

disorganized tiny cells grouped around the giant cells. Giant cells formed in the pericycle and cortical tissue were usually spherical, whereas those formed in vascular tissue were elongated. Mechanical damage was caused by developing females and egg masses, which was expressed as a semicircle of compressed small cells around the posterior part of the mature females. When pepper was inoculated with eggs from a root-knot nematode population previously attacking *Urena lobata*, giant cells were completely full of nuclei (usually over 40 per cell).

RESEARCH PAPERS — TRABAJOS DE INVESTIGACION

THE NON-TARGET EFFECTS OF PENTACHLORONITROBENZENE ON PLANT PARASITIC AND FREE-LIVING NEMATODES [EFECTOS ALEATORIOS DE PENTACHLORONITROBENCENO SOBRE NEMATODOS FITOPARASITOS Y DE VIDA LIBRE]. J.R. Adams, R. Rodríguez-Kábana, and P.S. King, Botany and Microbiology Department, Agricultural Experiment Station, Auburn University, Auburn, Alabama 36830, U.S.A.

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ABSTRACT

The effects of pentachloronitrobenzene (PCNB) on plant parasitic and free-living nematodes were studied *in vitro* and under greenhouse and field conditions. PCNB exerts a direct toxic effect on larvae of *M. incognita* (Kofoid and White) Chitwood, *Helicotylenchus dihystera* (Cobb) Sher, *Hoplolaimus galeatus* (Cobb) Sher, *Rotylenchulus reniformis* Linford and Oliveira, mononchoid and dorylaimoid nematodes and the microbivorous nematode *Pelodera chitwoodi* (Bassen) Dougherty. Toxic effects were most pronounced on mononchoid and dorylaimoid nematodes and on *P. chitwoodi*. Greenhouse studies indicated that PCNB can cause an increase or decrease in nematode numbers depending on the nematode species involved and the concentration of PCNB used. The nematode species most likely to increase in numbers when PCNB is applied are *Pratylenchus scribeneri* Steiner, *P. brachyurus* (Godfrey) Filip. and Schuur, Stek., *H. dihystera*, and *H. galeatus*. The effect on *M. incognita*, dorylaimoid, mononchoid and saprophagous nematodes was usually a decrease in numbers. In some instances nematode numbers increased at field application levels and decreased at higher levels of PCNB. Results from field studies generally coincided with those from greenhouse experiments.

Key Words: Soil fungicides, nematode control, cotton, *Gossypium hirsutum*, chloronitrobenzenes, quintozone, Terraclor®.

INTRODUCTION

Pentachloronitrobenzene (PCNB) is a selective soil fungicide principally manufactured by the Olin Corporation and marketed under the trade name Terraclor®. It is

used extensively in the Southeast and throughout the United States for the control of *Rhizoctonia solani* and *Sclerotium rolfsii*; the compound has been ineffective in controlling diseases caused by *Fusarium*, *Pythium*, and *Verticillium* (4). In addition, it has been found that the application of PCNB actually increases the incidence of disease caused by species of *Pythium* and *Fusarium* (3) and that this increase is probably due to the detrimental action of PCNB upon fungi competitive to these pathogens (2).

Relatively little work has been done to determine the effects of PCNB upon other soil-inhabiting organisms, especially nematodes. Boswell (1) found that PCNB increased the average numbers of *Pratylenchus brachyurus* per gram of peanut shells. A similar increase in numbers of *Pratylenchus penetrans* was reported by Rich and Miller (9) and Miller and Waggoner (5) following PCNB application to soils planted with strawberries. Other workers have reported that PCNB has nematicidal properties (7, 16, 17, 18). Similar compounds such as the tetrachloronitrobenzenes were shown recently to be nematicidal (11, 12). Because of the increasing use of PCNB in Alabama for control of Southern blight in peanuts and seedling diseases in soybeans and cotton, an in-depth study was performed to determine the action of PCNB on several plant parasitic and non-parasitic nematode species.

The objectives of this study were to determine: 1) the effects of PCNB on plant parasitic and free-living nematodes *in vitro*, 2) the effects of PCNB on nematodes in greenhouse pots at various rates, and 3) the effects of PCNB on nematode populations at various field sites.

MATERIALS AND METHODS

In Vitro Studies.

Technical grade PCNB (Olin Corporation) was dissolved in xylene and applied to attapulgitic clay granules at rates of 0, 1, 2, 4, 6, 8, and 10% (w/w). Approximately 2800 ug of each formulation was added to each of four 2-cm diameter syracuse dishes containing 1 ml water and five test nematodes. Amounts of PCNB present in each dish ranged from 0 ppm to 276 ppm. Nematodes were examined at intervals under a binocular dissecting microscope to determine the number of live nematodes. A nematode was considered to be dead if it gave no response to touch on the nerve ring area with a needle. Nematodes tested were larvae of *Meloidogyne incognita* (Kofoid & White) Chitwood and adults of *Rotylenchulus reniformis* Linford and Oliveira, *Helicotylenchus dihystera* (Cobb) Sher, *Hoplolaimus galeatus* (Cobb) Sher, *Pelodera chitwoodi* (Bassen) Dougherty, and monochoid and dorylaimoid nematodes.

Although PCNB is practically insoluble in water, it was necessary to determine if observed toxicity to nematodes was a result of a contact or solute effect. For this study, 2.5 g of the 0 and the 10 percent granules were added to 20 ml distilled water and allowed to stand for 48 hours. A no granule control of distilled water was treated in the same manner. The supernatant solution was drawn off and 1 ml of each solution added to each of four 2-cm diameter syracuse dishes. The spiral nematode *Helicotylenchus dihystera* was used in this study and the treatment was the same as that in the previous *in vitro* study.

Greenhouse Studies.

PCNB was applied to soil in greenhouse pots as the commercial preparation Terraclor® 10G (Olin Corporation). Two experiments were set up for each of two soil types. A Dothan sandy loam was obtained from a field in which cotton, corn, and soybeans had been rotated and a Norfolk sandy loam was obtained from a field which had been under cotton cultivation for a number of years. PCNB was applied in each

experiment at rates of 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.5, 1.0, and 2.0 grams/kg soil. In terms of kg/ha these rates were equivalent to 0, 56, 112, 224, 448, 896, 1120, 2242, and 4483 assuming that PCNB was applied broadcast and disked to a 15 cm depth. The first experiments for each soil type contained no plants and the other two were with soil planted to DPL-16 cotton. The pots containing no plants were sampled at two-week intervals for a period of 6 weeks so that the effects of PCNB on the nematodes could be determined with time.

The pots containing cotton were sampled after 8 weeks. Fifty cm³ of soil was taken from each pot and the nematodes extracted by the molasses flotation sieving method (13, 14). A subjective root-knot index was compiled from the roots of cotton plants in each pot after careful washing of the plant from the soil. The index was based on a 1-6 scale with 1 representing little or no galling and 6 very extensive galling. Phytotoxic effects were also assessed by determining the root and top weights of the plants.

Field Experiments.

Two field experiments were set up in separate locations in Alabama. Experimental sites chosen were located at the Wiregrass Substation at Headland, and the Sand Mountain Substation at Crossville. PCNB (Terraclor® 10G formulation) was applied broadcast and disked to a 15 cm depth. Plots were arranged in a randomized complete block design and PCNB was applied at rates of 0, 11, 22, 34, 45, and 56 kg active ingredient/ha. Eight replications of each treatment were employed. Plots were planted to Bragg soybeans immediately following application. The plots were sampled after 8 weeks by taking approximately twenty 15 cm deep x 2.5 cm diam. cores of soil at regular intervals from the root zone in each plot. Extraction procedures were the same as those used for the greenhouse studies. Yield data from the plots also were recorded.

Statistical Analyses.

All data were analysed following standard procedures for analysis of variance and by the least significant difference test (15). Unless otherwise specified differences cited in the text were significant at the 5% or lower level of probability.

RESULTS

In Vitro Studies.

In vitro granule studies indicated that PCNB is toxic to all nematodes studied, but lethal doses varied according to the species. The plant parasitic nematodes *H. dihystera* (Fig. 1A), *H. galeatus* (Fig. 1B), and *R. reniformis* (Fig. 1C), showed much more tolerance to PCNB than the microbivorous nematode, *P. chitwoodi* (Fig. 1D), or the free-living dorylaimoid and mononchoid nematodes which behaved similarly to *P. chitwoodi*. Larvae of the root-knot nematode, *M. incognita*, showed approximately half the tolerance of the other plant parasitic types (Fig. 1E).

The spiral nematode *H. dihystera* was not adversely affected by PCNB present in the aqueous supernatant obtained from the 10% PCNB attapulgitic granules. In fact, there were more nematodes alive in the PCNB-supernatant than in the supernatant from the granule and in the no-granule control.

Greenhouse Studies-Tests in unplanted soil

Dothan loamy sand. In this soil type, numbers of *M. incognita* larvae were reduced significantly at fungicide levels of 0.025, 0.05, 1.0, and 2.0 g/kg after 4 weeks (Table 1); no difference in numbers was noted after 2 and 6 weeks.

At the 2-week sampling date, numbers of *Helicotylenchus dihystera* had decreased at treatment rates of 0.1, 0.2, 1.0, and 2.0 g/kg (Table 1). After 4 weeks numbers of these nematodes were reduced at PCNB rates of 0.4, 1.0, and 2.0 g/kg, but were

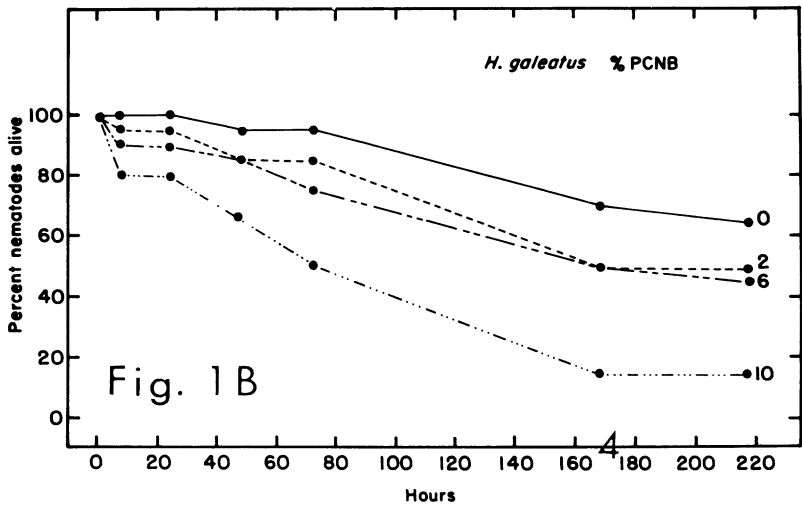
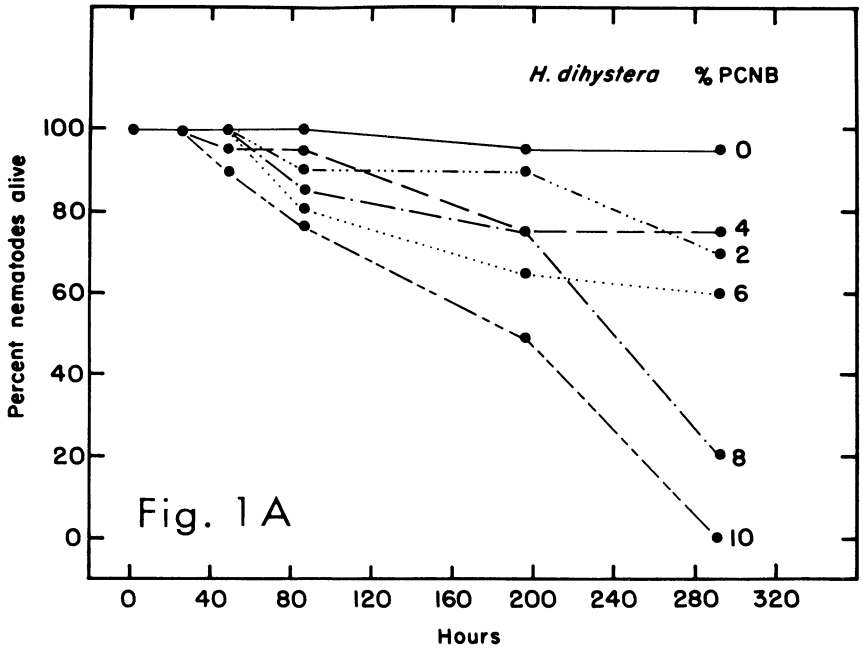
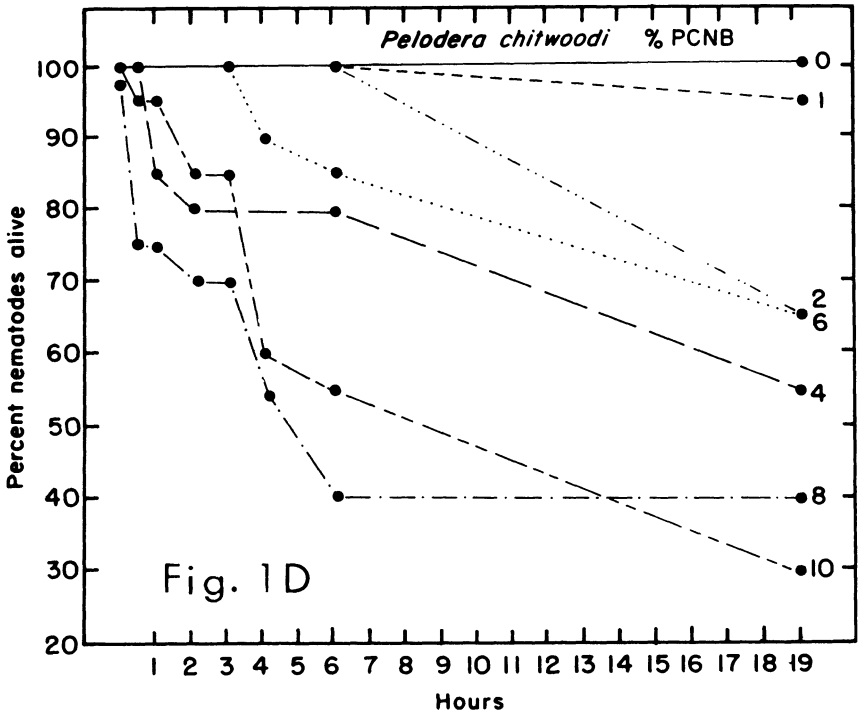
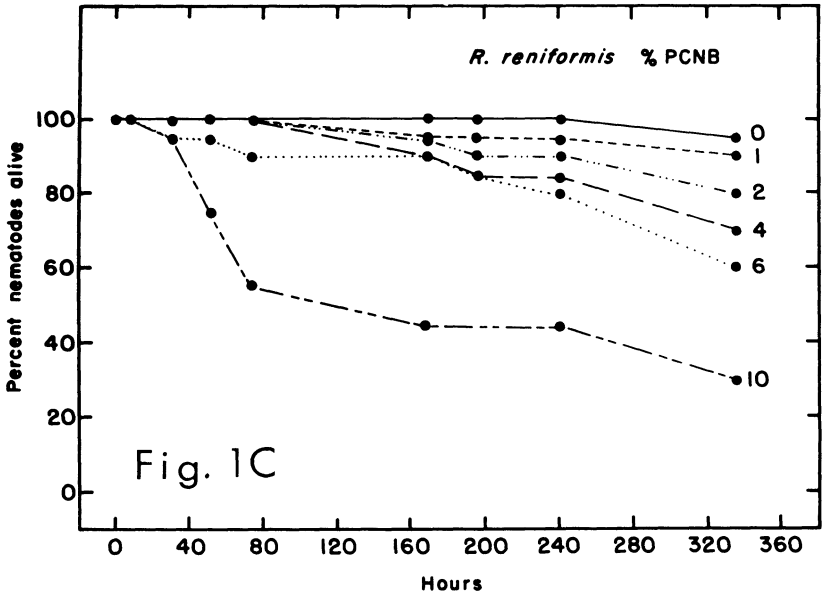


Fig. 1. *In vitro* effect of PCNB on selected nematode species: A. *Helicotylenchus dihystra*; B. *Hoplolaimus galeatus*; C. *Rotylenchulus reniformis*; D. *Pelodera chitwoodi*; E. *Meloidogyne incognita* larvae.



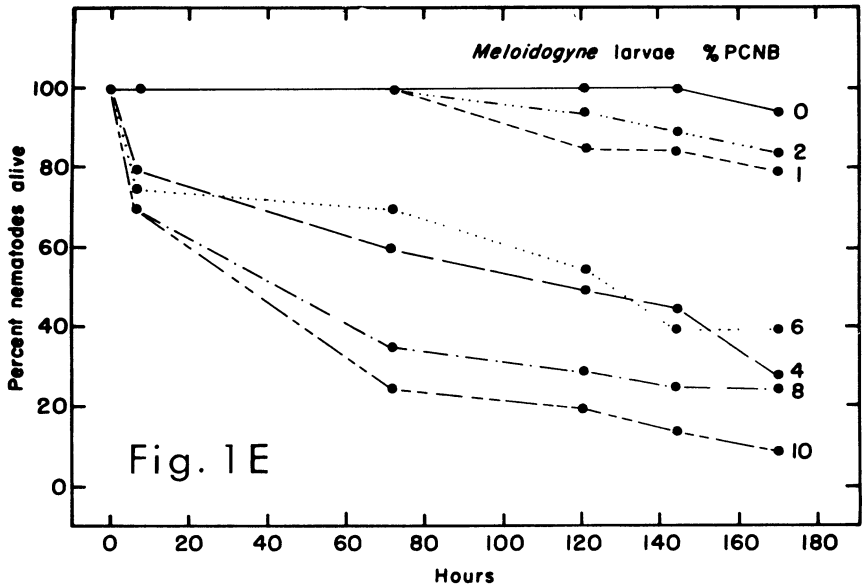


Table 1. The effects of PCNB on plant parasitic nematodes in a Dothan sandy loam at 2, 4, and 6 weeks.

PCNB g/kg soil	Numbers per 50 cm ³ soil					
	Larvae of <i>Meloidogyne incognita</i>			<i>Helicotylenchus</i> <i>dihystera</i>		
	2	4	6	2	4	6
0	13.3	10.875	6.3	60.4	155.5	112.2
0.025	9.4	3.4	4.4	47.1	240.5	169.9
0.05	15.4	6.6	5.5	73.0	212.0	126.8
0.10	9.4	8.6	4.8	33.9	163.9	90.8
0.20	9.6	10.9	3.9	33.3	121.6	72.0
0.40	9.4	8.5	5.6	49.5	102.6	82.1
0.50	11.9	6.6	3.8	43.5	121.6	65.4
1.00	4.9	4.5	3.3	11.1	85.6	57.9
2.00	4.3	1.8	1.8	16.9	64.5	35.1
LSD (P=0.05)	4.0	4.0	N.S.	21.2	43.6	28.9
LSD (P=0.01)	5.3	5.3	N.S.	28.2	58.0	38.4

Table 2. The effects of PCNB on *Hoplolaimus galeatus* in a Norfolk sandy loam after 2, 4, and 6 weeks.

PCNB g/kg soil	Numbers per 50 cm ³ soil		
	2	4	6
0	44.9	85.1	26.0
0.025	50.1	60.6	30.8
0.05	53.0	66.0	21.9
0.10	52.9	59.8	15.4
0.20	63.6	52.1	11.5
0.40	50.4	35.3	11.6
0.50	61.5	49.8	12.1
1.00	47.9	38.5	5.5
2.00	46.9	36.6	5.4
LSD (P=0.05)	N.S.	22.9	10.7
LSD (P=0.01)	N.S.	30.5	14.2

increased significantly at rates of 0.025 and 0.05 g/kg. Sampling after 6 weeks showed a significant reduction in numbers of *H. dihystera* at fungicide rates of 0.2 g/kg or above, and an increase in numbers at 0.025 g/kg.

Norfolk sandy loam. In this soil type numbers of *Helicotylenchus dihystera* and *M. incognita* larvae were too low to establish any significant trend. A reduction in the numbers of *H. galeatus* was observed at PCNB levels of 0.025 and 0.1 g/kg and above after 4 weeks (Table 2); however after 6 weeks, only treatment levels of 0.2 g/kg and above showed a decrease in their numbers.

Tests with cotton.

Dothan loamy sand. A significant reduction in the number of larvae of *M. incognita* was obtained with PCNB rates of 0.05, 0.1, and 0.4 g/kg and above (Table 3), and the root-knot gall index decreased significantly at treatment levels of 0.1 g/kg and above (Table 4); no phytotoxic effects of PCNB on cotton was indicated from root weights of the plants. Numbers of *H. dihystera* were reduced with treatments of 0.025 g/kg and higher rates (Table 3).

Norfolk sandy loam. Numbers of *M. incognita* larvae in this soil type were reduced by PCNB rates of 0.1 and 0.4 g/kg and above (Table 5). The root-knot index showed a significant reduction at levels 0.025 and 0.1 g/kg level or higher. No phytotoxic effect was evident from the root weights of the plants (Table 6).

H. dihystera was reduced in number at all treatment levels of PCNB (Table 5),

Table 3. The effect of PCNB on plant parasitic nematodes in a Dothan sandy loam planted to cotton.

PCNB g/kg soil	Numbers per 50 cm ³ soil	
	<i>Meloidogyne incognita</i>	<i>Helicotylenchus dihystrera</i>
0	41.9	293.1
0.025	25.1	170.6
0.05	10.0	123.1
0.10	9.0	80.9
0.20	24.1	55.3
0.40	3.6	66.1
0.50	10.0	59.0
1.00	2.3	36.5
2.00	4.0	36.4
LSD (P=0.05)	20.3	68.9
LSD (P=0.01)	27.1	91.8

whereas numbers of *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuur, Stek increased at levels exceeding 0.025 g/k (Table 5), and *H. galeatus* increased at 0.20 g/kg (Table 5).

Field Studies.

The following nematodes were observed to be present at the Wiregrass Substation: *Meloidogyne arenaria* (Neal) Chitwood, *Pratylenchus brachyurus*, *Criconemoides ornatus*, *H. dihystrera*, *Tylenchus* sp., and mononchoid, dorylaimoid, and saprophagous nematodes. At the Sand Mountain Substation *Pratylenchus* sp., *Helicotylenchus* sp., *Tylenchus* sp., and monochiod, dorylaimoid, and saprophagous nematodes were observed.

Little difference in nematode numbers was noted in the field studies at the rates of PCNB applied. The 56 kg/ha rate of PCNB in the field studies corresponded approximately with the 0.025 g/kg rate in the greenhouse studies.

At the Wiregrass Substation, numbers of *Tylenchus* sp. and dorylaimoid nematodes were reduced significantly at all treatment levels (Table 7). Mononchoid nematodes were reduced in number at PCNB rates of 11, 34, and 45 kg/ha.

Numbers of *Helicotylenchus* sp., were increased by the application of 11 kg/ha of PCNB at the Sand Mountain Substation (Table 8), while dorylaimoid nematodes were reduced in number at rates exceeding 11 kg/ha.

No difference in the yield of soybeans was obtained at any treatment level of PCNB at any of the experiment stations.

Table 5. The effects of PCNB on plant parasitic nematodes in a Norfolk sandy loam planted to cotton.

PCNB g/kg soil	Numbers per 50 cm ³ soil			
	Larvae of			
	<u>Meloidogyne</u> <u>incognita</u>	<u>Pratylenchus</u> <u>brachyurus</u>	<u>Hoplolaimus</u> <u>galeatus</u>	<u>Helicotylenchus</u> <u>dihystera</u>
0	102.3	6.3	24.4	26.9
0.025	80.1	22.1	18.7	4.4
0.05	112.6	60.0	28.0	0.6
0.10	29.4	58.0	11.3	0.1
0.20	36.7	38.3	68.4	1.6
0.40	14.9	43.4	34.9	0.0
0.50	5.0	47.1	18.9	0.3
1.00	14.9	53.7	31.0	0.1
2.00	1.3	43.6	16.7	0.4
LSD (P=0.05)	69.4	26.4	16.7	4.1
LSD (P=0.01)	92.4	35.2	22.2	5.5

and *H. galeatus*, all of which are in the family *Tylenchidae*.

Nematodes which showed a decrease in number most instances were *Meloidogyne* sp., and (data from greenhouse experiments not presented) dorylaimoid, mononchoid, and saprophagous nematodes. Dorylaimoid type nematodes usually decreased significantly at all rates of PCNB studied.

The increase in certain plant parasitic nematodes usually occurred at recommended field application levels of PCNB, with a corresponding decrease in their numbers occurring at the higher levels. The increase or decrease in numbers of nematodes of a particular species was not noticeably affected by either the soil type or crop plant studied.

Field studies indicated approximately the same results as the greenhouse studies. Numbers of *Tylenchus* sp., and dorylaimoid and mononchoid nematodes were observed to decrease upon PCNB application. At the Sand Mountain experiment station numbers of *Helicotylenchus* sp. increased at the 11 kg/ha rate (ca. field rate).

The increase in numbers of certain plant parasitic nematodes upon PCNB application is probably due to a number of factors. It is possible that the decrease in numbers of *Meloidogyne* sp., due to their greater susceptibility, causes less competition for root space and, therefore, less susceptible nematodes could increase in number. Also PCNB is much less toxic to species which tend to increase in number than to species predatory of other nematodes (mononchoid and some dorylaimoid nematodes), which would tend to favor an increase in the more PCNB-tolerant types. The greater

Table 4. Effect of PCNB on the galling of cotton roots by Meloidogyne incognita in a Dothan sandy loam.

PCNB g/kg soil	Root weight (g)	Root-knot ^x Index
0.	3.70	5.00
0.025	4.18	4.37
0.05	4.11	4.10
0.10	3.64	2.75
0.20	4.66	3.26
0.40	3.41	2.75
0.50	4.90	3.12
1.00	4.69	1.37
2.00	3.72	2.12
LSD (P=0.05)	N.S.	1.02
LSD (P=0.01)	N.S.	1.36

^x Each index is the average of 8 reps/treatment.

Index is based on a rating of 1-6 where degree of galling is determined with a scale based on the percent of the root system galled and where: 1 = 0-5%; 2 = 6-10%; 3 = 11-20%; 4 = 21-40%; 5 = 41-80%; 6 = 81-100%.

DISCUSSION

In vitro studies indicated that PCNB granules are toxic to plant parasitic and free-living nematodes. This toxic effect is much more pronounced in the free-living mononchoid and dorylaimoid nematodes than in the plant parasitic types. When compared to the other plant parasitic nematodes studied, *M. incognita* larvae were approximately twice as susceptible to the toxicity of PCNB.

The PCNB that was dissolved in water had no adverse effects on the spiral nematodes (*H. dihystra*), probably due to its very low concentration. This solution actually seemed to prolong the life of these nematodes over the controls.

Results of greenhouse studies indicated that PCNB application can cause an increase or decrease in numbers of a particular species of nematode. Whether a particular nematode will increase, decrease, or not change significantly in number upon PCNB application cannot be predicted. In some cases a particular species may increase in number, in another case a decrease is indicated. The nematode species most likely to show an increase in numbers are *P. scribneri*, *P. brachyurus*, *H. dihystra*,

Table 6. Effect of PCNB on degree of galling of cotton roots by Meloidogyne incognita in a Norfolk sandy loam.

Treatment rates g PCNB/kg soil	Root Weight	Root-knot x Index
0	4.36	6.00
0.025	5.28	4.00
0.05	5.79	4.57
0.10	5.91	2.29
0.20	4.83	2.57
0.40	5.00	2.29
0.50	4.61	1.57
1.00	4.69	1.29
2.00	4.36	1.00
LSD (P=0.05)	N.S.	1.52
LSD (P=0.01)	N.S.	2.03

x Each index is the average of 7 reps./treatment. Index is based on a 1-6 rating where degree of galling is determined with a scale based on the percent of the root system galled and where: 1 = 0-5%; 2 = 6-10%; 3 = 11-20%; 4 = 21-40%; 5 = 41-80%, 6 = 81-100%.

susceptibility of mononchoid and dorylaimoid nematodes to PCNB is probably due in part to their greater mobility through the soil which results in higher probability of contacting PCNB granules. It has been suggested that PCNB is toxic to certain nematode-trapping fungi which could also cause numbers of more tolerant nematodes to increase. Oostenbrink (8) found that organic manuring reduced infestation and reproduction of meadow nematodes and suggested that this was due to nematode-trapping fungi. Miller and Waggoner (6) found that PCNB, due to its greater persistence than the nematicide DBCP, decreased control of meadow nematode numbers. They suggested that PCNB might easily inhibit growth of a wide variety of organisms normally predaecous to *P. penetrans* (10).

Due to the fact that *P. scribneri* increased in the absence of a host, it is possible that PCNB in some way stimulates hatch of the eggs of this nematode and others in the family *Tylenchidae*.

A decrease in the incidence of other soil microorganisms, especially those for which the fungicide is intended, might cause a better environment for the less susceptible nematodes to live and reproduce.

Table 7. Effect of PCNB on nematodes in soil from a soybean field experiment at Headland, Alabama.

PCNB (kg/ha)	Nematodes per 50 cm ³ soil		
	Tylenchus	dorylaimoid	Mononchoid
0	13.6	23.0	15.7
11	9.2	12.1	7.2
22	8.9	9.7	9.7
34	8.0	10.4	3.6
45	5.6	10.4	4.4
56	6.2	9.6	10.6
LSD (P=0.05)	3.7	4.2	7.6
LSD (P=0.01)	5.0	5.6	10.3

Table 8. Effect of PCNB on numbers of nematodes in soil from a soybean field experiment at Crossville, Alabama.

PCNB (kg/ha)	<u>Helicotylenchus</u>	Dorylaimoids
	<u>dihystera</u>	
0	145.4	23.4
11	224.6	17.9
22	196.7	15.0
34	163.4	15.1
45	125.2	12.7
56	102.4	9.8
LSD (P=0.05)	68.4	6.4
LSD (P=0.01)	92.8	8.6

RESUMEN

Se estudiaron los efectos de pentacloronitrobenzeno (PCNB) sobre nematodos fitoparásitos y de vida libre con experimentos in vitro, de invernadero y de campo. PCNB ejerce una acción tóxica contra larvas de *Meloidogyne incognita* (Kofoid and White) Chitwood, *Helicotylenchus dihystera* (Cobb) Sher, *Hoplolaimus galeatus*

(Cobb) Sher, *Rotylenchulus reniformis* Linford and Oliveira, nematodos mononcoideos y dorilaimoideos, así como contra el microbívoro *Pelodera chitwoodi* (Bassen) Dougherty. El grado de toxicidad fue más acentuado con los nematodos mononcoideos y dorilaimoideos y con *P. chitwoodi* que con las otras especies. Estudios de invernadero indicaron que PCNB puede causar aumentos o reducciones en número de nematodos de acuerdo con la especie y la concentración de PCNB utilizada. Las especies de nematodos que son más propensas a aumentar en número seguida la aplicación de PCNB son: *Pratylenchus scribneri* Stiener, *P. brachyurus* (Godfrey) Filip. and Schuur, Stek., *H. dihystra*, y *H. galeatus*. El efecto sobre *M. incognita*, y sobre nematodos mononcoideos, dorilaimoideos y saprófitos fue de reducción en número. En algunos casos el número de nematodos aumentó con dosis normales de campo para luego disminuir con dosis más altas de PCNB. Los resultados obtenidos en el campo coincidieron en general con los de los experimentos de invernadero. *Claves: fungicidas del suelo, combate de nematodos, algodón, Gossypium hirsutum, cloronitrobenzenos, quintozene, Terraclor®.*

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