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Biodegradation of Carbofuran in Pretreated and Non-Pretreated Soils

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Carbofuran (2,2-dihydro-2,2-dimethyl-7-benzofuranylmethyl-carbamate) is a broad spectrum insecticide which is effective against soil insects in corn, rice, sugar cane, peanuts, cotton and pests on potatoes (Pandya, 1983; Williams et al., 1976). Decreased effectiveness of insect control in soils treated with carbofuran was observed after soils had been treated for a number of years (Ahmad et al., 1979; Caro et al., 1973; Felsot et al., 1981; Felsot et al., 1982). Loss of efficacy was not related to insect resistance or soil leaching (Felsot et al., 1982). Irreversible adsorption was not involved (Williams et al., 1976); however, the carbofuran levels in the soil were lower than expected (Felsot et al., 1981). Repeated applications of carbofuran increased the rate of degradation (Felsot et al., 1981; Kaufman et al., 1984).

The objective of these experiments was to study the potential of enhanced carbofuran degradation in two South Carolina soils which had been treated for several successive years. The degree of degradation and type of degradation products were also determined.

MATERIALS AND METHODS

Analytical grade carbofuran, ^{14}C-carbonyl carbofuran (Specific Activity 1.24 μCi/g), and five metabolites of carbofuran: 3-hydroxycarbofuran, 3-ketocarbofuran, 7-hydroxybenzofuran, 3-keto-7-hydroxybenzofuran, and 3-hydroxy-7-hydroxybenzofuran were obtained from FMC Corporation (Princeton, NJ).

The soil types tested were a Wagram soil (loamy, siliceous, thermic Arenic Paleudults) with a loamy sand texture, 0.6% organic matter and soil pH of 5.8; and a Varina soil (clayey, kaolinitic, thermic Plinthic Paleudults) with a sandy loam texture, 1.0% organic matter and soil pH of 6.1. The Wagram and Varina soil samples had an 8- and 3-year history, respectively, of annual

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applications of carbofuran and butylate [S-ethyl bis(2-methylpropyl) carbamothioate] at 2.2 and 6.7 kg ai/ha, respectively (Skipper et al., 1986). Plant vegetation was removed and random soil cores were collected from the top 10 cm. The soils were passed through a 2-mm mesh screen and stored in plastic bags at 25 to 27°C until tested. Wagram and Varina soil samples were also collected which had no previous pesticide treatment (hereinafter referred to as nonhistory soils).

Bellco biometric flasks contained 50 g dry weight (DW) of soil which was brought to 70% field capacity (FC), then fortified with a mixture of unlabeled or technical carbofuran and ^{14}C-carbofuran giving a final concentration of 30 ppm and a specific activity of 1.24 µCi per sample. The flasks were incubated at 25 to 27°C in the dark. The amount of $^{14}CO_2$ released was measured by combining 0.5 ml of the NaOH solution from the side arm, and 5.0 ml of Ready-solv scintillation fluid (Beckman). Radioactivity was determined using a Beckman LS-100C counter. Three replicates were used for each history soil and two replicates were used for each nonhistory soil. The experiment was repeated three times.

Technical carbofuran was also used in degradation studies. The experiment was set up in triplicate using double plastic ziplock bags. Each bag contained 200 g (DW) of soil that had been brought to 70% FC. The soil was fortified with 4 mg of carbofuran giving a final concentration of 30 ppm. Soil samples (10 g DW) were removed at time zero, and every other day, for 19 days. Soil was incubated at 25 to 27°C in the dark.

An induction experiment was established with the nonhistory Varina soil. The soil received three successive treatments, each one with 20 ppm carbofuran. Soil (400 g) was placed in double, large plastic ziplock bags and the moisture level adjusted to 70% FC. Technical carbofuran (8 mg) was added and the soil incubated in the dark at 25 to 27°C, for 30 days. After 30 days, 50 g (DW) of soil was removed and tested for carbofuran degradation ($^{14}CO_2$ evolution as described previously. The remaining 350 g of soil was treated with an additional 7 mg of technical carbofuran and incubated as described above. After 30 days (60 days cumulative), 50 g (DW) of soil was removed and tested for degradative potential. The remaining 300 g of soil was treated with an additional 6 mg of technical carbofuran and incubated for 30 days (90 days cumulative). At the end of the incubation period, a soil sample (50 g DW) was tested for degradation of carbofuran. The experiment was replicated three times.

Forty milliliters of HPLC grade ethyl acetate and 5.0 ml distilled water were added to soil samples (50 g DW). The mixture was placed on a reciprocal shaker and agitated for 45 minutes at 150 rpm. The organic phase was decanted, and the soil re-extracted using 40 ml HPLC grade ethyl acetate as described. Organic extracts were combined in a 250-ml round bottom flask and evaporated to dryness under partial vacuum on a rotary evaporator at 30°C. Radioactivity was measured by combining 0.5 ml of the

suspension with 5.0 ml of Ready-solv scintillation fluid (Beckman). Radioactivity was determined as described above.

When technical carbofuran was used, the resuspended extract was air-dried, dissolved in HPLC grade methanol and filtered (0.45 μm Gelman). A 10-μl aliquot of each 2.0 ml methanol solution was analyzed in duplicate with a Varian 5000 liquid chromatograph equippped with a CDS-111 microprocessor, 9176 recorder, and UV-50 detector (all Varian accessories). Detector settings were: 254 nm, 8 nm band width, and 0.2 nm absorbance range. A micropack, MCH-10 (30 cm x 4 nm) column with matching precolumn was used. The solvent system was $CH_3OH:H_2O$ (60:40) at a flow rate of 2 ml min^{-1} and a chart speed of 0.25 cm min^{-1}.

RESULTS AND DISCUSSION

Carbofuran rapidly disappeared in soils collected from the fields previously treated with carbofuran for 3 and 8 years in succession (referred to as history soils). No carbofuran was detected by HPLC in the history soils after 12 days (Fig. 1). In the nonhistory soils (Fig. 1), the amount of carbofuran recovered remained virtually the same over the 12-day sampling period.

The amounts of applied ^{14}C-carbofuran degraded and evolved as $^{14}CO_2$ in the history soils during the 30-day incubation period ranged from 57 to 65% (Fig. 2). In the nonhistory soils 25 to 35% $^{14}CO_2$ was evolved from the applied ^{14}C-carbofuran (Fig. 2). Total $^{14}CO_2$ recovery ranged from 65 to 99%. The trend of carbofuran degradation in the Wagram and Varina soils was similar (Fig. 1 and 2).

Degradation products were identified as 3-ketocarbofuran and 3-hydroxycarbofuran by HPLC cochromatography from the second set of experiments and by TLC from the third set of experiments. They occurred at 2 days in the carbofuran treated history soils and were present in the nonhistory soils after 3 days. The products, 3-ketocarbofuran and 3-hydroxycarbofuran, had retention times of 2.88 minutes and 2.21 minutes, respectively, compared to 3.89 minutes for carbofuran. The 3-hydroxy product appeared in smaller concentratons and less frequently than 3-ketocarbofuran. This may be due to the rapid conversion of 3-hydroxycarbofuran to 3-keto-carbofuran.

In the history soils the products did not persist once carbofuran was no longer detected by HPLC analysis (Fig. 3). In the nonhistory soils only 3-ketocarbofuran was detected. The 3-hydroxy derivative was not consistently detected. Product disappearance may be due to adsorption or further microbial breakdown (Ou et al., 1982; Rajagopal et al., 1984). Previous studies have indicated ring cleavage of carbofuran was negligible (Rajagopal et al., 1984). Proposed pathways of carbofuran degradation show carbofuran degradation to 3-hydroxycarbofuran then 3-ketocarbofuran which is further broken down into a soil-bound residue, or carbofuran is metabolized to 7-hydroxybenzofuran

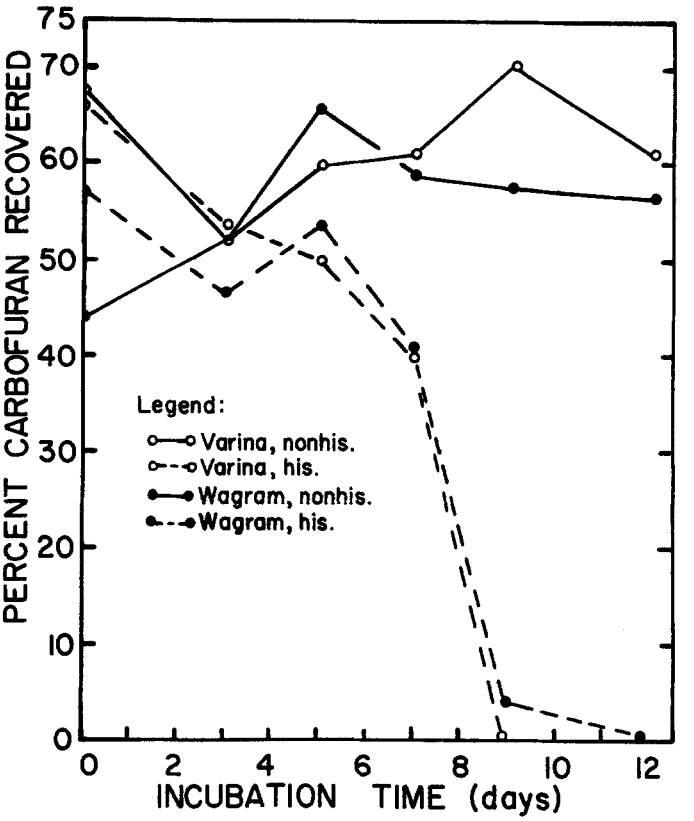

Figure 1. Carbofuran degradation in previously treated (history) and nontreated (nonhistory) soils. Data presented as percent carbofuran recovered from an in vitro incubation with a Wagram and Varina soil.

then degraded to a soil bound residue (Rajagopal et al., 1984). The latter pathway probably occurs in flooded soils since large amounts of 7-hydroxybenzofuran accumulate in flooded soils treated with carbofuran (Venkateswaralu and Sethunathan, 1978, 1979).

Induction occurs when a chemical is applied periodically to the same area of land. After each successive application the numbers of microorganisms that are able to degrade the chemical increases. After a period of time the chemical is degraded by the microbes before it can be effective against its target. An induction experiment was conducted using repeated applications of carbofuran to determine the effect on the degradative rate of the chemical in a nonhistory soil.

The first application of carbofuran to a nonhistory soil exhibited a pattern of $^{14}CO_2$ evolution not different from that of the control (Fig. 4) and was similar to that observed with nonhistory soils (Fig. 2). The second application had an initial lag period

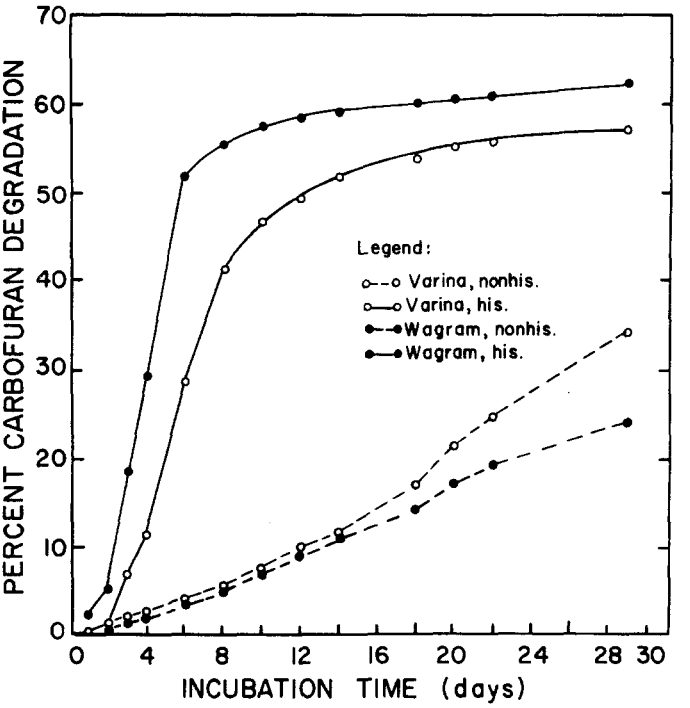

Figure 2. Degradation of carbofuran to CO_2 in previously treated (history) and nontreated (nonhistory) soils. $^{14}CO_2$ evolution was quantified, and the percent ^{14}C-carbofuran degraded with incubation time is presented for the Wagram and Varina soils.

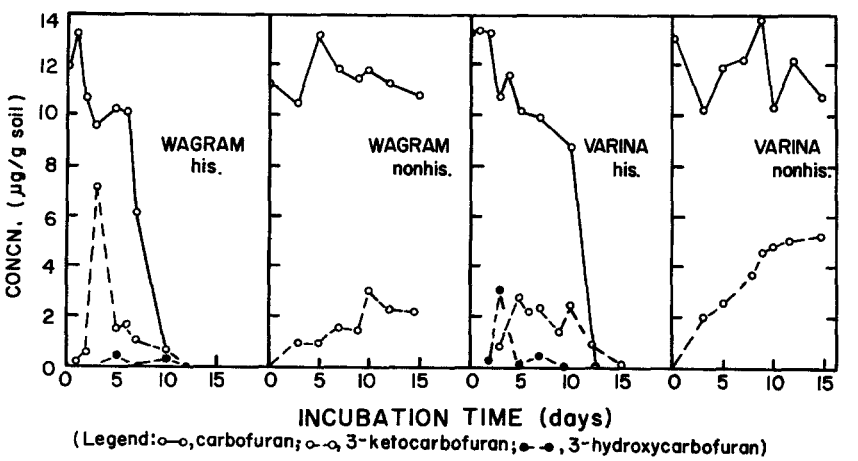

Figure 3. Quantities of carbofuran, 3-ketocarbofuran and 3-hydroxy-carbofuran detected in history and nonhistory Wagram and Varina soils.

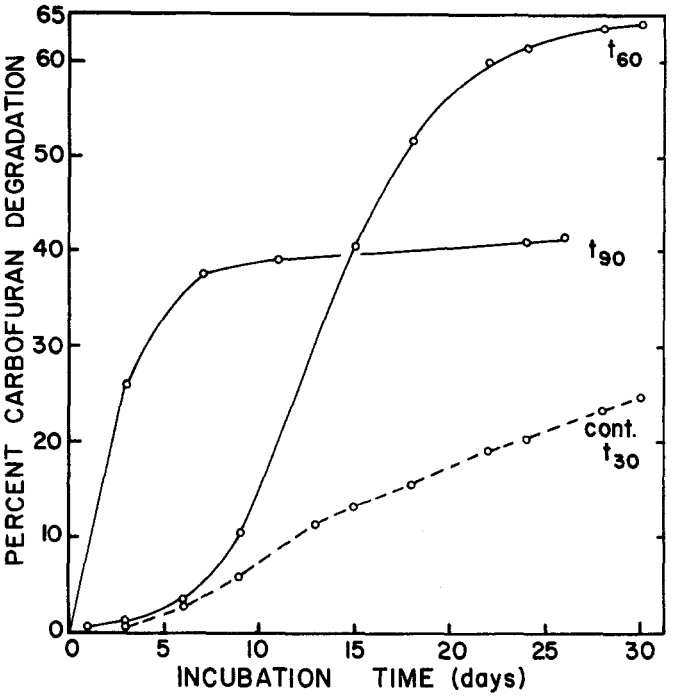

Fig. 4. Effect of successive carbofuran treatments (each 20 ppm) on degradation in a nonhistory Varina soil. The soil was treated at day zero, after 30 and 60 days and analyzed for $^{14}CO_2$ evolution from ^{14}C-carbofuran at 30 (t_{30}), 60 (t_{60}) and 90 (t_{90}) presented as percent carbofuran degraded.

until the sixth day of incubation, then the carbofuran was rapidly degraded. By the third application of carbofuran, the lag phase had disappeared and the degradative rate was faster than the previous, history-soil rates (Fig. 2). Previous field studies conducted in the Midwest have indicated poor insect control after 2 to 4 years of carbofuran use (Felsot et al., 1981). Other pesticides have also exhibited poor control after 2 to 3 years (Obrigawitch et al., 1982; Skipper et al., 1986; Wilson, 1984).

Enhanced biodegradation occurred in the two South Carolina soils, Wagram and Varina, when tested in vitro, that had a previous history of carbofuran exposure. HPLC analysis and TLC confirmed the presence of two metabolites, 3-ketocarbofuran and 3-hydroxy-carbofuran. The two products detected have also been present in previous studies of aerobic degradation of carbofuran (Gorder et al., 1982; Ou et al., 1982; Ragab et al., 1983; Williams et al., 1976). A nonhistory Varina soil was induced to degrade carbofuran at a rate equivalent to history soils after three successive applications of carbofuran. A similar pattern of degradation may occur under field conditions.

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