

# Effect of the Nematocides Nemagon and DD on Mineralization, Nitrification, Soil Microbial Population, and Soil Fertility Status of Two Tropical Soils<sup>1</sup>

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## ABSTRACT

Nitrification was inhibited in an acid soil by 60 p/m or more of Nemagon; the  $\text{NH}_4^+$  oxidizing bacteria being affected but not the *Nitrobacter*, as  $\text{NO}_2^-$  accumulation was not observed. Nitrogen mineralization was not affected even at 20,000 p/m of Nemagon and an increase in available P, Mn, and a rise in pH occurred in both acid and alkaline soils. Extractable zinc increased in the acid soil.

DD was extremely toxic to the nitrifying bacteria; much more so than Nemagon. It inhibited  $\text{NH}_4^+$  oxidizing bacteria but not the *Nitrobacter*. Nitrogen mineralization was progressively depressed with increasing levels of DD. It resulted in a decrease in pH and extractable P. Mn increased in both the acid and alkaline soils, whereas the effect on Fe and Mg were inconsistent. Bacteria, actinomycetes and fungi were suppressed drastically. At lower levels bacterial population was increased, indicating a possible role in detoxification of DD.

## INTRODUCTION

Soil fumigants are chemical pesticides widely employed to control nematodes. Many such chemicals are not entirely selective. Along with control of nematodes other microorganisms may be affected. This side effect may or may not be desirable from the point of view of crop production.

The nematocides Nemagon (1,2-dibromo-3-chloropropane) and DD (a 50-50 mixture of 1,3-dichloropropene and 1,2-dichloropropane) have been used extensively to control nematodes on pineapple in Puerto Rico. DD also has been used for this purpose in Hawaii, where soil side effects, such as restricted nitrification (2, 6) and altered availability of Mn, P, and Fe (5) have been reported.

Due to restricted nitrification, DD application results in increased soil ammonium. Pineapple plants, however, do not seem to be adversely affected as they use ammonium as well as nitrate forms of nitrogen (4). A DD application results in increased ammonium nutrition leading to faster growing, greener and more succulent plants. Crops able to utilize  $\text{NH}_4^+$ -N may grow normally, but such crops as tomato, celery and lettuce, which

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require  $\text{NO}_3^-$ -N, may be adversely affected if grown on fields treated previously with DD (1).

The effects of Nemagon on soils have not been published. The side effects of DD as noted in Hawaiian soils might not occur in Puerto Rico because of differences in climate and soil conditions.

The effects of Nemagon and DD on microbial populations, soil nitrogen mineralization, nitrification, and certain soil plant nutrients were therefore determined in two Puerto Rico soil types.

#### MATERIALS AND METHODS

Composite surface soil samples (0–20 cm) were collected from three sites: a) Bayamón sandy clay loam from a cultivated pineapple field, b) Bayamón sandy clay loam from a virgin site, and c) San Antón loam from a virgin site. The first two soils are acid and were collected from a pineapple growing area in Manatí. The San Antón loam, collected from the

TABLE 1.—Some characteristics of the soils used

Soil type	pH	Organic matter	Sand	Silt	Clay	CEC
			<i>Percent</i>			<i>Me/100 g</i>
Bayamón silty clay loam (cultivated)	4.1	1.5	56	9	35	5.1
Bayamón silty clay loam (virgin)	4.3	1.9	61	12	27	6.7
San Antón loam (virgin)	7.7	5.1	42	33	25	34.1

southern coast of the Island near Salinas, is alkaline and well known for its high fertility. Some of the important characteristics of these soils are given in table 1.

The soils were air-dried in a greenhouse and passed through a 2 mm sieve. Soil samples of 350 g each were adjusted first to near neutral pH by addition of  $\text{H}_2\text{SO}_4$  or  $\text{Ca}(\text{OH})_2$ . To each soil sample, 3 ml of soil inoculant from a known rapidly nitrifying soil was applied to ensure the presence of nitrifiers. The inoculant was obtained by shaking 500 g of soil with 1,000 ml of distilled water and filtering it through a filter paper. Half the samples received 100 p/m N as  $(\text{NH}_4)_2\text{SO}_4$  in 2 ml of aqueous solution and the remainder served as controls. Upon drying, the samples were mixed thoroughly to distribute the additives uniformly throughout the soil.

Nemagon levels were 0, 6 (the recommended field rate,) 60 and 600 p/m. DD levels were 0, 200 (the recommended field rate), 2,000 and 20,000 p/m, on an active ingredient basis.

Nemagon was applied as follows: The 350 g soil samples, after pH adjustment and N treatment, were poured into glass bottles 17.8 cm tall and

5 cm in diameter. The necessary amount of Nemagon in 2 ml distilled water solution was injected 15 cm deep in the center of the soil by a hypodermic syringe. Care was taken to inject the chemical at the above mentioned depth in order to simulate field treatment. As the needle was withdrawn enough distilled water was injected at three sites near the edge of the bottle so as to attain quickly field capacity. Water was injected using a 30 ml Manopet syringe to which a needle 15 cm long was attached.

DD is insoluble in water. It thus was placed in bottles having only a 2.5 cm layer of soil, the remainder of the soil sample being poured in afterward. Water was injected as in Nemagon soils to attain field capacity.

After applying the treatments, the mouths of the bottles were covered with "Parafilm" which prevents moisture escape but permits gaseous exchange. The treatments were replicated three times and were incubated in the laboratory at  $23 \pm 1^\circ \text{C}$ . A set of treatments were separated at fixed intervals for determination of pH, mineral nitrogen, and other soil plant elements. After the soil was removed and air-dried for a day in the greenhouse, a 50 g soil sample from each treatment was extracted with 250 ml of 1.0 N KCl. Ammonium nitrogen in the extract was determined by distillation with MgO into 2 percent boric acid, followed by titration with standardized HCl. Nitrite nitrogen was determined colorimetrically by the method of Prince (3). A second distillation with Devarda's alloy of the aliquot used earlier for  $\text{NH}_4^+$  determination gave the value for  $\text{NO}_2^- + \text{NO}_3^-$ , from which  $\text{NO}_3^-$  was obtained by subtracting the value for  $\text{NO}_2^-$ . The pH was determined by glass electrodes in 1:1 soil:water suspension.

Calcium, magnesium and potassium were extracted from a 20 g soil sample with neutral ammonium acetate and analyzed in the Beckman flame photometer. Iron was extracted from a 20 g soil sample with neutral ammonium acetate. A 100 ml aliquot was evaporated to dryness and digested with nitric and perchloric acids until a white residue was obtained. A Beckman DU spectrophotometer was used to make the analysis. Phosphorus was extracted from a 20 g soil sample by means of a sodium acetate solution of pH 4.8 and analyzed in a Technicon Autoanalyzer. Zinc and manganese were extracted from a 20 g soil sample, with redistilled water and analyzed in a Jarell-Ash atomic absorption spectrophotometer.

Microbial population in the soil samples was determined by dilution plate technique. Soil bacteria and actinomycetes were grown on sodium albuminate agar medium and soil fungi were grown on Martin's Rose Bengal medium with streptomycin.

## RESULTS

The virgin Bayamón soil was slightly higher in pH, organic matter content, and cation exchange capacity than soil collected from a nearby pineapple field (table 1) as expected. San Antón soil, one of the most fertile

soils of the Island, had an alkaline pH and was much higher in organic matter content and cation exchange capacity than the two acid soils.

Effects of the three rates of Nemagon on nitrification in the three soils are shown in table 2. In the two untreated acid soils nitrification was almost complete in 8 weeks whereas in the untreated San Antón soil nitrification was completed in only 2 weeks. There was no significant difference in nitrification between the two acid soils, indicating that cropping or cultivation did not change their nitrifying capacity. When 6 p/m of Nemagon was applied to the Bayamón soil it strongly inhibited the nitrifying bacteria, as evidenced by a smaller amount of  $\text{NO}_3^-$ -N after 8 weeks. At 60

TABLE 2.—Effect of Nemagon (p/m) on nitrification (p/m of  $\text{NO}_3^-$ -N) of 100 p/m added  $\text{NH}_4^+$ -N

Soil type	Nemagon added	$\text{NO}_3^-$ -N formed after (weeks)			
		2	4	8	16
		<i>P/m</i>			
Bayamón silty clay loam (cultivated)	0	0	47.3	97.5	93.2
	6	0	2.9	26.7	85.5
	60	0	0	4.1	2.3
	600	0	0	6.4	0
Bayamón silty clay loam (virgin)	0	3.6	37.6	95.1	104.4
	6	0	6.4	34.8	75.2
	60	0	0	0	56.6
	600	0	0	0	0
San Antón loam (virgin)	0	100.0	100.6	106.2	90.5
	6	88.7	91.9	99.6	92.4
	60	26.3	81.6	89.9	91.2
	600	0	18.1	22.5	17.2

p/m of Nemagon, no nitrification took place up to 16 weeks in the cultivated Bayamón soil. Nitrification had started by this time in the virgin soil, suggesting that the effect of the chemical was disappearing in this soil, thus permitting reestablishment and activation of nitrifying bacteria. In the San Antón soil the recommended field rate had hardly any effect on nitrification. Nemagon at 60 p/m inhibited nitrification significantly, and at 600 p/m very little nitrification occurred even after 16 weeks of incubation. Apparently ammonium-oxidizing bacteria were affected but not the *Nitrobacter*, as no  $\text{NO}_2^-$  accumulation was observed.

Nitrogen mineralization is the production by the heterogeneous microbial population of mineral nitrogen such as  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  (which are easily available for plant use) from the essentially unavailable organic forms of nitrogen in the soil. The results (table 3) show, in general, that application of Nemagon did not affect nitrogen mineralization, thereby

TABLE 3.—*Effect of Nemagon on N mineralization*

Soil type	Nemagon added	Mineral N <sup>1</sup> formed after (weeks)	
		2	16
		<i>P/m</i>	
Bayamón silty clay loam (cultivated)	0	118	135
	6	116	146
	60	108	122
	600	124	102
Bayamón silty clay loam (virgin)	0	56	74
	6	50	71
	60	54	76
	600	37	72
San Antón loam (virgin)	0	113	149
	6	105	139
	60	117	141
	600	102	135

<sup>1</sup> Mineral N = NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup>; no NO<sub>2</sub><sup>-</sup> observed.

TABLE 4.—*Effect of Nemagon on soil fertility status 8 weeks after application*

Soil type	Nemagon added	P	Mn	Zn	Mg	Fe	pH
		<i>P/m</i>					
Bayamón silty clay loam (cultivated)	0	3.2	1	0.16	100	1	5.8
	6	4.0	1	0.18	100	1	5.9
	60	5.0	1	0.20	188	1	6.6
	600	5.4	24	0.63	100	1	6.6
Bayamón silty clay loam (virgin)	0	3.0	trace	0.12	138	1	6.1
	6	3.0	trace	0.25	113	1	6.0
	60	3.0	1	0.13	113	1	6.2
	600	4.0	27	0.40	113	1	6.5
San Antón loam (virgin)	0	56.6	trace	0.08	1031	2	7.1
	6	57.4	trace	0.13	1031	2	7.2
	60	61.6	trace	0.08	1044	2	7.1
	600	60.6	3	0.08	1050	2	7.2

suggesting that nonspecific soil microbes are quite tolerant to this nematocide.

Increased Nemagon raised the pH and available phosphorus in each soil (table 4). A marked increase in available Mn was noted in the two acid soils receiving 600 p/m of Nemagon; but there was little increase in the San Antón soil. Similarly, a significant Zn increase was noticed in the acid soils receiving 600 p/m of Nemagon, but no such increase occurred in the San Antón soil. Magnesium and Fe levels remained unaffected by Nemagon.

Nitrification almost ceased for 8 weeks in all the soils after receiving 200

TABLE 5.—Effect of DD on nitrification of 100 p/m applied  $NH_4^+$ -N

Soil type	DD added	NO <sub>2</sub> -N formed after (weeks)		
		2	4	8
		<i>P/m</i>		
Bayamón silty clay loam (cultivated)	0	11.2	63.5	89.5
	200	2.8	1.4	0.4
	2,000	0	0	0
	20,000	3.1	0	0
Bayamón silty clay loam (virgin)	0	1.6	0	84.6
	200	0.9	0	4.3
	2,000	1.6	0	0.3
	20,000	0	0	1.8
San Antón loam (virgin)	0	79.4	88.1	93.0
	200	0	0	2.8
	2,000	0	0	2.8
	20,000	0	0	0

TABLE 6.—Effect of DD on N mineralization

Soil type	DD added	Mineral N <sup>1</sup> formed after (weeks)		
		2	4	8
		<i>P/m</i>		
Bayamón silty clay loam (cultivated)	0	50	55	67
	200	43	58	61
	2,000	34	40	42
	20,000	34	41	40
Bayamón silty clay loam (virgin)	0	45	68	67
	200	44	64	71
	2,000	21	36	41
	20,000	23	32	33
San Antón loam (virgin)	0	78	106	128
	200	74	109	124
	2,000	67	88	105
	20,000	66	75	79

<sup>1</sup> Mineral N =  $NH_4^+$  +  $NO_3^-$ ; no  $NO_2^-$  observed.

p/m DD (table 5). During the same period in the untreated acid soils nitrification had progressed 85 to 90 percent, but in the alkaline soil it was completed in 2 weeks. The ammonium oxidizing bacteria appeared to be inhibited but not the *Nitrobacter* as no accumulation of  $NO_2^-$ -N occurred.

Whereas Nemagon had no effect on N mineralization, DD progressively inhibited the process (table 6).

Research conducted in this laboratory has consistently shown that San Antón soil has a very high nitrifying and detoxifying capacity. DD has been the most toxic of the pesticides tested in this laboratory to nitrifying

TABLE 7.—Changes in soil microbial population 8 weeks after application of DD

Soil Type	DD added	Bacteria	Actinomycetes	Fungi
	<i>P/m</i>	× 100,000	× 100,000	× 1,000
Bayamón silty clay loam (cultivated)	0	134	6	33
	200	248	18	3
	2,000	5	0	0
	20,000	0	0	0
Bayamón silty clay loam (virgin)	0	82	98	49
	200	57	43	3
	2,000	1	1	0
	20,000	1	0	0
San Antón loam (virgin)	0	116	30	50
	200	240	31	25
	2,000	204	0	0
	20,000	0	0	0

TABLE 8.—Effect of DD on soil fertility status

Soil type	DD added	pH	P	Mn	Fe	Mg	Zn
Bayamón silty clay loam (cultivated)	0	6.4	6	0	0.52	50	0
	200	6.7	6	0	1.04	38	trace
	2,000	6.3	5	0	0.50	38	trace
	20,000	5.5	2	24	0.97	38	trace
Bayamón silty clay loam (virgin)	0	6.4	7	0	1.50	100	0
	200	6.7	9	0	0.95	100	0
	2,000	6.4	8	18	1.50	138	0
	20,000	5.8	4	240	2.00	200	trace
San Antón loam (virgin)	0	7.2	64	0	1.1	988	0
	200	7.2	64	0	0.6	1000	0
	2,000	7.1	65	0	0.3	1088	0
	20,000	7.0	59	61	0.5	1100	0

bacteria and heterogenous microbial population such as N mineralizers. It was thus considered essential to investigate its effects in detail on the different classes of soil microbes. The results of microbial count recorded 8 weeks after DD application to soils are given in table 7. The DD eliminated all types of microbes in every soil when applied at the 2000 p/m level. This effect was more drastic in acid than alkaline soils. At the 200 p/m rate the bacterial population increased indicating that bacteria may be using this chemical in their metabolism, thus instrumental in its detoxification. Bacteria were suppressed at rates higher than 200 p/m, however.

Analyses of soil fertility elements in soil samples 8 weeks following DD application showed that pH and available P decreased with high rates of DD (table 8). This is in contrast to the findings of Smith (5), who reported in-

creased pH and available P following DD application. At high levels DD increased available Mn.

### RESUMEN

A dos suelos ácidos (arcilla arenosa Bayamón) y a uno alcalino (San Antón lómico) se les aplicaron los nematocidas Nemagon y DD, el primero a concentraciones de 0, 6, 60 y 600 p/m y el segundo a las de 0, 200, 2,000 y 20,000 p/m. Muestras de los suelos tratados se incubaron en el laboratorio en condiciones de humedad equivalentes a las de campo, a una temperatura de  $23^{\circ} \pm 1^{\circ}$  C. Se analizaron las muestras a distintos intervalos para detectar la presencia de nitrógeno mineral y otros elementos nutritivos y además determinar el efecto sobre la mineralización del nitrógeno, la nitrificación del  $\text{NH}_4^+\text{-N}$  añadido, el estado de fertilidad del suelo y la población microbiana.

Aplicado a razón de 60 p/m en el suelo ácido, el Nemagon inhibió la nitrificación. Este efecto fue aumentando progresivamente a medida que subía el nivel de Nemagon. Las bacterias oxidantes del amonio se afectaron, pero no así las *Nitrobacter*, ya que no se observó acumulación de  $\text{NO}_2^-$ . Tampoco hubo efecto alguno sobre la mineralización del nitrógeno. La aplicación de Nemagon aumentó el contenido en P y Mn asimilables y también subió el pH. En los suelos ácidos también aumentó el contenido en Zn extractible.

El DD produjo efectos extremadamente tóxicos sobre las bacterias nitrificantes, mucho más que los que tuvo el Nemagon. Restringió a las bacterias oxidantes del amonio, pero no así a las *Nitrobacter*. Suprimió, en proporción a la cantidad usada, la mineralización del nitrógeno. La aplicación de DD resultó en descenso en pH, así como en el contenido de P extractible. En todos los suelos el nivel de Mn aumentó, pero con respecto a Fe y Mg los resultados fueron inconsistentes. DD suprimió drásticamente a las bacterias, los actinomicetes y los hongos del suelo. Aplicado a menores concentraciones, el DD produjo un aumento en la población bacteriana, lo cual indica, posiblemente, que las bacterias juegan un papel importante en la desintoxicación del DD.

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