Efficacy of 1,2-Dibromo-3-chloropropane for Control of Meloidogyne javanica as Influenced by Concentration, Exposure Time and Rate of Degradation

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Abstract: Laboratory experiments were conducted by applying 1,2-dibromo-3-chloropropane (DBCP) to sealed vials of soil infested with *Meloidogyne javanica*. A minimum initial concentration of 0.25 μg of DBCP/g of oven-dry soil killed all nematodes within 35 days. A concentration of 1.0 μg/g killed all nematodes within 28 days. The rate of degradation of this chemical was determined by treatment of steamed and nonsteamed dry soil in open and sealed vials. Extraction of the chemical, followed by quantification by gas chromatography, showed approximately 100% of the amount applied recovered after 14 days in sealed vials without soil. With soil present, approximately 10% of the amount of chemical applied was recovered. *Key Words:* root knot, DBCP, chemical control, fumigant, bioassay.

Marks et al. (5) and Johnson (2) reported that 1,2-dibromo-3-chloropropane (DBCP) is absorbed rapidly by nematodes but is lost slowly. This indicates that maintenance of high concentrations of DBCP in soil for prolonged periods of time is unnecessary, so long as the time of exposure is sufficient to result in a lethal concentration of toxicant within the nematode. Since the concentrations of DBCP in fumigated soil continuously change as a result of loss to the atmosphere, downward and upward movement and degradation; a high percentage of the deaths probably result from relatively small concentrations maintained in soil for long periods of time.

Since DBCP is not readily hydrolyzed in aqueous environments, its persistence in soil should be limited by its volatility rather than by chemical or biological conversion in the soil. However, the work of Castro and Belser (1) indicates that a significant biological dehalogenation does occur in soil with DBCP being converted slowly to *n*-propanol, chloride and bromide.

Originally this study was to determine DBCP concentrations and exposure times required to eradicate *Meloidogyne javanica* (Treub) Chitwood, from soil. Elaborate precautions were taken to prevent escape of DBCP from the container for the duration of the test. Nevertheless, the concentration decreased steadily throughout the test. This decrease was attributed to degradation.

An experiment was conducted to determine the rate at which DBCP is degraded in a closed system; to determine whether DBCP is degraded in sterilized soil; and to determine the rate at which DBCP is lost to the atmosphere from soil and from open containers. This would provide important information on the residual nature of DBCP in soil by explaining the disappearance of DBCP that cannot be attributed to loss by vaporization.

MATERIALS AND METHODS

The soil used in this test was a 1:1 mixture

Received for publication 7 February 1973.

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of washed sand and a silt loam mixed in a twin-shell blender. It contained 69, 25 and 6% sand, silt and clay, respectively. The moisture equivalent was 12%.

M. javanica were cultured on tomato *Lycopersicon esculentum* Mill. 'Rutgers' in a greenhouse in the same soil mixture. Soil from pots containing second-stage larvae was screened through a 2.0-mm sieve to remove extraneous root material. The screened soil was added to noninfested soil and mixed thoroughly in first a concrete mixer and then in a twin-shell blender. After mixing, the soil contained approx. 2.5 second-stage larvae/cc; moisture was 6.8%.

INFLUENCE OF CONCENTRATION OF DBCP AND TIME OF EXPOSURE ON CONTROL OF MELOIDOGYNE JAVANICA: Aliquots (650 g) of this soil were weighed and placed in 400-ml small-mouth jars. The filled jars were placed in a 6 C cold room to chill. Technical DBCP was diluted with acetone and added to the soil in the cold room to prevent loss by vaporization. The quantity of DBCP added per jar was 0, 0.05, 0.125, 0.25, 0.50, 0.75 or 1.0 μg of DBCP/g of oven-dry soil. This was equivalent to 0, 1.0, 2.5, 5.0, 10.0 or 20.0 μg of DBCP/g of soil water.

DBCP was added to the soil by making a hole midway in the soil mass with a glass rod and adding the diluted solution with a 1.0-ml volumetric pipette. The control soil received 1.0 ml of acetone.

Immediately after the addition of the DBCP mixture, the jars were capped with Bakelite lids with plastic liners and sealed with Teflon tape. Each jar was inverted so that the air space would be on the bottom and was stored in the laboratory for the duration of the test at 21-24 C.

Three replicate jars from each treatment were assayed at weekly intervals for 7 weeks. Bioassays for *M. javanica* were made weekly. However, the concentration of DBCP in the jars was determined only at 14-day intervals.

In order to minimize the loss of DBCP when the jars were opened at the end of the exposure period, they were placed in a 6 C cold room for 12 hr. This was not done unless the concentration of DBCP was to be determined. DBCP was extracted from 30-40 g of soil removed from each jar and placed in 125-ml glass-stoppered bottles. The balance of soil in each jar was used in the bioassay for *M. javanica*.

DBCP was extracted from the soil by adding 25 ml of redistilled hexane plus 10 ml of distilled water to each bottle. The bottles were stoppered and shaken vigorously for 30 min on a reciprocating shaker. The supernatant hexane, containing the DBCP, was decanted into 5-ml glass-stoppered volumetric flasks. The analyses were made using the gas chromatographic methods described by Johnson and Lear (4).

Bioassays were made by placing 350 ml of soil from each jar into clay pots 7.6 cm in diam. The soil was covered with 1-2 cm of sand and the pots placed in a greenhouse where the soil was allowed to "air" for 1 week. After 1 week, 2- to 3-week-old tomato seedlings were transplanted, one to each pot. The seedlings were removed from the pots and examined for galls 4 weeks after planting. Due to continuous galling on some root systems, it was impossible to determine the precise number of galls. Therefore, the higher gall counts must be considered close estimates. Galls per plant are reported as the mean of three replicates.

RATE OF DEGRADATION OF DBCP IN SOIL: The soil used in this experiment was a 1:3 mixture of washed sand and Yolo fine sandy loam mixed in a twin-shell blender. It contained 70, 16 and 14% sand, silt and clay, respectively. The moisture equivalent was 13.1%.

After mixing, the percent moisture was 9.7%. Twenty-five grams of soil were weighed into 20-ml glass vials; other vials were left empty. Technical DBCP was diluted with acetone and added to all vials at the rate of 10.0 μg of DBCP/g of oven-dry soil (228 μg DBCP/vial). The solution of DBCP, added to each vial with a 100-ml volumetric pipette, was deposited in the center of the soil mass by first punching a hole in the soil with a glass rod or, if the vial was empty, directly on the bottom of the vial.

Treatments were as follows: (i) open vial without soil; (ii) open vial with soil; (iii) closed vial with soil; (iv) closed vial with sterilized soil; (v) closed vial without soil. The closed vials were immediately capped with Bakelite caps with aluminum foil liners and sealed with teflon tape after the addition of the DBCP mixture. The open vials were left uncapped; however, the hole in the soil made for the addition of DBCP was closed. The vials were stored at 21 to 24 C.

Three replicate vials were assayed for DBCP each time extractions were made. The first extraction was made within 1 hr after treatment. The average value of DBCP obtained

TABLE 1. Percent of DBCP recovered from soil in sealed jars.

Time after treatment	DBCP recovered[a] (%)	Adjusted recovery[b] (%)
1 hour	95	100
1 week	83	87
3 weeks	64	67
5 weeks	50	53
7 weeks	37	39

[a]Mean percent DBCP recovered from six treatments (three replicates/treatment).
[b]Adjusted to allow for DBCP that could not be extracted from the soil.

from each treatment at this extraction was used to determine the maximum percent recovery that could be expected from each treatment and, thus, was the basis for comparison of succeeding extractions. Since the percentage of DBCP recovered from all closed vials was near 100, the initial reported value was adjusted to 100% for convenience. Subsequent percentages take this adjustment into account. At the first extraction, most of the DBCP had already vaporized from the open vials and the percent recovery was not adjusted to 100 as with the closed vials. The second extraction was made 24 hr after application and subsequent extractions were made at weekly intervals for 14 weeks.

Extractions from soil were made by placing the contents of each vial in 125-ml glass-stoppered bottles and adding 25 ml of redistilled hexane plus 10 ml of distilled water. The bottles were stoppered and shaken vigorously for 30 min on a reciprocating shaker. The supernatant hexane, containing the DBCP, was decanted into 5-ml glass-stoppered volumetric flasks. Extractions from vials without soil were made by washing the vial with 10 ml of redistilled hexane. The analyses were made using the gas chromatographic method described by Johnson and Lear (4).

RESULTS AND DISCUSSION

Since this study was made in a confined system, it was assumed the nematodes would be in intimate association with DBCP in an unchanging environment. However, extraction and quantification of DBCP from the soil indicated that the concentration of DBCP inside the jars decreased at a relatively steady rate (Tables 1 and 2).

Since elaborate precautions were taken to prevent loss of DBCP by vaporization, it must have degraded in the soil. At the end of 7 weeks only 39% of the original material was present. The DBCP was degraded at the approximate rate of 12% of the remaining DBCP per week (Tables 1 and 2).

INFLUENCE OF CONCENTRATION OF DBCP AND TIME OF EXPOSURE ON CONTROL OF MELOIDOGYNE JAVANICA: At that rate of degradation there would still be

TABLE 2. Percent of DBCP recovered from open and closed containers with and without soil.

Time after treatment	DBCP recovered[a] (%)				
	Open vial no soil	Open vial + soil	Closed vial + soil	Closed vial + sterilized soil	Closed vial no soil
1 hour	6.3	44.9	100 [b]	100 [b]	100 [b]
1 day	0	14.3	99.0	99.3	100
1 week	0	10.7	95.6	95.4	100
2 weeks	0	10.0	92.3	98.3	100
3 weeks	0	9.7	83.2	87.6	98.2
4 weeks	0	9.2	65.7	71.9	100
5 weeks	0	8.9	52.8	58.8	100
6 weeks	0	9.3	38.5	42.7	95.5
7 weeks	0	9.0	29.9	33.4	97.0
8 weeks	0	9.1	13.9	16.5	100
9 weeks	0	9.4	10.2	13.3	98.3
10 weeks	0	9.5	2.8	11.0	99.3
11 weeks	0	9.0	2.7	10.8	95.9
12 weeks	0	8.6	3.4	11.0	100
13 weeks	0	9.0	2.9	10.3	95.5
14 weeks	0	8.5	2.4	9.1	99.7

[a]Mean of three replications.
[b]Adjusted to allow for the DBCP that could not be extracted from the soil.

TABLE 3. Influence of concentration of DBCP and time of exposure on the death of *Meloidogyne javanica* as measured by the number of galls on tomato roots.

Treatment (μg DBCP/g oven-dry soil)	Weeks after application						
	1	2	3	4	5	6	7
0	245[a]	190	105	65	50	16	15
.050	220	180	75	45	30	9	7
.125	170	125	80	30	21	1	0
.250	225	120	60	25	9	0	0
.500	200	130	35	15	5	0	0
.750	240	110	20	6	0	0	0
1.000	160	85	15	0	0	0	0

[a]Galls per plant. Mean of three replicates.

approximately 3.5% remaining at the end of 6 months (26 weeks). It would take almost 9 months (36 weeks) to reduce the level to 1.0% of the original.

Although all populations declined over the 7-week period in the confined system (Table 3), the nematodes in the soil containing higher rates of DBCP were eradicated in a shorter period of time. This experiment indicates that a minimum initial concentration of 0.125 μg of DBCP/g of oven-dry soil will kill all *M. javanica* within 7 weeks; however, only 0.05 μg of DBCP/g of oven-dry soil was recovered at the final assay (39% of the original concentration). Since all concentrations of DBCP were decreasing throughout the test, it is probable that a continuous concentration of 0.125 μg would have killed the nematodes in less than 7 weeks. Higher rates of DBCP eradicated the nematodes within a shorter period of time. All nematodes were killed within 28 days when 1.0 μg of DBCP/g of oven-dry soil was initially added to the jars. With concentrations less than 0.125 μg DBCP/g of oven-dry soil the nematodes were not eradicated after 7 weeks of exposure.

Johnson and Lear (3) reported that an exposure period of 3-4 weeks was necessary for DBCP to eradicate *M. javanica* and *M. incognita* at all rates used. Even though the concentrations of DBCP used in their study were from 5 to 50 times greater than the highest concentrations in this study, the same trend is evident. Greater concentrations of DBCP will kill nematodes more rapidly. However, the end result with low concentrations will be the same if the exposure period is long enough.

Since nematodes rapidly absorb DBCP but release it more slowly (2, 5) the presence of a high concentration of DBCP in soil is unnecessary if the exposure period is long enough for a lethal concentration of toxicant to be absorbed. Lethal concentrations of DBCP within a nematode may be attained in two ways. The first, a relatively high concentration for a short period of time as occurred in soil treated with 1.0 μg of DBCP/g of oven-dry soil. Under these conditions the amount of DBCP within the nematode rapidly reaches a lethal concentration. The second, a low concentration maintained over a long period of time, which occurred in soil treated with 0.05 and 0.125 μg of DBCP/g of oven-dry soil. If absorption continued to exceed elimination, a lethal concentration would eventually be attained and the ultimate results would be the same. An examination of data (Table 3) indicates that this happened.

The results of this study do not necessarily reflect what is happening in the field since these nematodes were nonfeeding. Under field conditions where the nematodes are actively feeding, the rate of absorption and elimination of DBCP may be different. If the rate of elimination is greater and the absorption is about the same, a lethal internal concentration may never be reached at the lower rates of application.

RATE OF DEGRADATION OF DBCP IN SOIL: The results (Table 2) show that when DBCP is added to open vials without soil, it quickly evaporates and no residue was detectable 1 day after treatment. Virtually all of the DBCP added to capped vials without soil was present at the end of 14 weeks, indicating that the method of sealing the vials prevented loss of DBCP by vaporization.

DBCP added to soil in open vials was lost to the atmosphere until approximately 9-10% of the original DBCP remained in the soil. It took about 2 weeks to reach the 10% level. After that, the level remained steady for the duration of the test. Since the soil was too dry for microorganisms to effectively degrade the DBCP, that which remained was in all probability sorbed to the soil particles. This is very interesting because it shows that DBCP can remain in dry soil for relatively long periods of time. It is possible that the sorbed DBCP could be washed down with irrigation water.

Although soil in closed vials appeared to degrade DBCP somewhat faster than sterilized soil in closed vials, there were no significant differences between the treatments. This

probably can be accounted for by the failure to completely sterilize the soil or by the addition of contaminants at the time the solution of DBCP was added. The DBCP was degraded in the closed system to levels below soil in open vials.

It was not the purpose of this paper to determine the nature of the interaction of DBCP with the soil. However, the degradation of DBCP can probably be attributed to the action of soil microorganisms in the closed systems as suggested by Castro and Belser (1).

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