Control of Rhizoctonia solani by Pentachloronitrobenzene Accumulated from Soil by Bean Plants

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ABSTRACT

Roots and hypocotyls of 10 plant species tested accumulated pentachloronitrobenzene to levels ca. 0.1 - 2.0 times those in the surrounding soil. The concentration of PCNB in those portions of bean and muskmelon seedlings emerging from soil decreased rapidly after emergence. No translocation of PCNB to upper parts of the plants was evident, and it disappeared rapidly when added directly to cotyledons or filter papers. Bean seedlings grown for 4-7 days in soil treated with PCNB at concentrations up to 30 μg/g were inoculated with *Rhizoctonia solani*, then placed in moist chambers or transplanted into soil without PCNB. The severity of disease in plants containing PCNB was significantly less than in plants not previously exposed to the fungicide. At the time of inoculation the concentration of PCNB in the outer cells of the susceptible hypocotyl was 3.5-10.0 times higher than in the inner cells. In culture similar concentrations restricted growth of the fungus by 50%. PCNB was concentrated 10-fold above the ambient soil concentration in peat particles recovered from a peat-loam soil mixture treated with PCNB. Increasing the organic matter content of soil or sand reduced the accumulation of the fungicide by bean plants and the level of protection against *R. solani*.

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The fungicide pentachloronitrobenzene (PCNB) is commonly used against certain soil-borne plant pathogens, including *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Streptomyces scabies*. The fungicide is strongly sorbed by biological materials. Mycelia of *Rhizoctonia solani* sorbed PCNB from soil to levels ca. seven times that initially applied to soil (6). More PCNB was extracted by aqueous leaching from soil from which organic matter had been removed by H_2O_2 treatment than from nontreated soil, but the sorptive capacity was restored to the H_2O_2-treated soil by the addition of 1% dry mycelium of *R. solani* (6). More recently Katan and Lockwood (5) detected PCNB concentrations in alfalfa hay particles seven to eleven times greater than the 10 μg PCNB/g applied to the soil in which they were incubated. Cellulose filters and chitin particles likewise concentrated PCNB from soil (Katan and Lockwood, *unpublished results*). These results suggest the possibility that belowground plant parts might also sorb PCNB, and that the accumulated fungicide might contribute to disease control. Therefore, we investigated the possible accumulation of PCNB by tissues of plants grown in PCNB-treated soils, the significance of this phenomenon in control of *R. solani* infecting bean seedlings, and the effect of soil organic matter on both sorption and control. A brief report of these results have been published (2).

MATERIALS AND METHODS.—*Plants and soil mixtures.*—Plants of the following species were used: Bean, *Phaseolus vulgaris* L. 'Sanilac' and 'Brittle Wax'; Corn, *Zea mays* L. 'Michigan-400'; Cotton, *Gossypium hirsutum* L. 'Acala SJ-1'; Cucumber, *Cucumis sativus* L. 'National Pickling'; Muskmelon. *Cucumis melo* L. 'Hales Best'; Oats, *Avena sativum* L. 'Clinton'; Onion, *Allium cepa* L. 'Downing Yellow Globe'; Pea, *Pisum sativum* L. 'Miragreen'; Soybean, *Glycine max* (L.) Merr. 'Hark'; and Wheat, *Triticum aestivum* L. 'Little Club'. Seeds were surface-sterilized in 0.5% NaClO for 10 min, thoroughly rinsed, and air-dried. Seeds were sown in soil and sand mixtures to a depth of ca. 2 cm, placed in a greenhouse at 22 ± 5 C with a 16 hr photoperiod, and watered daily.

Steamed Conover loam soil (4) was sieved (3mm × 3mm mesh size) and stored air dry. For most experiments the loam soil was mixed with silica sand (3:2, w/w) and will be referred to as loam-sand mix. The greenhouse potting soil was a mixture of equal volumes of loam, sand, and peat. A loamy-sand field soil (10) was used in experiments conducted in Rehovot, Israel, and will be referred to as Rehovot soil. When testing the effects of organic matter, peat particles of 20 to 40-mesh size (420-840 μ) were mixed with sand or loam-sand mix on a volume basis (v/v) with the peat concentration being expressed as the percent of the total volume of the mixture. In investigating the partitioning of PCNB in soil, peat particles (20 to 40-mesh) were mixed with an equal volume of loam soil of less than 40-mesh size. For PCNB analysis of each fraction, the peat was recovered from the mixture by aqueous washing on a 40-mesh sieve. Recovery of peat particles from sand-peat mixtures was achieved by floating off the more bouyant peat particles onto an 80-mesh sieve.

PCNB extraction and measurement.—PCNB was used as a 75% wettable powder unless stated otherwise, and its concentration is expressed as μg active material per cc or g of mix. Concentrations

were usually expressed on a volume basis to eliminate the effect of differing mixture densities. PCNB concentrations of 10-30 μg/g or cc, which correspond to normal field rates, were used in all experiments. Concentrations of sorbed PCNB are given as μg/g fresh weight of plant material, and μg/cc or g of dry loam soil or peat particles, respectively. After fresh weight determination plant tissues were ground in a mortar. Triturated tissue, filter paper, loam soil, and peat particles were extracted with hexane:isopropanol (3:2, v/v) by vigorous shaking on a mechanical shaker for 30 min. After extraction the hexane layer was decanted and the isopropanol removed from this layer by partitioning with distilled water. The peat particles were then oven dried at 60 C before volume and weight determinations were made.

PCNB in hexane extracts was detected and quantitatively measured by injecting 2-8 μl samples into a Varian Aerograph Model 600-D gas chromatograph with an electron capture detector. The stainless steel column (5 ft × 1/8 in) contained 3% SE-30 on Chromosorb W (60- to 80-mesh). The carrier gas, nitrogen, was adjusted to a flow rate of 60 ml/min. The injector tube, column, and detector were maintained at 275, 155, and 175 C, respectively. PCNB concentrations were determined by comparing peak heights of samples with those of a standard curve based on 2-8 μl injections of a 0.1 μg technical grade PCNB/ml hexane solution.

Disease studies.—An isolate of *Rhizoctonia solani* Kuhn pathogenic to bean was maintained on potato-dextrose agar at 22 C. Bean seedlings grown for 4-7 days in soil with or without PCNB were inoculated by three methods: (i) the below ground portions of seedlings were dipped into suspensions of the pathogen containing 2, 5, and 10 g (fresh weight) mycelia/100 ml for 60 sec. Mycelia used for the suspensions was still-cultured for one week in potato-dextrose broth before being thoroughly washed with distilled water and then comminuted for 60 sec in a Waring Blendor. After inoculation, seedlings were transplanted into loam-sand mix for 16-20 days or placed in a covered moist chamber on moist paper toweling for 3-5 days. (ii) comminuted mycelia prepared as above were separated into "propagules" of various sizes on sieves. Three propagules of the 20- to 40-mesh size were placed on the hypocotyl of each seedling in a moist chamber. (iii) seedlings were transplanted into soil naturally infested with *R. solani.* Noninoculated controls were included in all experiments. Each experiment included three to five replicate pots per treatment of four to ten plants per replicate.

Moist chambers were kept in controlled environment chambers at 21 C, and 100% relative humidity, with a 16 hr photoperiod of both incandescent and fluorescent light at 6.3×10^4 ergs/cm^2 sec. Transplanted seedlings were returned to the greenhouse.

Four criteria were used to estimate disease severity: (i) percent of infected plants, (ii) average number of lesions per plant, (iii) average lesion size, and (iv) a disease severity index. The index was a visual rating of plants from 0 to 5, zero denoting healthy and five those seedlings with hypocotyls completely girdled by lesions.

Pathogenicity was confirmed by plating pieces of

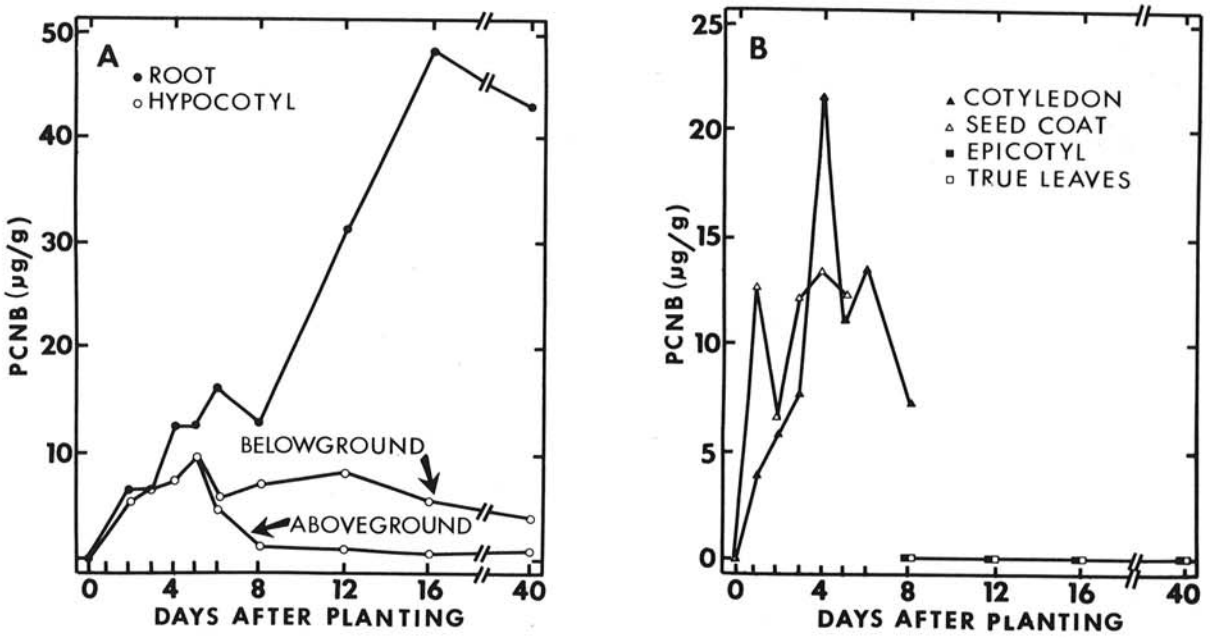

Fig. 1. The concentration of PCNB (μg/g fresh weight of tissue) in selected parts of bean plants at various times after sowing in loam-sand mix containing 25 μg PCNB/cc; **A)** roots and hypocotyls, **B)** seed coats, cotyledons, epicotyls, and true leaves.

TABLE 1. Accumulation of PCNB by the belowground portions of ten agricultural plant species one week after planting in loam-sand mix containing 25 μg PCNB/cc

Crop	Variety	PCNB (μg/g)[a]
Cotton	Acala SJ-1	18.8
Soybean	Hark	13.0
Bean	Sanilac	12.8
Pea	Miragreen	12.2
Corn	Michigan-400	6.0
Cucumber	National Pickling	6.0
Muskmelon	Hales Best	5.4
Onion	Downing Yellow Globe	3.5
Oats	Clinton	3.0
Wheat	Little Club	2.0

[a]Per gram fresh weight of tissue.

surface sterilized (0.5% NaClO for 60 sec) diseased hypocotyl tissue onto 2% water agar and incubating at 24 C until distinctive *Rhizoctonia* hyphae were observed.

All experiments were done at least twice with similar results.

RESULTS.—*Accumulation of PCNB from soil by plants.*—The concentration of PCNB in selected parts of bean seedlings was determined at various times after sowing seeds in loam-sand mix containing 25 μg PCNB/cc. PCNB in roots increased steadily after germination, reaching a plateau of ca. 40 μg/g near the sixteenth day (Fig. 1-A). Upon emergence of the cotyledons, the hypocotyl was divided into its above and belowground portions. The level of PCNB in the belowground portion remained at ca. 8 μg/g, a concentration near that of the intact hypocotyl just prior to emergence. However, that in the aboveground portion declined rapidly after emergence. Similar reductions in PCNB were recorded for the cotyledons after emergence (Fig. 1-B). No PCNB was detected in either the epicotyls or true leaves. Trends similar to those for bean were observed for muskmelon grown in loam soil with 10 μg PCNB/g (99% technical grade).

TABLE 2. The effect of organic matter on the accumulation of PCNB by the belowground portions of plants one week after planting in various soil mixtures containing 25 μg PCNB/cc

Soil mixture	PCNB, μg/g fresh weight of tissue		
	Bean	Muskmelon	Pea
Sand	65.2	13.9	23.4
Sand + 2% Peat (v/v)	53.6	11.0	28.5
Sand 10% Peat (v/v)	13.7	6.4	13.5
Loam-sand (3:2, w/w)	6.3	5.5	12.2
Loam-sand + 10% Peat (v/v)	3.6	1.9	7.8
Greenhouse potting soil (loam soil: sand: peat, 1:1:1, v/v/v)	1.8	1.4	3.5

When the belowground portion of the hypocotyl was sectioned into its outer and inner portions, the former had a PCNB concentration several times that of the latter. The concentration ratios for 4 to 7-day-old bean and muskmelon seedlings grown in loam soil with 10 μg PCNB/g (99% technical grade) were 10.0 ± 2.6 and 3.5 ± 0.5, respectively. A concentration of ca. 12 μg/g was detected in the outer portion of bean hypocotyls from 7-day-old seedlings harvested from loam-sand mix containing 25 μg PCNB/cc, with only 3 μg/g detectable in the inner portion. Wright (12) found that PCNB was confined almost totally to the peels of tubers of potato plants grown in PCNB-treated soil.

The rapid decline of PCNB from cotyledons and the aboveground portions of the hypocotyl did not appear to be the result of PCNB-metabolite formation as new peaks including that corresponding to the known metabolite, pentachloroaniline, were not observed on gas chromatograms. Results suggested that the loss might be due to vaporization of PCNB. Only 1% of 2 μg PCNB applied directly to bean cotyledons as a water suspension remained after 3 days. Similarly, less than 2% of 1 μg PCNB applied to 1.0 cm^2 pieces of filter paper exposed to both light and darkness was recovered after 3 days.

The belowground portions of ten species of agricultural crops accumulated PCNB from loam-sand mix containing 25 μg PCNB/cc (Table 1). Concentrations in 1-week-old seedlings ranged from 2.0 to 18.8 μg/g.

Effect of soil organic matter on PCNB accumulation.—Seeds of bean, pea, and muskmelon were sown (i) in sand with 0, 2, and 10% peat particles (v/v), (ii) in loam-sand mix with and without 10% peat, and (iii) in greenhouse potting soil. Each soil or mixture contained 25 μg PCNB/cc. Increasing organic matter content reduced the amount of PCNB accumulated by the belowground portions of 7-day-old seedlings of each crop (Table 2). The presence of 2% peat in sand had little effect in reducing sorption, but 10% peat reduced sorption to one-quarter to one-half that in sand alone. The addition of 10% peat to loam-sand, a mixture already containing considerable organic matter and which allowed low sorption of PCNB by seedlings, further reduced the accumulation of PCNB by plants grown in this mixture. Plants grown in greenhouse potting soil, which is one-third peat by volume, sorbed very little PCNB.

To determine directly if PCNB was being concentrated in soil organic matter, loam soil (< 40 mesh) was mixed with an equal volume of peat particles (20- to 40-mesh) and 10 μg PCNB/cc was added. After one week the peat fraction contained 12.0 μg PCNB/cc which was ca. ten times more than the 1.3 μg/cc in the loam portion, whereas at zero time the concentrations in these portions were 6.9 and 6.4 μg/cc, respectively. Fifty times more PCNB was present in the peat than in the loam fraction when concentrations were calculated on a dry weight basis.

Control of R. solani by PCNB in bean.—Sanilac bean seeds were sown in loam-sand mix with and

without 25 μg PCNB/cc. Seedlings were inoculated one week later by dipping the root system and part of the hypocotyl into suspensions containing 2, 5, and 10 g comminuted mycelia of *R. solani* per 100 ml, and then transplanted into loam-sand without PCNB for 17 days. The sorbed PCNB reduced the average number of lesions per seedling but had a greater effect on reducing the average lesion size (Table 3 and Fig. 2). At the highest inoculum concentration, 10 g/100 ml suspension, lesion size but not lesion number was suppressed. The amount of protection decreased as inoculum concentration increased. Disease severity indexes expressed as percentages of controls without PCNB were 46, 56, and 61 for seedlings inoculated with 2, 5, and 10 g mycelia/100 ml, respectively. The average concentration of PCNB in the belowground portions of the hypocotyls at the time of inoculation was 5.5 μg/g, a concentration greater than that required to restrict the radial growth of the pathogen by 50% on potato-dextrose agar. Similar protection was afforded 'Sanilac' bean seedlings transferred to moist chambers for 3-5 days.

In other experiments Brittle Wax bean seedlings were grown for 4-7 days in Rehovot soil containing 0, 10, and 30 μg PCNB/g prior to inoculation. Each seedling was inoculated with three 40-mesh size propagules of *R. solani* and incubated in moist chambers. The respective percentages of diseased plants were 75, 45, and 36 and the average numbers of lesions per seedling were 1.5, 0.9, and 0.5 for the three PCNB concentrations. Similarly treated Brittle Wax seedlings transplanted into naturally infested soil for 16 days had 95, 60, and 30% diseased plants, and disease severity indexes of 3.4, 1.5, and 0.8 for the three concentrations, respectively.

Effect of soil organic matter on protection.—The effect of soil organic matter on protection was determined by growing Sanilac bean seedlings in sand containing 0, 2, and 10% peat particles (v/v), each with and without 25 μg PCNB/cc. After 7 days, seedlings were inoculated by dipping the roots and hypocotyl in a 10 g/100 ml mycelial suspension followed by incubation in moist chambers or in loam-sand soil without PCNB. Disease severity increased with increasing peat contents in the sand (Table 4). Significant protection resulted from the 0 and 2% sand-peat mixtures but not from the mixtures containing 10% peat. Disease severity indexes expressed as percentages of controls without PCNB were 50, 63, and 96%, respectively, for the three treatments. Results similar to these were obtained from inoculated seedlings placed in moist chambers (Fig. 3).

The amount of PCNB sorbed by the peat particles separated by flotation from 170 cc of the 2 and 10% mixtures was determined at 0 and 7 days. After 7 days 465 and 1,010 μg PCNB, respectively, was sorbed by the peat recovered from the 2 and 10% mixtures. Even at zero time the peat particles from those same two mixtures contained 125 and 730 μg PCNB. Increased peat content apparently reduced the availability of PCNB to bean seedlings via sorption.

In summary, the level of disease protection was

TABLE 3. Disease severity of 'Sanilac' bean seedlings grown in loam-sand mix with and without 25 μg PCNB/cc for one week before inoculation with *Rhizoctonia solani*, then transplanted into loam-sand mix without PCNB for 17 days

Inoculum density[b]	Treatment	Disease severity[a]							
		Diseased plants		Lesions per hypocotyl		Lesion size		Disease severity index[c]	
		no.	*%*	*no.*	*%*	*mm²*	*%*	*rating*	*%*
0	No PCNB	0		0.0		0.0		0.0	
	PCNB	0	0	0.0	0	0.0	0	0.0	0
2	No PCNB	24		3.1		8.1		2.4	
	PCNB	20	83	2.1	68*	2.5	31*	1.1	46*
5	No PCNB	24		3.5		9.6		2.5	
	PCNB	24	100	2.3	66*	6.0	63*	1.4	56*
10	No PCNB	24		3.7		12.8		3.1	
	PCNB	24	100	3.8	103	5.7	45*	1.9	61*

[a]Each value is the mean for 24 plants.
[b]Grams of comminuted mycelium per 100 ml suspension.
[c]0 = healthy, 5 = hypocotyl completely girdled by lesions.
[d]* = significance at 0.05 level.

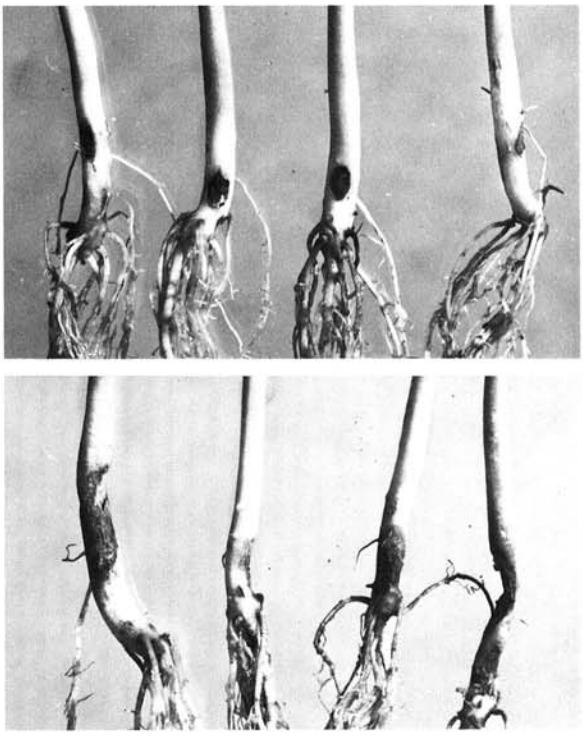

Fig. 2. Bean seedlings grown in loam-sand mix with (top) and without (bottom) 25 μg PCNB/cc for one week before inoculation with 10 g comminuted *Rhizoctonia solani* mycelia per 100 ml suspension, then transplanted into loam-sand mix without PCNB for 17 days.

TABLE 4. Disease severity of 'Sanilac' bean seedlings grown in three different sand-peat mixtures with and without 25 μg PCNB/cc for one week, then transplanted into loam-sand mix without PCNB for 17 days after inoculation with *Rhizoctonia solani*

Sand-peat mixture	Treatment	PCNB	Disease severity[a]							
			Diseased plants		Lesions per hypocotyl		Lesion size		Disease severity index[b]	
		μg/g[c]	no.	%	no	%	mm²	%	rating	%
Sand	No PCNB	0.0	16		3.8		9.5		2.2	
	PCNB	18.4	12	75	1.3	34*[d]	4.3	45*	1.1	50*
Sand + 2% peat (v/v)	No PCNB	0.0	16		2.8		10.7		2.4	
	PCNB	16.3	16	100	2.1	75	5.5	51*	1.5	63*
Sand + 10% peat (v/v)	No PCNB	0.0	16		3.1		11.3		2.3	
	PCNB	4.3	16	100	2.6	84	8.8	78	2.2	96

[a]Each value is the mean for 16 plants.
[b]0 = healthy, and 5 = hypocotyl completely girdled by lesions.
[c]μg PCNB per gram fresh weight of the belowground portion of hypocotyls.
[d]* = significance at 0.05 level.

directly related to the concentration of PCNB accumulated by the belowground or inoculated portion of the hypocotyl of bean (Table 3) and inversely related to the amount of PCNB sorbed by the peat particles (Table 4 and Fig. 3).

DISCUSSION.—PCNB was accumulated to various levels in roots and hypocotyls of bean and muskmelon when grown in PCNB-treated soil and frequently reached concentrations in excess of those in the soil itself. Its sorption by each of ten plant species tested indicates the general nature of the phenomenon. PCNB does not appear to be translocated as it was restricted to those aerial portions of the plant once in contact with the soil and was never detected in the true leaves or epicotyls. In contrast, Kuchar et al. (8) found PCNB, as well as traces of several metabolites, in the aerial portions of 7 and 14-day-old cotton seedlings grown in soil containing 300 ppm PCNB, a concentration more than 10-fold in excess of that used in our work.

Sorbed PCNB provided partial protection against *R. solani* infecting bean seedlings by suppressing both size and number of lesions. The high concentration of PCNB in the outer cells of the hypocotyl probably reduced both the number of successful penetrations by the pathogen, and the rate at which the pathogen invaded healthy tissue. Ko and Oda (7) observed a 50% reduction in growth rate of *R. solani* in soil treated with 100 ppm PCNB, and concluded that control of this pathogen by PCNB was due to

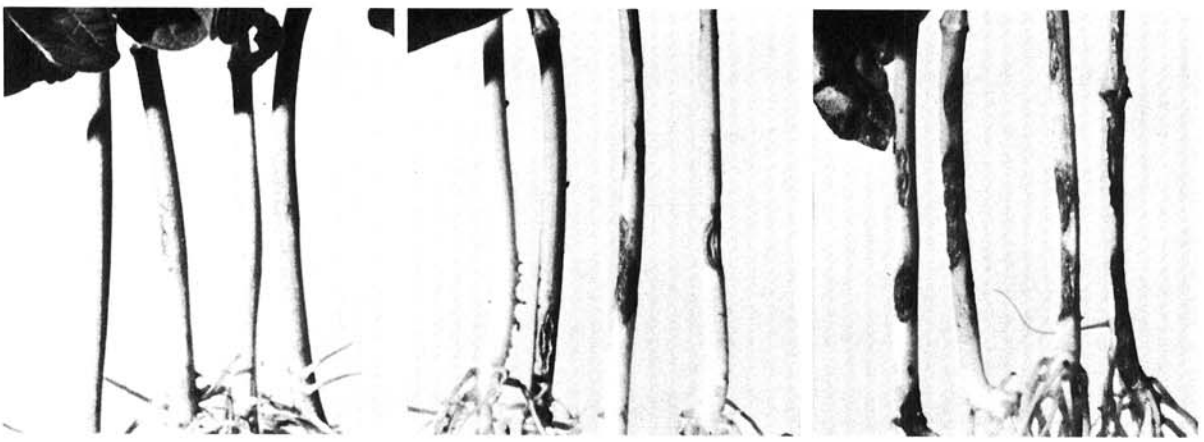

Fig. 3. Symptoms on bean seedlings inoculated with *Rhizoctonia solani* and placed in a moist chamber for 3-5 days, after a one-week treatment in sand (left), sand + 2% peat (center), and sand + 10% peat (right). All mixtures contained 25 μg PCNB/cc.

suppression of its growth. The results of Weinhold et al. (11) indicate that reducing the growth rate of *R. solani* by lowering the nutrient status of the pathogen in the infection court decreases its virulence. We obtained less protection at the higher inoculum concentrations (Table 4), an observation consistent with that of Martinson (9) who found that the greater the inoculum density the more PCNB was required to control damping-off of radish by *R. solani*.

PCNB was accumulated to maximum levels in the outer cells of the hypocotyl, that portion of the plant most susceptible to the pathogen, wherein it may afford protection to the infection court. The protective effect of PCNB sorbed by root systems and hypocotyls might be very important in disease control under field conditions. In our investigation bean seedlings were in contact with the fungicide only during the 4- to 7-day treatment period, whereas under natural conditions the plant would be in continuous contact with PCNB, providing an opportunity for continued sorption of the fungicide during exposure to the pathogen. In contrast with our results, Ko and Oda (7) found no reduction in the incidence of infection of beet seeds incubated in PCNB-treated soil for 12 hr prior to transplanting into soil infested with *R. solani*. In our results, disease incidence was reduced only slightly or not at all by PCNB sorbed by hypocotyls, whereas disease severity was markedly affected. Ko and Oda (7) did not measure disease severity, nor did they determine whether their short exposure period was sufficient to permit sorption of PCNB by the beet seeds.

The sorption of PCNB by soil organic matter resulted in low accumulation of the fungicide by plants, and low protection from *R. solani*. Much higher rates of PCNB were required to control Rhizoctonia disease in potatoes in muck than in clay (12) or in mineral soil (3). Similarly, Alexander (1) found that a PCNB drench controlled *R. solani* on poinsettia in sand but failed to do so in soil containing organic matter.

PCNB accumulated by plants might selectively alter microbial populations in the rhizosphere and outer cells of the root system in favor of PCNB-tolerant pathogens. No shifts in populations were noted by Farley and Lockwood (4) in PCNB-treated soil in the absence of an energy source. However, in the presence of a nutrient source, such as may be provided by plant roots and their exudates, PCNB-insensitive microorganisms increased in number while sensitive ones decreased. It was proposed that disease accentuation and/or exchange may result from the reduction in nutrient competition by suppressing PCNB-sensitive microorganisms. Similar shifts occurred upon saprophytic colonization of PCNB-containing plant debris. Katan and Lockwood (5) observed significant increases in *Fusarium* and *Pythium*, both PCNB-insensitive organisms colonizing particles of alfalfa hay which had concentrated PCNB 7- to 11-fold from PCNB-treated soil. There was a concomitant decrease in the populations of PCNB-sensitive microorganisms.

PCNB was readily sorbed by plant tissues in contact with treated soil, and the amount accumulated by the hypocotyls of bean seedlings was sufficient to provide protection against *R. solani*. Soil organic matter, via sorption of PCNB, reduced the amount available for plant accumulation and thus decreased the level of protection.

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