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Enhanced Microbial Degradation of Carbofuran in Soils with Histories of Furadan® Use

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Since 1975, numerous Illinois corn producers have reported poor control of the northern and western corn rootworms with Furadan 10G (carbofuran insecticide). Problem fields, located in central and northern Illinois, were usually characterized by a 2 to 4 year history of continuous carbofuran use. Although a low level of insect resistance to carbofuran has been found in western corn rootworm populations at several sites in Illinois (CHIO et al. 1976; FELSOT 1981), rootworm resistance alone does not adequately explain the failure of Furadan 10G to protect the corn root system from larval feeding (FELSOT 1981). Since soil insecticides are usually applied at planting time and are not actually needed until the rootworm eggs hatch 3 to 5 weeks later (depending on the planting date), it was hypothesized that poor rootworm control may result from lack of chemical persistence. This paper reviews the results of a study conducted in 1977 to assess carbofuran persistence in soils with and without histories of Furadan 10G use. Also, we report the isolation of a bacterium (*Pseudomonas* sp.) which can degrade carbofuran in pure culture.

MATERIALS AND METHODS

Soils: In September, 1976, soils were taken from four corn fields with a history of Furadan 10G use and poor performance (problem soils) and two corn fields with a history of phorate use (Table 1). Random samples were taken in each field with a 1 x 6 in. core sampler and bulked. Samples were also collected from contiguous fence row areas that were under sod, a field that had never been treated with pesticides, and a basement crawl space under a house built in 1935. The soils, mostly silt loams, were passed through a 3-mm mesh screen and stored moist at 0-4°C until tested.

Preliminary Screening of Soils for Carbofuran Degradation: Fifty grams of each soil were placed in flasks and individually fortified with 0.5 g of Furadan 10G giving a nominal concentration of 1000 ppm. After addition of 5 mL of distilled water, each flask was capped with

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TABLE 1

Recovery of carbofuran from soils with and without histories of Furadan use.

Soil Code	Location	Insecticide History[1/]	% Carbofuran Recovery Day 10	Day 20	Day 30
1	Orangeville, Stephenson Co.	Thimet 1972; Furadan 1974-75	47	15	0
2	Baileyville, Stephenson Co.	Furadan 1972-75	52	12	0
3	Rockgrove, Stephenson Co.	Furadan 1972-75	51	6	0
6	Lanark, Carroll Co.	Thimet 1972-73; Furadan 1974-75	42	0	0
4	Prophetstown, Whiteside Co.	Thimet 1972-75	73	48	21
5	Tampico, Whiteside Co.	Thimet 1972-75	94	50	8
13	Champaign, Champaign Co.	Chlordane	69	58	76
14	Arthur, Moultrie Co.	None	93	91	95

1/ In 1976 fields no. 1-6 were treated with Counter 15G insecticide, but small plots within the fields were treated with Furadan 10G to study rootworm feeding damage. Carbofuran and phorate are the active ingredients of Furadan and Thimet formulations, respectively.

aluminum foil and incubated at ambient room temperature. Soil moistures ranged from 34 to 37%. At 10, 20, and 30 days after addition of Furadan 10G, the entire contents of the flask were extracted and the concentration of carbofuran determined.

Problem soils were also autoclaved at 125°C for 2 h on 2 consecutive days. Sterile water was added to make a slurry before injecting carbofuran in acetone through a cotton plug to give a nominal 1000 ppm concentration.

Persistence of Carbofuran in Soils and Nutrient Broth Inoculated with Problem Soil Liquors: Soils (50 g) without OP/carbamate histories (nos. 13 and 14) were inoculated with 5 mL of soil liquors made by adding distilled water to a mixture of the problem soils (1:2 soil to water ratio; referred to as "problem soil liquor"). These soils were then fortified with 0.5 or 0.25 g of Furadan 10G, respectively, and extracted at 10, 20, or 30 days after incubation at ambient room temperature. Soils inoculated with distilled water served as controls.

In a similar experiment, 50 g of a sterilized problem soil (no. 6) or 10 mL of sterile Difco® nutrient broth were inoculated with 1 mL of problem soil liquor and fortified with 2.0 or 0.5 mg of carbofuran in acetone, respectively. Sterile soil and broth inoculated with 1 mL distilled water served as controls. Samples were analyzed for carbofuran over the next 30 days after incubation at ambient room temperature.

Isolation and Identification of Pure Bacterial Cultures from Soil: Pure cultures of bacteria were isolated from soils by the serial dilution plate method. One gram of soil was added to 100 mL of sterile water and agitated. Serial dilutions of 10^{-2} and 10^{-3} were made in sterile petri dishes containing Difco® nutrient agar. Plates were incubated at 30°C for 3 days, and then individual colonies having different characteristics were selected and streaked singly on nutrient agar. Only the bacterial isolates capable of degrading carbofuran were identified. The methods given by BUCHANAN & GIBBONS (1974), SKERMAN (1954), and STANIER et al. (1966) were used.

Selection of Bacterial Isolates Capable of Degrading Carbofuran: Each bacterial isolate was checked for its ability to degrade carbofuran in nutrient broth. Test tubes containing 10 mL of sterile Difco® nutrient broth were simultaneously inoculated with the bacterial isolate and 500 µg of carbufuran. Carbofuran concentration was determined after incubation at 30°C for 30 days.

Those cultures degrading carbofuran were selected for further study.

The bacterial isolate most effective in degrading carbofuran in the preliminary screening was added as above to 10 mL of broth along with enough carbofuran to give a final concentration of 500 ppm. These tubes were incubated at 30°C. At 1, 4, 8, 10, 15, 20, and 30 day intervals carbofuran concentration was determined. By altering both pH and incubation temperature, the effect of these two variables were examined.

To determine the effect of the age of bacterial cultures, broth was inoculated with the bacterium and incubated at 30°C for 30 days before carbofuran was added. Carbofuran concentration was then determined at daily intervals.

Analytical: Soils were extracted with equal volumes of ethyl acetate by soaking overnight in the incubation flasks. Nutrient broths were extracted with 5 mL ethyl acetate using a vortex mixer. Aliquots were taken directly from the organic phases in each case for gas chromatographic analysis. The GC conditions were: alkali flame ionization detector; 10% OV 101 in a 76 cm x 2 mm i.d. glass column; column temperature, 160°C; N_2 carrier gas, 25 mL/min.

RESULTS AND DISCUSSION

Carbofuran was rapidly lost in soils collected from fields with at least a three year history of Furadan use and reported control failures in 1976 (Table 1). Less than 20% of the initial insecticide added was recovered 20 days after fortification and only traces of carbofuran were recovered at 30 days. Soils collected from fence row areas of problem fields gave unexpectedly similar results. In soils with a history of phorate use, 50% of the added carbofuran was recovered after 20 days and 8 (soil no. 5) to 21% (soil no. 4) was recovered after 30 days. Recovery was even greater (44-83%) from fence row soils from phorate fields. The concentration of carbofuran in soils no. 13 and 14 (without OP/carbamate histories) was essentially unchanged over the 30 day incubation period. These observations are significant because rootworm egg hatch occurs in early June, and larvae are readily visible by mid-June. Since corn planting and insecticide application occur between late April and late May in Illinois, a toxic dose of insecticide must be present approximately 25 to 55 days after treatment.

When soils capable of degrading carbofuran were sterilized before Furadan 10G was added, no degradation occurred.

Ninety days after carbofuran introduction an insignificant amount of carbofuran had been lost. However, when a soil-water mixture from soils capable of degrading carbofuran was added to sterile soil no. 6 or soil no. 13 and 14, carbofuran was degraded within 30 days (Table 2). Likewise, when problem soil liquor was added to sterile nutrient broth, carbofuran was also significantly degraded (Table 2).

Two bacterial isolates from problem soils were capable of degrading carbofuran both in pure culture and when added to sterile soil. One of these isolates, identified as *Achromobacter* sp. degraded carbofuran in broth and when added to sterile soils, but the rate of degradation was much slower than that of the second isolate, a *Pseudomonas* sp. All of the experiments in this study were conducted with the *Pseudomonas* sp. isolate. This isolate was a short, gram-negative rod, polar-flagellated, that produced a slight yellow pigment, but did not fluoresce.

TABLE 2

Recovery of carbofuran (ppm) from soils no. 6, 13, 14 and from nutrient broth inoculated with soil liquor from Furadan-treated soils

	DAY			
TREATMENT	0	10	20	30
Soil no. 13				
distilled H_2O	-	690 ± 110	580 ± 60	760 ± 90
inoculated	-	880 ± 90	310 ± 30	14 ± 25
Soil no. 14				
distilled H_2O	480 ± 20	460 ± 10	460 ± 10	480 ± 20
inoculated	450 ± 10	410 ± 10	43 ± 8	7 ± 6
Soil no. 6				
sterile	440 ± 10	-	440 ± 50	410 ± 20
inoculated	450 ± 30	-	26 ± 3	0 ± 0
Broth				
sterile	-	38 ± 2	40 ± 2	36 ± 3
inoculated	-	32 ± 3	24 ± 1	2 ± 1

When the *Pseudomonas* sp. isolate and carbofuran were simultaneously added to sterile broth, less than 0.5% of the carbofuran was present after incubation for 30 days at 30°C. Under these conditions degradation of carbofuran approximated first order kinetics (R^2 - 0.92, k_r = -0.058/day) (Figure 1). The pH optima for carbofuran

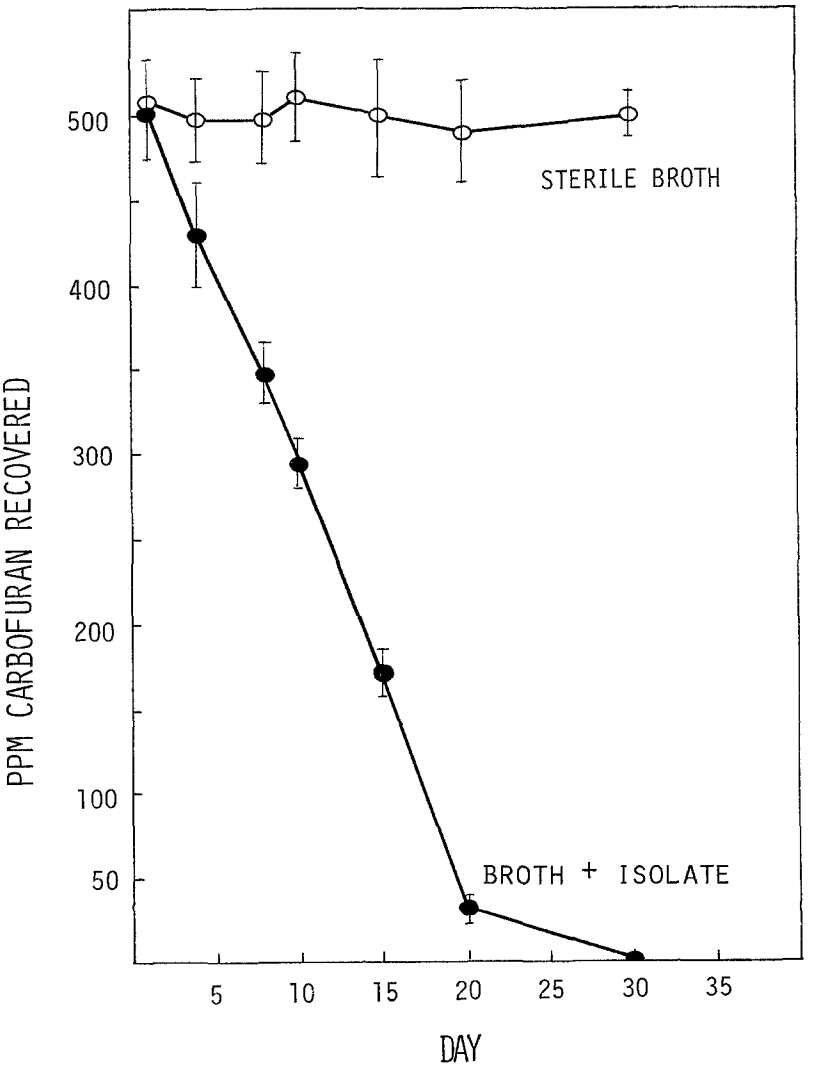

FIGURE 1. DEGRADATION OF CARBOFURAN IN BROTH FRESHLY INOCULATED WITH PSEUDOMONAS ISOLATE.

degradation was 6.8 and optimal degradation occurred between 28 and 32°C. No degradation occurred after 30 days at 40 or 16°C.

The rate of carbofuran loss in nutrient broth was directly proportional to the time interval of bacterial incubation before insecticide addition. When the *Pseudomonas* sp. isolate was incubated in broth for 30 days at 30°C before carbofuran was added, 99.5% of the carbofuran was degraded 48 h after introduction.

An unexpected decrease in carbofuran persistence associated with poor control of phylloxera in a vineyard soil was reported by WILLIAMS et al. (1976). Rapid degradation of carbofuran was shown to be caused by strains of *Actinomycetes* isolated from the soil. However, it was not clear whether the soil studies had a history of Furadan use. Two recent studies attempted to correlate lack of carbofuran persistence in soil with failure to adequately control corn rootworms, but no relationships were found between history of insecticide use and persistence (AHMAD et al. 1979; GORDER et al. 1980).

SIDDARAMAPPA et al. (1978) reported equal persistence of carbofuran in both untreated and repeatedly treated rice soils, and no microbial degradation of the insecticide was observed after repeated surface applications in the field. On the other hand, VENKATESWARLU et al. (1977) isolated a bacterium from flooded rice soils that in pure culture completely degraded carbofuran within 40 days.

Pseudomonas spp. isolated from soil have been shown to degrade the carbamate insecticides carbaryl and propoxur (ZUBERI & ZUBAIRI 1971, GUPTA et al. 1975). Current work in our lab is exploring the ability of the *Pseudomonas* sp. isolate to degrade other carbamate insecticides, whether enhanced Furadan degradation occurs under field conditions, and if so, the environmental parameters influencing such degradation.

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