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Effect of Fenitrothion on Microorganisms which Degrade Leaf-Litter and Cellulose in Forest Soils

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Fenitrothion, an organophosphorus insecticide (HATTORI et al. 1976), has been used for the past ten years to control the spruce budworm (Choristoneura fumiferana) in North American forests. Only recently have reports appeared regarding the impact and fate of fenitrothion on the forest environment (ROBERTS et al. 1977). Recent studies have indicated that qualitatively, fenitrothion does not affect forest soil microflora (SPILLNER et al. 1979) even when applied in massive doses (SALONIUS 1972). Therefore, fenitrothion would not appear to be detrimental to the general population of forest soil microbes. The most important populations of forest soil microbes are the leaf-litter degraders and more specifically, the cellulose-degraders (SIU 1951). The current studies were conducted to determine the effect of fenitrothion on these important groups of microorganisms.

MATERIALS AND METHODS

Chemicals. Sumithion® 8EC [supplied by Stauffer Chemical Company, Richmond, CA, comprised of 81.9% Sumithion technical (95% purity), 8.1% Socal #2, 10% Atlox 3409F] was combined with water to yield stock solutions, 13.7 µg a.i./mL and 5.0 µg a.i./µL, used, respectively, in the leaf-litter and cellulose degradation studies.

[^{14}C]Cellulose (ICN Chemical) uniformly ring-labeled (50 µCi @ 4.5 µCi/mg) was combined with 1 mL water in a tissue grinder and ground into a slurry. The [^{14}C]cellulose slurry was combined, through repeated rinsing of the tissue grinder, with 5 g cellulose and ca. 30 mL water in an Omni-mixer and was blended at high speed for 15 min. The resulting slurry was centrifuged (20,000 x G), the supernatant solution removed, and the pellet ([^{14}C]cellulose) air-dried. The specific activity of the resulting [^{14}C]cellulose was 0.0112 $\pm$ 0.0008 µCi/mg.

Soils and Leaf-Litter. Organic soil and leaf-litter obtained from the University of Maine forest were used. The organic soil represents a 5.0-cm layer of material lying below the leaf-litter and over the mineral soil. Physical properties were as follows: sand 74%, silt 23%, clay 3%, organic matter 44.4%, pH 7, and field capacity 115 g H_2O/100 g dry soil.

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Treatment of Leaf-Litter. Leaf-litter (450 g) was placed in a 5-gal drum and mechanically rotated while spraying with 30 mL of stock solution (411 µg a.i.). After mixing for 10 min the litter was separated into 150 g portions and each portion was placed in a 1-gal incubating chamber which was coupled to a gas trap containing 115 mL 10% KOH. Three control chambers (150 g litter), 3 control sterile chambers (150 g litter sterilized by autoclaving at 130 C 15 psi for 20 min on two successive days), an empty chamber, and the fenitrothion-treated leaf-litter chambers were then placed in a dark growth chamber held at 28 C. Each flask was attached to an individual outlet of a manifold coupled to a vacuum pump which pulled CO_2-free air through each metabolism chamber/gas trap apparatus. The CO_2 trapped in the KOH solution was precipitated as $BaCO_3$ by adding 150 mL of a 0.5N $BaCl_2$/0.5N NH_4Cl solution. The precipitate was then collected on filter paper, washed with water and acetone, air-dried, and weighed.

Treatment of Forest Organic Soil with [^{14}C]Cellulose and Fenitrothion. Experiments were run using the soil metabolism flasks described by BARTHA & PRAMER (1968). A constant oxygen tension was maintained over the soil to help keep the microbes viable. CO_2 resulting from microbial activity was trapped in the side arm containing 20 mL 0.5N NaOH. Experiments were conducted in a dark growth chamber at 30 C. NaOH was sampled periodically for radiochemical, titrimetric, and gravimetric analysis.

Into each flask was combined ca. 0.5 g [^{14}C]cellulose either 10 µL (50 µg) or 50 µL (250 µg) of the Sumithion 8EC solution described previously, 16 mL of water, and 33 g of soil. The final moisture content of the soil was 133 g water/100 g dry soil. On a wet soil basis, cellulose fortification was 1% while fenitrothion treatments were 1 or 5 ppm. Fenitrothion and [^{14}C]cellulose-treated soils were prepared in duplicate.

Control experiments included a blank flask (no soil), a flask containing untreated soil, and flasks containing sterilized soil. Sterilization was achieved by autoclaving flasks containing soil and [^{14}C]cellulose (no fenitrothion) for 1.0 hr at 15 psi and 120 C on two consecutive days. Flasks containing sterilized soil were coupled together and maintained separately from the unsterilized experiments. Aseptic procedures were followed in the sterilized soil experiments.

Microbial Analysis. Standard microbial procedures were used to determined the viable populations of bacteria, yeast, fungi, and actinomycetes. One gram of each leaf-litter sample was mixed with physiological saline in a blender. The samples were serially diluted and plated onto (a) plate count agar to detect bacteria, (b) soluble starch-casein agar to detect actinomycetes, and (c) potato

dextrose agar with rose bengal to detect fungi. One gram of each sample was also oven-dried to determine moisture content.

Radiochemical Analysis. Standard radioanalytical procedures were followed.

RESULTS

Forest Leaf-Litter Degradation. Gravimetric determinations of CO_2 were made 11 and 14 days after treatment of the leaf-litter (Table 1). A slight increase in CO_2 evolution was detected from the fenitrothion-treated litter after 11 days, but after 14 days, the treated and untreated litter had similar rates of respiration. Furthermore, microbial analysis (Table 2) revealed similar populations of microflora in untreated and treated forest litter.

Ten days after initiation of this study a white mycelial growth was visible in the sterilized chambers. Contamination was also evident from the high level of CO_2 evolved in comparison to CO_2 trapped in the empty control chamber. Microbial analysis also showed an increase in organisms in the sterilized leaf-litter after 14 days (Table 2). However, the microbial populations in the sterilized leaf-litter was <6% of the populations in the nonsterilized leaf-litter.

TABLE 1. Gravimetric determination of CO_2 (g) from forest leaf-litter metabolism chambers.

Treatment	Day 0-11	Day 12-14
Untreated	18.5 ± 2.1	7.9 ± 1.8
1 ppm Fenitrothion	23.7 ± 0.3	7.6 ± 1.3
Sterilized	0.9 ± 0.5	2.7 ± 1.2

TABLE 2. Microorganisms ($x10^{-7}$) per gram of wet leaf-litter.

Sample	bact.[a/]	actino-mycetes	fungi	yeast
Sterilized, day 0	0.06	0.06	0.03	trace
Untreated, day 0	12.	3.8	0.03	0.3
Sterilized, day 14	0.75	0.4	0.075	trace
Untreated, day 14	9.1	6.4	4.2	0.1
Treated, day 14	12.	6.4	3.1	0.3

a/ The bacterial population includes the actinomycetes count.

Cellulose Degradation. As in the leaf-litter study, an indication of the effect of fenitrothion on the general microbial population was obtained by comparing CO_2 evolution from treated soils with that from the untreated soils. The addition of cellulose to the soil resulted in significant enhancement of biological activity as evidenced by a 43 - 66% increase in CO_2 evolution from the fortified, as compared to the unfortified, soils. The average (of duplicate runs) accumulated CO_2 is shown in Figure 1. Furthermore, respiration in soils containing 1 or 5 ppm fenitrothion is not significantly different (90% confidence limits) from soils fortified only with cellulose.

[^{14}C]Cellulose degradation was measured by the evolution of $^{14}CO_2$. The quantitative accountability of carbon-14 (100 $\pm$ 10%) indicates trapping of $^{14}CO_2$ was efficient throughout the study. Comparison of [^{14}C]cellulose degradation in the variously treated soils is made in Figure 2 where the cumulative average $^{14}CO_2$ evolution of the replicate soil treatments is plotted against the sampling time after treatment. No significant difference (90% confidence limits) between treated (1 and 5 ppm) and untreated [^{14}C]cellulose-fortified soils were observed.

DISCUSSION

The respiration rate of leaf-litter microbes increased substantially from 11 - 14 days in comparison to the initial rate for 0 - 11 days (Table 1). This indicates an acclimation period was required for the microbes to reach equilibrium in the apparatus. The initial stimulation of microbial respiration in leaf-litter 11 days after treatment with fenitrothion may have been due to aeration of the leaf-litter during mechanical incorporation of the fenitrothion which accelerated the microbes toward an equilibrium state. This is supported by the fact that similar respiration rates and populations of microbes were found in both untreated and treated leaf-litter after 14 days. These results suggest that 1 ppm fenitrothion has no significant effect on degradation of forest leaf-litter by microorganisms.

The enhanced respiration (Figure 1) of forest organic soils fortified with 1% cellulose indicated either an enrichment and/or stimulation of cellulytic decomposing microbes in the soil. This enhanced activity facilitated observation of the effects of added fenitrothion on this segment of the microbial community. Fenitrothion at 1 or 5 ppm did not significantly affect microbial respiration. This result is not surprising in view of the report that fenitrothion at concentrations of 274 - 640 ppm in forest soils was not inhibitory to the microflora (SALONIUS 1972). From statistical analysis it was possible to determine that 25% inhibition of microbial respiration would have been significant.

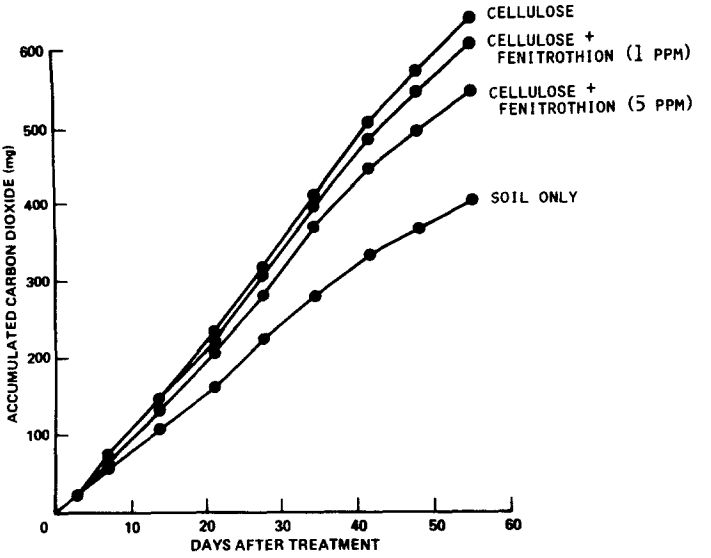

Figure 1. The average accumulated evolved CO_2 from [^{14}C]cellulose-fortified and nonfortified soils

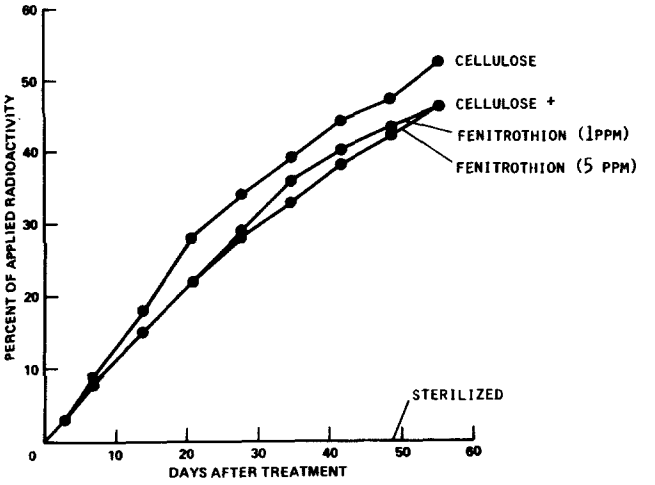

Figure 2. The average accumulated evolved $^{14}CO_2$ from [^{14}C]cellulose fortified soils.

A direct measure of [^{14}C]cellulose degradation was obtained by monitoring the evolution of $^{14}CO_2$ (Figure 2). This is a technique which has been used and substantiated by other workers (RAMANUJAM et al. 1977). The absence of $^{14}CO_2$ evolution from sterilized soils (positive controls) indicated microbial activity was responsible for the cellulose degradation observed. Comparison of cellulose degradation in treated and untreated soils indicated that 1 and 5 ppm fenitrothion is not inhibitory to cellulose-degrading microorganisms in this forest soil.

Taking into account that residues of fenitrothion under actual use conditions are ca. 0.05 ppm in forest soils (YULE & DUFFY 1972, BUSSEY 1978), the data from these studies indicate that fenitrothion and its soil metabolites (SPILLNER et al. 1979) 1/ will not be detrimental to leaf-litter or cellulose-degrading microorganisms in this forest soil.

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1/ In separate studies 50% degradation of [ring-^{14}C] fenitrothion occurred in 3 days in forest organic soil. The major metabolites were 3-methyl-4-nitrophenol, an incorporated fraction, and $^{14}CO_2$.