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Nematode Persistence after Fumigation: A Methodological Problem[1]

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With the loss of DBCP as a fumigant nematicide, increasing emphasis has been placed on the use during crop growth of other fumigant nematicides which traditionally have been used only before planting. At planting and postplant applications of ethylene dibromide alone and in combination with chloropicrin have been used successfully for peanut production in the southeastern United States (12,13). Even when methyl bromide is used, planting may occur 2–3 days after fumigation (3,14). These trends indicate that in some cases it may be desirable to assay nematode populations within a short time after fumigation, rather than at the recommended 2–3 weeks after fumigation (2).

An unusual problem arose in a chemical test recently conducted at the Agricultural Research and Education Center (AREC) in Homestead, Florida, when nematode counts were made within 7 days after fumigation. Initially this fumigation experiment appeared to be unsuccessful, with few or no differences in nematode counts between fumigated and nonfumigated plots when assayed up to 2 weeks after fumigation. However, the anticipated differences in counts were recorded at 6 weeks and later in the season. It is possible that nematodes already dead in the soil might have been unknowingly included in the initial counts since, after the extraction process, all nematodes were routinely killed by heating in a water bath at 55–60 C for 10 minutes in order to facilitate the counting process. Many manuals dealing with nematological methods (1,5,15) recommend killing nematodes by heat before fixing and identifying, but often it is not clear whether such a step is to be included between the extraction and the counting processes or whether the nematodes are to be counted live. The following experiment was conducted to determine whether the methods used could cause a misinterpretation of the experimental results due to inflation of heat-killed nematode counts by fumigant-killed animals.

The experiment was established on raised beds of Rockdale series soil (6) with pH = 7.5 at the AREC in Homestead, Florida. The experimental design was a randomized complete block, replicated four times, with four treatments. Each treatment represented a common commercial practice in south Florida: (i) methyl bromide (98%) + chloropicrin (2%), applied as 280 kg Dowfume MC-2/ha and covered immediately with a thin plastic mulch; (ii) sodium methyl dithiocarbamate (32.7%) drenched onto the soil as 933 liters Vapam in 51 kl of water/ha and left uncovered; (iii) plastic mulch without fumigation; and (iv) check, without plastic mulch or fumigation. All treatments were applied on 22 April 1983, and all plastic was removed on 25 April 1983. Each plot, 15 m long × 1 m wide, was sampled for plant-parasitic nematodes 3, 4, 5, 7, 12, and 17 days after treatment. Each sample consisted of soil collected with a hand trowel from 20 locations within the plot. In the laboratory, each sample was passed through a 4.0-mm sieve to remove rocks and plant debris, and a 100-cm^3 subsample was processed for nematodes by a combination of decanting and sieving followed by centrifugation (9,10). Numbers of live (based on motility) and total (live and dead) nematodes were determined after the extraction process with no killing and fixing step between. Resultant numbers for each date were analyzed by an analysis of variance followed by Duncan's multiple-range test.

Rotylenchulus reniformis Linford and Oliveira and *Helicotylenchus dihystera* (Cobb)

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TABLE 1. Numbers of live and total (live and dead) *Rotylenchulus reniformis* and *Helicotylenchus dihystera* per 100 cm^3 of soil at various times after fumigation.

Treatment	Days after fumigation*											
	3		4		5		7		12		17	
	Live	Total	Live	Total	Live	Total	Live	Total	Live	Total	Live	Total
R. reniformis												
Methyl bromide	1 a	269 a	0 a	199 a	0 a	135 a	5 a	100 a	1 a	12 a	0 a	0 a
Vapam	0 a	168 a	0 a	178 a	0 a	140 a	0 a	56 a	0 a	5 a	0 a	0 a
Check, plastic mulch	178 b	180 a	141 b	116 a	101 b	105 a	242 b	270 b	140 b	154 b	129 b	158 a
Check, unmulched	152 b	156 a	122 b	125 a	159 b	169 a	149 b	155 a	231 c	236 b	111 b	125 a
H. dihystera												
Methyl bromide	0 a	59 a	0 a	15 a	0 a	19 a	0 a	15 a	0 a	1 a	0 a	1 a
Vapam	0 a	35 a	0 a	50 a	0 a	25 a	0 a	25 a	0 a	2 a	0 a	0 a
Check, plastic mulch	41 b	42 a	44 b	50 a	18 ab	21 a	15 b	26 a	18 b	20 b	15 b	18 b
Check, unmulched	45 b	48 a	46 b	52 a	26 b	32 a	10 b	10 b	26 b	30 b	14 b	18 b

* Data are means of four replications. Means in columns followed by the same letter are not significantly ($P = 0.05$) different, according to Duncan's multiple-range test.

Sher were found in the experimental plots (Table 1). The efficacy of the soil fumigants was apparent in the live numbers of both species within 3 days after fumigation and persisted throughout the experiment. Examination of only the total numbers (live and dead) of each species would give quite a different impression of the results, since differences among treatments were not apparent until 7–12 days after fumigation. Yet these are the results that would have been obtained if the nematodes had been routinely heat-killed between the extraction and counting steps, since all nematodes extracted, living or dead, would be counted in that case. Bodies of dead nematodes were preserved intact in the soil up to 7–12 days after fumigation, with decomposition evident only after that time. Evidently the organisms which decompose dead nematodes are also killed or suppressed by fumigation, resulting in a time lag before recolonization similar to that observed for bacteria involved in nitrification (7). The warm conditions under which this experiment was conducted would favor a rapid increase of such microorganisms; a longer lag period could be expected under cooler conditions. This effect should be considered if nematode counts must be made within 3 weeks of fumigation; live counts should be obtained without prior killing of nematodes in the samples. A vital stain (8) may be of some benefit in distinguishing live and dead nematodes. A method which requires live nematodes, such as sieving followed by Baermann funnels (4), could also be used, although tests with this method under the current conditions recovered fewer live nematodes than did the centrifuge method. Compared to centrifuge methods, Baermann funnel methods have the additional drawback of often giving more variable numbers. If funnel extraction methods are used for those samples taken soon after fumigation, it is useful to develop regression equations relating numbers obtained by the funnels to those obtained by the more usual centrifuge methods (11).

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