the stand was poor at two locations, and hot weather at flowering resulted in poor yields for all plots. Nevertheless, substantial

TABLE 1. Residual Dexon [sodium-*p*-(dimethylamino)benzenediazosulfonate] present in Wisconsin soils up to 2.5 years after treatment

Plot location	Dates		Dexon application rate, lb./acre				
	Treatment	Sampling	60	30	15	7.5	0
Arlington	10/68	7/69		1.89[a]	1.56		0.42
	5/69	5/69			6.40	2.08	0.64
	5/69	7/69			1.67	0.72	0.17
Sun Prairie	10/66	5/69	1.90	1.28			0.34
	10/66	7/69	0.79	0.59			0.27
	10/68	7/69		1.17	1.67		0.22
Janesville	10/68	6/69		5.30	2.98		0.73
	10/68	7/69		2.73	3.22		0.36
Beaver Dam	10/68	7/69		2.69	2.13		0.30
South Beaver Dam	10/68	7/69		2.99	1.37		0.27
	5/69	7/69			2.09	1.00	0.59
Marshfield	6/69	6/69			5.24	2.61	0.61

[a] μg Dexon/g oven-dry soil. Values given are averages of three replicate assays on each sample from plots treated in 1968 and 1969 and from replicate plots treated in 1966.

TABLE 2. The effect of prior treatment of field plots with Dexon [sodium-*p*-(dimethylamino)benzenediazosulfonate] on yield of peas at three Wisconsin locations in 1969

Plot location	Treatment		Adjusted yield[a]	
	Date	lb./acre	lb./acre	% of control
Arlington (poor stand)	10/68	30	1,750	656
		15	1,356	508
		0	267	100
	5/69	15	909	485
		7.5	320	284
		0	187	100
Sun Prairie	10/68	30	1,250	520
		15	700	290
		0	240	100
South Beaver Dam	10/68	30	2,253	216
		15	2,776	265
		0	1,045	100
	5/69	15	1,921	147
		7.5	1,606	123
		0	1,309	100

[a] Adjusted to equivalent yield at tenderometer value of 100(2).

increases in yield were effected by treatments applied at Arlington and Sun Prairie where root rot was most severe. At South Beaver Dam, where root rot was less severe, total yields were better, and the effect of treatment was less.

In 1970, yields were again determined for the Arlington plots treated in 1968 and in 1969. Yields of peas from plots treated in fall 1968 at 30, 15, and 0 lb./acre were 2,670, 2,000, and 1,450 lb./acre, respectively. Plots treated at 15, 7.5, and 0 lb./acre in spring 1969 yielded 1,976, 1,554, and 1,450 lb./acre, respectively. The mean increases of 33% 2 years after the 15-lb./acre treatment and 84% 2.5 years after the 30-lb./acre treatment indicate the magnitude of beneficial effects that may be expected where root rot is severe.

A more direct estimate of the population and activity of *A. euteiches* in soil may be made by determining root rot severity (8) for plants grown in pots in which optimum soil moisture for disease development can be provided. Soil collected from each of the Arlington plots at harvest was assayed in August under conditions provided by plant-growth rooms, and a second set of samples collected in October 1969 was assayed under greenhouse conditions. Plants were washed free of soil 3 weeks after planting, and root rot indices and dry wt of plant growth determined. Data for the two sampling dates were averaged and are presented in Table 3. The same pattern prevailed for all sites; lower root rot indices and higher plant yields were associated with Dexon levels equal to or greater than 1 μg/g of soil.

Residues of 3-30 μg/g soil could be expected with application rates of 7.5-60 lb. Dexon/acre. It is clear, therefore, that the amount recovered was only a fraction of that initially added to the soil. The rate at which the chemical disappears from the soil was determined in the laboratory. Silt loam soil from the Arlington plots was treated at a rate equivalent to 30 lb. Dexon/acre, and rates of disappearance of the chemical as influenced by three soil moisture levels were determined (Fig. 1). Dexon disappeared from the soil so rapidly during the initial 30 min when the

TABLE 3. Residues of Dexon [sodium-*p*-(dimethylamino)benzenediazosulfonate], root rot severity, and plant growth in soil collected in August 1969 from field plots previously treated with Dexon

Plot location	Date of treatment	Rate of application	Residual Dexon in soil[a]	Root rot severity index[b]	Dry wt/100 plants
		lb./acre	μg/g	%	*g/100 units*
Arlington	10/68	30	1.89	38	18.5
		15	1.56	48	13.4
		0	0.42	68	11.8
Arlington	5/69	15	1.67	26	16.5
		7.5	0.72	64	8.5
		0	0.17	78	9.3
Sun Prairie	10/68	30	1.67	42	10.6
		15	1.17	54	9.6
		0	0.22	79	9.0
Janesville	10/68	30	2.73	44	16.0
		15	3.22	32	18.6
		0	0.36	94	6.4
South Beaver Dam	10/68	30	2.99	15	17.6
		15	1.37	50	17.3
		0	0.27	98	3.9
	5/69	15	2.09	24	15.4
		7.5	1.00	46	16.2
		0	0.59	90	7.4
Beaver Dam (North)	10/68	30	2.69	25	17.4
		15	2.13	30	15.5
		0	0.30	77	11.1

[a] μg/g oven-dry soil.
[b] Index values range from 0 for healthy plants to 100 when all plants are dead.

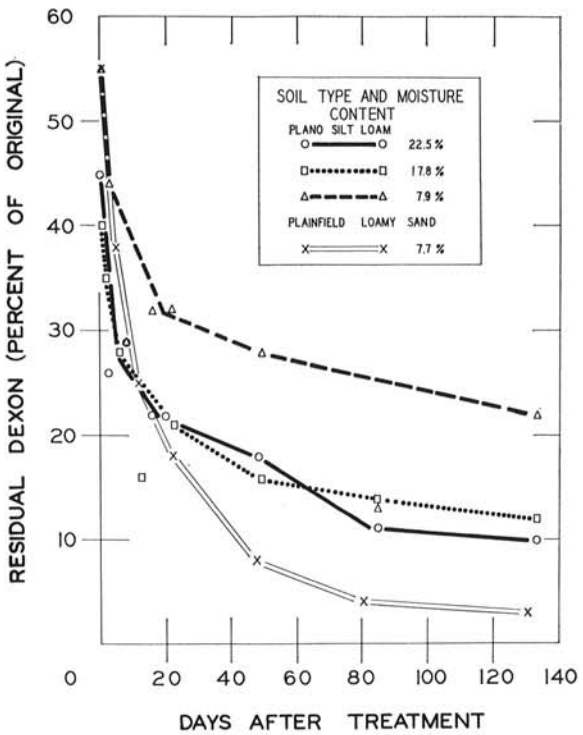

Fig. 1. Persistence of Dexon [sodium-*p*-(dimethylamino)benzenediazosulfonate] in soil treated at the rate of 30 lb./acre as influenced by soil type (Arlington Plano silt-loam and Hancock-Plainfield sand) and soil moisture content.

soil was being mixed that 30-50% of the amount added to the soil could not be detected in the first sample. The initial loss for a sandy loam (Fig. 1) was less, but by 80 days only 5% of the Dexon initially present could be detected. Subsequent loss of Dexon from both soil types occurred at a much slower rate. It was apparent, however, that Dexon levels of field soil samples must have been approached soon after treatment of the soil, and that these levels persisted for a relatively long period.

An indication of the minimum level at which residual Dexon is effective in terms of biological activity towards *A. euteiches* was obtained by determining the concentration at which formation of zoospores was inhibited. Test solutions were applied to microcultures that had been activated for asexual sporulation by the standard washing technique (7). All preparations and operations were carried out with minimum exposure to light, and solutions not being used were kept in the dark. Spore formation was partially inhibited at 1.5 μg Dexon/ml, and only 2 of 11 test cultures showed a trace of sporulation at 3 μg/ml. Sporulation was not affected at 0.75 μg/ml. Activity of Dexon in association with soil was determined by adding 8 g of soil to 10 ml of test solution and shaking the suspension for 30 min in the dark. The supernatant was separated by centrifugation at 27,000 *g* and tested for Dexon activity by the microculture method. In many tests, a substantial number of spores were produced when the original test solution contained 3 μg/ml, and frequently sporulation was not affected when the original concentration was 1.5 μg/ml, indicating that addition of soil effected partial inactivation of the toxicant.

The effect of residual Dexon was also determined by planting peas at intervals after soil was treated and determining root rot severity. Arlington silt loam soil, heavily infested naturally with *A. euteiches,* was treated with Dexon at rates of 30, 15, 7.5, 3.75, and 1.87 μg/g soil, and peas were planted in soil from each treatment 3, 10, 21, and 42 days later. Plants were grown in the greenhouse at ca. 22 C, and the soil was saturated at least every 2nd day. The soil was washed from the roots with high-pressure sprays 30-40 days after planting, and root rot severity and fresh wt of shoots and roots were determined. The effect of the treatments (Fig. 2) tended to be greater when planting was made 3 days after treatment than when it was made after 42 days. This was most evident with 7.5 and 15 μg Dexon/g of soil. Only the 30- and 15-μg/g treatments reduced the severity index below the 50% level, where serious damage would be expected. In terms of plant wt, the 7.5 μg/g level was nearly as effective as the higher dosages. The threshold level for effective treatment was near the 7.5 μg/g level. The difference between effectiveness on root rot severity and effect on plant wt occurred because root rot development at the 7.5 μg/g level was sufficiently slow for foliage symptoms to occur. Had the experiment been continued through flowering and pod production, the threshold would have been nearer the 15 μg/g level.

Discussion.—The amount of Dexon required to inhibit *A. euteiches* depended on the biological process involved and on the type of assay used. Hills & Leach (3) showed that the ED_{50} for linear growth of mycelium on a solid medium over a period of 78.5 hr was 23 μg/ml. When the effect on dry wt of mycelium produced in shake cultures was determined, the ED_{50} was 9.5 μg/ml. In the tests on *A. euteiches* zoospore production reported above, nearly complete inhibition occurred at 1.5 μg/ml. This can be related to the situation in the soil if it is assumed that (i) the available Dexon in the soil is in the soil solution; and (ii) the soil moisture content is 25%. At a level of 2.5 μg Dexon/g of soil, the concentration of Dexon in the soil solution would be about 10 μg/ml. Field threshold levels of ca. 1 μg/g of dry soil would provide a soil solution concentration of about 4 μg/ml. This would only partially control mycelial growth, but should inhibit asexual spore formation. Given the possibility of diverse inocula and types of soil, and of nonuniform distribution of chemical, the field and laboratory data agree reasonably well.

The practical conclusion from these results is that it is necessary to provide and maintain residue levels of ca. 2 μg Dexon/g of dry soil if pea root rot due to *A. euteiches* is to be controlled.

The results of the persistence studies clearly demonstrate that the rate of degradation of Dexon in soil

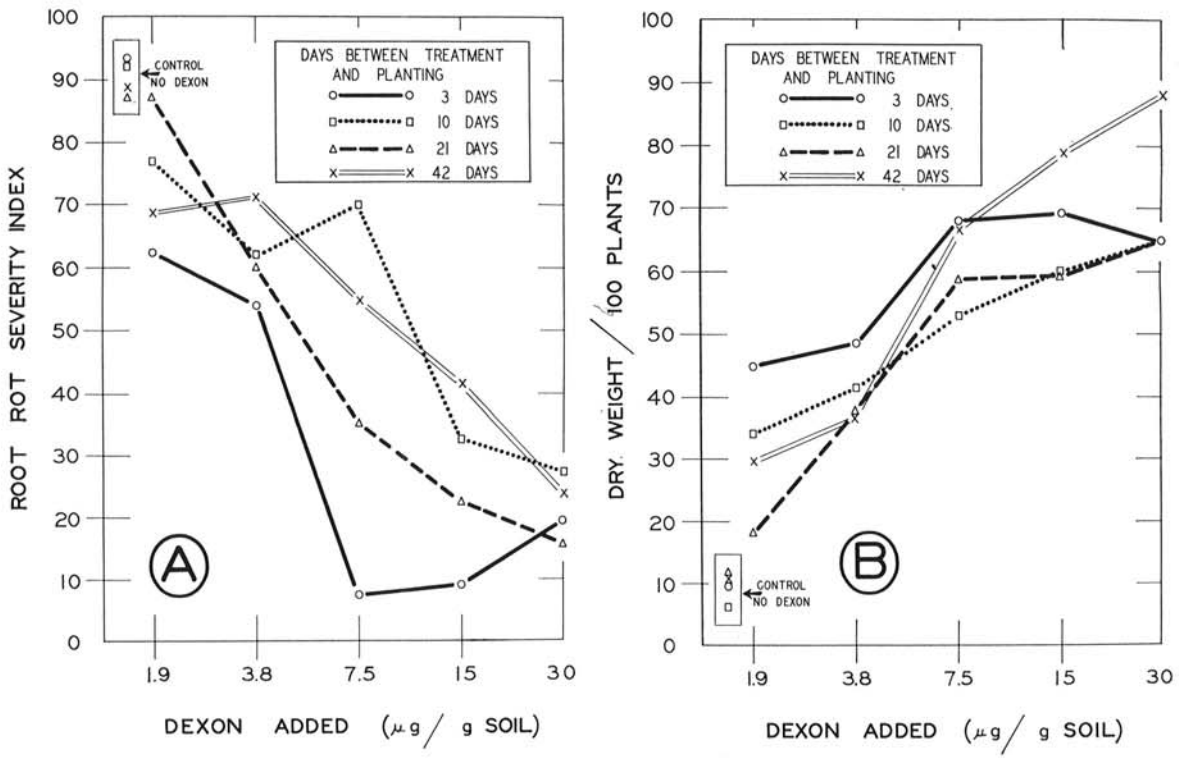

Fig. 2. Effect (under greenhouse conditions) of length of interval between application of Dexon [sodium-*p*-(dimethylamino)benzenediazosulfonate] to soil and planting on **A)** root rot caused by *Aphanomyces euteiches;* and **B)** fresh wt of pea plants grown in soil treated with 0-30 µg Dexon/g soil (equivalent of 0-60 lb. Dexon/acre).

was rapid during the first few hours and days, then became slower so that low levels of the chemical persisted for some time. The actual rate varied with soil type and moisture content, but the trend was the same in all soils. It is evident that assays of soils sampled soon after treatment and then held in storage have to be interpreted carefully, as the Dexon level would decrease while the samples were being stored. However, in samples taken more than 90 days after treatment, loss during subsequent storage of the samples would not be great and the values determined for the various samples should be indicative of relative levels of residual Dexon.

Data such as those above provide a basis for estimating approximate residue levels resulting from any given treatment. One can assume that 50% of the Dexon added would be gone by 1 day, 70% by 1 week, 80% by 3 weeks, and 90% by 3 months; however, this relationship would be affected by such soil factors as composition, temperature, and moisture. On this basis, 30 lb. Dexon/acre (60 µg/g) added in the fall prior to planting would be reduced to around 2 µg/g by spring. The latter level would have a nominal effect on pea root rot. That this treatment effected excellent control under field conditions indicates that Dexon is not only fungistatic, as suggested by Hills & Leach (3) and Kahn & Baker (4), but also that it effects a reduction in inoculum levels. The persistence of the effect of treatment into the second planting season after treatment probably reflects an initial reduction in active propagules and a delayed buildup of inoculum in subsequent plantings of susceptible crops.

The change in effect of the 7.5-lb./acre rate with date of planting in the greenhouse tests and the relative ineffectiveness of the lower rates is explicable, as the soil residues would be expected to drop below the 1 µg/g level within the first 3 weeks after treatment.

The results of Kahn & Baker (4) are also explicable if one assumes that they were dealing with mycelial growth, and that 10 µg Dexon/g soil were required to provide 100% protection for cucumber against *Pythium*. The dosage of 25 µg/g but not the two higher doses would fall below the required level of Dexon during the brief period of their experiment. The potato disc assay, which is less sensitive to Dexon than is the damping-off test [not more so as the authors indicate (4)], apparently required between 10 and 15 µg Dexon/g soil to reduce the recovery of *Pythium* by 50% when inoculum was used at the 4% rate. The difference in sensitivity between the potato disc and the damping-off assay probably reflects the difference in time available for the absorption of an inhibitory dose of the toxicant.

There is thus some rationale in the use of Dexon as a soil treatment. It must be present at the site of infection at or above a critical level (1-2 µg/g of soil for *A. euteiches*). For pea root rot control, a uniform distribution of Dexon through the soil is required, as the entire root system is susceptible to infection.

Broadcast treatments of 7.5 lb. or less Dexon/acre will provide such short periods of protection as to be ineffective. Only when this rate is applied to the seed coat or to a furrow, or otherwise restricted to an infection site, is the concentration of Dexon sufficiently high to be effective and practical.

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