Degradation of Carbofuran by Soil Microorganisms

by I. H. WILLIAMS, H. S. PEPIN, and M. J. BROWN
Research Station, Agriculture Canada
Vancouver, B.C. V6T 1X2

Carbofuran (2,2 dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate) is a useful soil insecticide effective against a broad range of soil inhabiting pests (APPLE et al. 1969; BERRY 1971; TAPPEN 1966). Normally it has a reasonably long half-life of up to 50 weeks in neutral or acid soils (GETZIN 1973). In alkaline soils hydrolysis occurs at the carbamate linkage causing rapid degradation, probably to the phenol.

Recently when this insecticide was used for the control of phylloxera in a vineyard planted in gravelly loam of pH 6.0, it proved less effective than was anticipated. Analysis of the soil for carbofuran also showed a lower concentration than would have been expected from the rate of application used.

On fortifying an untreated sample of this particular soil with a known amount of carbofuran and analysing at intervals over a period of several weeks recovery was found to decrease with time. A test for possible irreversible adsorption by the clay content of the soil proved negative and this led to the logical alternative of possible microbial degradation. Some evidence of this had been reported previously (GETZIN 1973).

This paper describes experiments which confirm this hypothesis and identify the more active organisms involved.

EXPERIMENTAL

Measurement of microbial degradation: The effect of microbial degradation was measured by comparing the rate of release of $^{14}CO_2$ from sterile and non-sterile samples of the same soil treated with ^{14}C carbonyl labelled carbofuran. The radiolabelled material was supplied by F.M.C., Middleport, N.Y. Label purity of greater than 99.7% was determined by TLC. Activity was 3.54 mCi/mmol. A standard solution containing 1 µCi/ml in acetone was prepared from this material. Untreated vineyard

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 2 © 1976 by Springer-Verlag New York Inc.

soil containing 12% moisture was screened through a 2 mm mesh sieve and 50 g aliquots of this were transferred to two 8 oz. screw-cap jars. One was autoclaved for two 1 hr. periods on successive days. An equivalent of 2 ppm of ^{14}C-labelled carbofuran was added to each sample from the standard solution. Jars were tumbled over night in a Fisher-Kendall mixer.

The biometric apparatus used in the experiment consisted of two 300 mm x 20 mm ID chromatographic columns equipped with stopcocks and $ 24/40 female joints at the top. Fitted to these were adapters joined to two absorption tubes connected in series. The latter consisted of 20 ml scintillation vials containing 6 ml of 0.5N sodium hydroxide solution.

The columns were sterilized and the fortified soil samples transferred to them under aseptic conditions. Air supplied by an aquarium pump was moisturized and freed of atmospheric CO_2 by passage through a scrubber containing 0.5N sodium hydroxide. This air was allowed to percolate slowly upward through the columns, its flow being controlled by a by-pass valve on the scrubber and by the individual column stopcocks. Samples containing any evolved $^{14}CO_2$ were collected at weekly intervals over a period of eight weeks. After removing the absorption tubes 10 ml of phase combining solution (PCS Amersham/ Searle) was added to each and the absorbed ^{14}C counted in a scintillation spectrometer (Picker Nuclear Liquimat Model 650). After eight weeks the soil was removed from the columns and analysed for total ^{14}C by igniting 1 g samples in an induction furnace, collecting the $^{14}CO_2$ evolved and measuring it by scintillation counting. All counting was corrected for background and quenching.

Isolation of soil microorganisms: Organisms used in the study were isolated from samples of furadan-treated soil from Oliver, B.C. One gram of each sample was placed in 100 ml of sterile water in a 250 ml erlenmeyer flask and shaken 30 minutes on a rotary shaker. A dilution series, giving a final dilution of 1:1,000,000 was set up, 1 ml of the final dilution being pipetted and swirled on the surface of each of 10 water agar (WA) plates. Some isolations were made directly from soil crumbs placed on potato dextrose agar (PDA) plates. All WA plates and half the PDA plates were incubated at room temperature. The rest of the PDA plates were incubated at 3°C. All developing colonies were transferred to PDA for purification. Stock cultures were stored on oatmeal agar slants.

Evaluation of individual microorganisms in degrading carbofuran: Pure cultures of eight different micro-

organisms were studied. Exactly 0.1 ml of standard ^{14}C-carbofuran solution was added to each of a series of sterilized Warburg flasks and the solvent evaporated under aseptic conditions. To each was added 5 ml of one of the cultures to be studied. A slow stream of moisturized, CO_2-free air generated as previously described was passed over each culture and any released $^{14}CO_2$ was absorbed in 0.5N sodium hydroxide as in the previous experiment and counted in the same manner. The activity of each culture was assessed on the basis of the amount of CO_2 released during a three day period as related to the dried weight of the particular organism involved.

Effect of temperature on the degradation of soil-applied carbofuran and its 3-hydroxy metabolite: Samples of two types of soil from the area being investigated were fortified at the 1 ppm level with carbofuran and 3-hydroxycarbofuran. After thorough mixing of the samples 20 g aliquots were removed and analysed. The remainder of one set was maintained at 20°C while the other set was immediately transferred to the cold room and maintained at -16°C. At periods of 4, 8, and 12 weeks further aliquots were removed from both sets and analysed.

RESULTS AND DISCUSSION

In Fig. 1 relative rates of release of $^{14}CO_2$ from sterile and non-sterile soil are plotted. From this it is evident that under the conditions of the experiment the rate of degradation of carbofuran during the second week was forty times greater in the non-sterile soil than in the sterile soil. Analysis for total ^{14}C in the soil after eight weeks incubation showed that the amount of carbofuran remaining in the non-sterile soil had decreased to less than 60% of the amount originally present. In the sterile soil no measurable loss had occurred.

Fig. 2 illustrates the relative activity of various microorganisms in degrading carbofuran. Here it is seen that some of the actinomycetes are particularly active. An attempt is being made to have these identified though this is proving difficult.

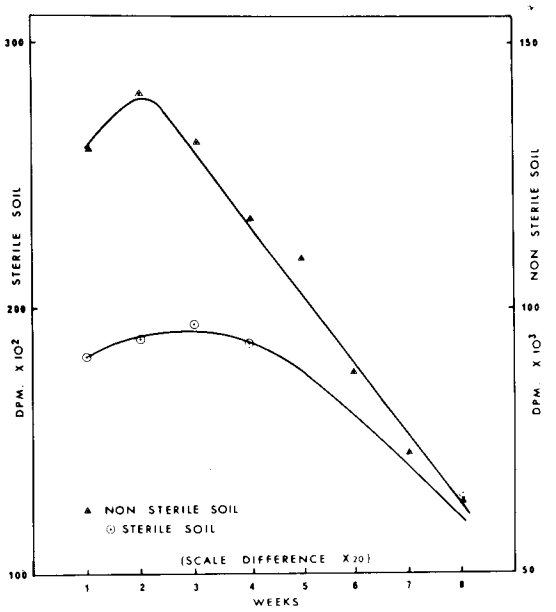

Figure 1. Comparison of rates of degradation of sterile and non-sterile soil fortified with ^{14}C-labelled carbofuran.

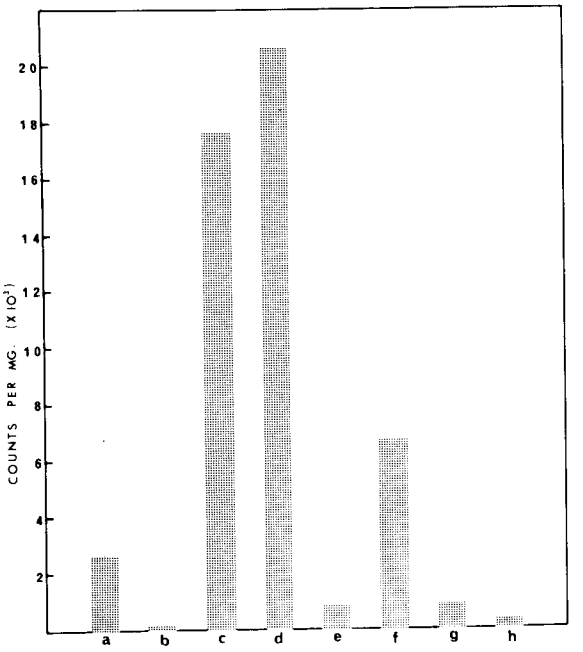

Figure 2. Relative effectiveness of a number of microorganisms in degrading carbofuran over a three day period. (a) actinomycete, (b) penicillium, (c)(d)(e)(f) actinomycetes, (g) penicillium, (h) unidentified.

TABLE I

Effect of Temperature on the Degradation of Carbofuran and 3-Hydroxycarbofuran in Two Okanagan Soils

Storage period (weeks)	Soil No.*	Recovery (% of original conc. present)			
		Storage at 20°C		Storage at -16°C	
		Carbofuran	3-OH Carbofuran	Carbofuran	3-OH Carbofuran
0	1	82.8	90.7	82.8	90.7
	2	81.5	91.9	81.5	91.9
4	1	50.5	ND**	75.0	83.0
	2	58.3	ND	76.8	67.6
8	1	24.4	ND	57.1	82.7
	2	34.3	ND	72.5	53.5
12	1	ND	ND	63.3	86.0
	2	7.8	ND	63.4	76.6

* 1 - Westbank
2 - Oliver

** none detected

Table I shows the effect of temperature on the rate of degradation of carbofuran and 3-hydroxycarbofuran in soil. It is obvious from this that serious losses can occur between the time of sampling the soil and its eventual analysis if it is not kept at sub zero temperatures during this period. Of particular interest is the very rapid disappearance of 3-hydroxycarbofuran. Whether the same microorganisms responsible for the degradation of carbofuran are also responsible for the disappearance of this metabolite has yet to be determined.

From this study it is evident that in a soil containing high levels of actinomycetes rapid degradation of carbofuran may be expected. This should be borne in mind not only in storing samples but also in assessing the efficacy of this insecticide in controlling soil insects. What in some cases may appear to be possible build-up of resistance by the insect for which control is desired may in fact be a lowering of the insecticidal concentration to an ineffective level by microbial degradation.

Further studies will be carried out to determine whether other carbamates are degraded in similar manner by the same organisms.

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