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Effect of Zectran on Microbial Activity in a Forest Soil

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ABSTRACT

When Zectran or No. 2 fuel oil was applied to soil in concentrations far greater than those that could result from current operational application rates, neither material adversely affected soil microbial activity. We conclude from these data that low-volume applications of Zectran as an insecticide for forest use poses no hazard to soil microbes.

The insecticide Zectran^{1/} applied aerially at a nominal rate of 0.15 pound per acre was as effective as malathion or DDT applied at 1.0 pound per acre to control western spruce budworm (*Choristoneura occidentalis* Freeman) in Montana field tests (Buffam, Meyer, and Miskus 1967). On ponderosa pine foliage in the laboratory, Zectran proved more toxic than DDT against larvae of a pine tussock moth (*Dasychira* sp.) (Lyon, Brown, and Richmond 1967).

The chemical nature of Zectran suggests it may be much less persistent in the forest ecosystem than DDT (Hammer 1962). Zectran levels in plant foliage decrease rapidly after application (Pieper and Miskus 1967). Thus, it is a promising chemical for use against forest tree defoliators previously controlled by aerial applications of DDT (Anonymous 1967).

In determining the safety of chemicals proposed for use in the forest environment, we are interested in effects of the chemicals on soil microbes which influence soil fertility. Soil respiration, as indicated by carbon dioxide (CO₂) evolution, is a good general index of the activity of microflora involved in organic matter decomposition (Casida 1968). This index was used in our laboratory experiments to measure effects of Zectran on the microflora found in a coastal Oregon forest soil. (See details in table 1 and "Materials and Methods.")

Zectran applied to soil without tree litter or oil carrier had no apparent effect on CO₂ evolution (table 1). Tree litter applied to soil without Zectran or oil carrier also had no apparent effect (table 2). Further, there appeared to be no interaction between Zectran and tree litter (table 3).

^{1/} The Dow Chemical Company trademark for 4-dimethylamino-3,5-xylyl methylcarbamate.

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Table 1.--*Effect of Zectran on evolution of CO₂ for 70 days
from Astoria silty clay loam*

Soil treatment comparisons	Total CO ₂ as carbon	Difference ^{1/}
-----Milligrams-----		
1. No treatment	111	
vs.		
Zectran at 1 p.p.m.	107	4
2. No treatment	111	
vs.		
Zectran at 10 p.p.m.	104	7
3. Zectran at 1 p.p.m.	107	
vs.		
Zectran at 10 p.p.m.	104	3

^{1/} First two comparisons not included in tests of significance; difference in third comparison is not significant at the 95-percent level of probability.

Table 2.--*Effect of tree litter on evolution of CO₂ for 70 days
from Astoria silty clay loam*

Soil treatment comparisons	Total CO ₂ as carbon	Difference ^{1/}
-----Milligrams-----		
1. No treatment	111	
vs. alder litter	105	6
2. No treatment	111	
vs. conifer litter	122	11
3. All treatments with alder litter	153	
vs. all treatments with conifer litter	159	6
4. All treatments without litter	148	
vs. all treatments with litter	156	8

^{1/} First two comparisons not included in tests of significance; differences in third and fourth comparisons are not significant at the 95-percent level of probability.

Table 3.--*Effect of Zectran in combination with tree litter on evolution of CO₂ for 70 days from Astoria silty clay loam*

Soil treatment comparisons	Total CO ₂ as carbon	Difference ^{1/}
-----Milligrams-----		
1. Zectran at 1 p.p.m. + alder litter	110	
vs. Zectran at 10 p.p.m. + alder litter	96	14
2. Zectran at 1 p.p.m. + conifer litter	100	
vs. Zectran at 10 p.p.m. + conifer litter	113	13

^{1/} Differences were not significant at the 95-percent level of probability.

Comparisons based on means of all treatments combined (table 2) indicate that mean CO₂ evolution for all treatments combined is substantially higher than means of treatments which included only Zectran or tree litter. Thus, the oil carrier is indicated as having some role in increasing microbial respiration. The comparisons shown in table 4 demonstrate that the carrier indeed is responsible for a substantial increase in microbial respiration. There was no significant difference in CO₂ production between the rates of carrier used, but means of all treatments containing the carrier were significantly greater than means of all treatments that did not include the carrier.

We next looked for interactions between Zectran and the carrier. The CO₂ index was not significantly different between Zectran in carrier at 1 part per million (p.p.m.) and at 10 p.p.m. (table 5). We do see, however, that when Zectran in carrier is compared with the carrier only, presence of Zectran apparently causes a significant decrease in CO₂ evolution. While this decrease is significant relative to levels obtained with carrier only, the absolute value for CO₂ production is still substantially higher than values measured in the absence of any carrier.

In our study of interactions between the carrier and tree litter (table 6), there was no significant difference between carrier rates of 1 and 10 p.p.m. in combination with conifer litter. However, when alder litter and carrier were together, the 10-p.p.m. carrier rate led to a highly significant reduction

Table 4.--*Effect of acetone-fuel oil carrier on evolution of CO₂ for 70 days from Astoria silty clay loam*

Soil treatment comparisons	Total CO ₂ as carbon	Difference ^{1/}
-----Milligrams-----		
1. No treatment vs. carrier at 1-p.p.m. rate ^{2/}	111 183	72
2. No treatment vs. carrier at 10-p.p.m. rate	111 192	81
3. Carrier at 1-p.p.m. rate vs. carrier at 10-p.p.m. rate	183 192	9
4. All treatments without carrier vs. all treatments with carrier	106 194	88**

^{1/} First two comparisons not included in tests of significance; difference marked ** is significant at the 99-percent level of probability.

^{2/} Carrier rates of 1 and 10 p.p.m. are equal to those used when Zectran at 1 and 10 p.p.m. was dissolved in carrier.

Table 5.--*Effect of Zectran in combination with acetone-fuel oil carrier on evolution of CO₂ for 70 days from Astoria silty clay loam*

Soil treatment comparisons	Total CO ₂ as carbon	Difference ^{1/}
-----Milligrams-----		
1. Zectran at 1 and 10 p.p.m. + carrier vs. carrier only at 1- and 10-p.p.m. rates ^{2/}	169 188	19*
2. Zectran at 1 p.p.m. + carrier vs. Zectran at 10 p.p.m. + carrier	167 170	3

^{1/} Difference marked * is significant at the 95-percent level of probability.

^{2/} Carrier rates of 1 and 10 p.p.m. are equal to those used when Zectran at 1 and 10 p.p.m. was dissolved in carrier.

Table 6.--*Effect of acetone-fuel oil carrier in combination with tree litter on evolution of CO₂ for 70 days from Astoria silty clay loam*

Soil treatment comparisons	Total CO ₂ as carbon	Difference ^{1/}
-----Milligrams-----		
1. Carrier at 1-p.p.m. rate + conifer litter	187	
vs.		
carrier at 10-p.p.m. rate + conifer litter	197	10
2. Carrier at 1-p.p.m. rate ^{2/} + alder litter	201	
vs.		
carrier at 10-p.p.m. rate + alder litter	170	31**

^{1/} Difference marked ** is significant at the 99-percent level of probability.

^{2/} Carrier rates of 1 and 10 p.p.m. are equal to those used when Zectran at 1 and 10 p.p.m. was dissolved in carrier.

in CO₂ evolution. Red alder litter has a high content of phenolic compounds, some of which are inhibitory to micro-organisms (Li et al. 1969). We hypothesize that the higher rate of acetone-fuel oil carrier released phenolic compounds from the alder litter and that some inhibition of microbial activity thus resulted. Again, the reduction we noted as a result of this interaction appears not to be substantial in comparison with CO₂ evolution rates from all treatments that did not contain the carrier.

When we looked for interactions between all three treatments used in this study--Zectran, litter, and carrier--we found no significant difference between Zectran in carrier at 1 p.p.m. versus 10 p.p.m. in the presence either of alder or conifer litter (table 7).

Finally, in determining the relative effect of acetone and of fuel oil in the carrier mixture, we found no significant differences between untreated controls and fuel oil at either the 1- or 10-p.p.m. rate; nor was there any difference between fuel oil alone at 1- versus 10-p.p.m. rates (table 8). In contrast, presence of acetone at the 1-p.p.m. rate significantly increased CO₂ evolution over that measured from untreated soil. Acetone at the 10-p.p.m. rate also significantly increased CO₂ evolution, but the absolute amount of increase was substantially less than that noted with the 1-p.p.m.

Table 7.--Effect of Zectran, tree litter, and acetone-fuel oil carrier on evolution of CO₂ for 70 days from Astoria silty clay loam

Soil treatment comparisons	Total CO ₂ as carbon	Difference ^{1/}
-----Milligrams-----		
1. Zectran at 1 p.p.m. + alder litter + carrier	187	
vs.		
Zectran at 10 p.p.m. + alder litter + carrier	202	15
2. Zectran at 1 p.p.m. + conifer litter + carrier	200	
vs.		
Zectran at 10 p.p.m. + conifer litter + carrier	193	7

^{1/} Differences not significant at 95-percent level of probability.

Table 8.--Effect of fuel oil and of acetone on evolution of CO₂ for 70 days from Astoria silty clay loam^{1/}

Soil treatment comparisons ^{2/}	Total CO ₂ as carbon	Difference ^{3/}
-----Milligrams-----		
1. Soil alone	81	
vs. fuel oil at 1-p.p.m. rate	78	3
2. Soil alone	81	
vs. fuel oil at 10-p.p.m. rate	84	3
3. Fuel oil at 1-p.p.m. rate	78	
vs. fuel oil at 10-p.p.m. rate	84	6
4. Soil alone	81	
vs. acetone at 1-p.p.m. rate	166	85*
5. Soil alone	91	
vs. acetone at 10-p.p.m. rate	121	40*
6. Acetone at 1-p.p.m. rate	166	
vs. acetone at 10-p.p.m. rate	121	45*

^{1/} These data are from a separate study. Soil age after collection and environmental temperature were different from that in the main study, accounting for the generally lower absolute values for total CO₂ as carbon.

^{2/} Rates are equal to those used for acetone-oil carrier in Zectran treatments at 1 and 10 p.p.m..

^{3/} Differences marked * are significant at the 95-percent level of probability.

rate. We conclude from these comparisons that acetone, not fuel oil, was responsible for increases in CO₂ evolution noted whenever the acetone-fuel oil carrier was included in treatments.

Thus, when Zectran or No. 2 fuel oil was applied to soil in concentrations far greater than those that could result from current operational application rates (Buffam, Meyer, and Miskus 1967), neither material adversely affected soil microbial activity. We conclude from these data that low-volume applications of Zectran as an insecticide for forest use poses no hazard to soil microbes.

MATERIALS AND METHODS

In general, an Astoria silty clay loam soil was subjected to different treatments which included Zectran, forest tree litter, and an acetone-oil carrier mixture (table 9). Treated soil was incubated for 70 days, and microbial activity was determined from the amount of CO₂ evolved.

A bulk sample of the A11 horizon of Astoria silty clay loam was taken beneath a conifer stand--Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and Sitka spruce (*Picea sitchensis*)--at the Cascade Head Experimental Forest near Otis, Oreg. A detailed list of chemical and physical properties of this soil is included in Franklin et al. (1968). For the soil used in the present study, organic matter was 28 percent, total carbon by dry combustion was 16 percent, and Kjeldahl nitrogen was 0.41 percent.

Fifty-gram portions of sieved (10-mesh) soil, oven-dry basis, were transferred into 250-milliliter widemouth Erlenmeyer flasks. Each treatment (table 9) was replicated three times. In treatments which contained forest litter or Zectran powder, these additives were mixed with the soil before it was transferred to the flasks. Distilled water was then added in sufficient amounts to bring moisture content of the treated soil to 50 percent of water-holding capacity (81 percent, dry weight basis).

For treatments which included Zectran in an oil carrier, a stock solution was prepared by dissolving 2 grams of the insecticide in 20 milliliters acetone, then adding 80 milliliters No. 2 fuel oil. Subsequent dilutions were made so that 1 milliliter additions of the solution to 50 grams of soil would yield 1 or 10 p.p.m. actual Zectran. Treatments which included the carrier alone or carrier in combination with litter were in amounts of the carrier equivalent to those in which Zectran had been included.

The tree litter used was collected from the Cascade Head Experimental Forest near where the soil was collected for the study. Litter was air dried

Table 9.--Description of treatments to Astoria silty clay loam
and total CO₂ evolved over 70 days of incubation

Soil treatment ^{1/}	Total CO ₂ as carbon
	Milligrams
None--control	111
Z1	107
Z1, conifer	100
Z1, alder	110
Z10	104
Z10, conifer	113
Z10, alder	96
Conifer	122
Alder	105
Z1, oil	167
Z1, oil, conifer	200
Z1, oil, alder	187
Z10, oil	170
Z10, oil, conifer	193
Z10, oil, alder	202
Oil(1)	183
Oil(10)	192
Oil(1), conifer	187
Oil(10), conifer	197
Oil(1), alder	201
Oil(10), alder	170

^{1/} Z1 or Z10 = Zectran at 1 or 10 p.p.m.; conifer or alder = type of forest litter; oil(1) or oil(10) = oil without Zectran but in same amount as used when Zectran in oil carrier was at 1 or 10 p.p.m. Oil carrier was a 1:4 mixture of acetone and No. 2 fuel oil.

at room temperature and ground to pass through a 60-mesh sieve. The alder litter contained 51.74 percent carbon and 2.31 percent nitrogen; the conifer litter, 51.85 percent carbon and 0.84 percent nitrogen.

Each flask containing soil and additives was connected to a CO₂-free, water-saturated air supply. The exit connection from the flask was inserted into a tube containing 1N NaOH to absorb CO₂ evolved from the soil by microbial activity. The entire respiration setup was incubated at 25° ± 1° C. for 70 days. The tubes of NaOH were replaced at intervals of 1, 4, 8, 14, 28, 42, and 70 days. Amount of CO₂ absorbed by the NaOH was determined by double titration on a Beckman automatic titrator. Details of the apparatus and methods are given in Bollen and Lu (1961) and Bollen and Glennie (1961).

Resulting data were subjected to two analyses of variance in which treatment degrees of freedom were partitioned into an orthogonal set of comparisons (tables 1-8).

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