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Effect of Corn Plants and Rhizosphere Populations on Pesticide Degradation

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Numerous chemical, physical, and biological factors are known to affect the degradation of pesticides in soils. Among them, microbial degradation is one of the most important. A number of studies have elucidated the importance of interacting microbial communities in the degradation of xenobiotic compounds. Lappin et al. (1985) described a five-member microbial community, isolated from the rhizosphere of wheat, which was capable of growth on mecoprop [2-(4-chloro-2-methylphenoxy) propanoic acid] as the sole carbon and energy source. None of the pure cultures were capable of growing on mecoprop alone.

Synergism probably can be found not only among members of microbial communities, but also between higher plants and microorganisms. Data from studies of plant-microbe interactions in the rhizosphere implicate its microbial community as an important exogenous line of defense for plants against potentially harmful organic compounds in soil (Walton et al., 1994).

The soil environment is extremely important for microbial activity. Soil microbial populations are affected by organic matter content, pH, moisture, aeration, and temperature. Some of these factors, in turn, are influenced by the proximity to the zone of plant root activity, the rhizosphere. Concentration and diversity of microorganisms in rhizospheres are much higher than in the bulk soil. Within four weeks a microenvironment was created around corn or wheat roots, characterized by an accumulation of root-derived organic materials (Merckx et al., 1986). Exudation and sloughing in wheat can reach 22% of total plant production (Milchunas et al., 1985). According to Haller and Stolp (1986), about 25% of organic matter flowing to the root system was excreted into the rhizosphere. Such exudates consist of up to 65% sugars, 33% organic acids, and 2% amino acids (Kraffczuk et al., 1984).

Considering the great amount of carbon photosynthetically absorbed by crops (up to 9 t ha^{-1} for corn, 3.7 t ha^{-1} for wheat; Buyanovsky and Wagner, 1986), rhizosphere microorganisms have access to significant amounts of readily

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available organic carbon. This creates an entirely different environment for pesticide degradation than that in bulk soil.

Some findings emphasize the importance of rhizospheres in biological degradation of pesticides. Sandmann and Loos (1984) found that populations of microorganisms able to degrade 2,4-D [(2,4-dichlorophenoxy)acetic acid] in rhizospheres of crops can be many times higher than in surrounding control soils. High, stimulated populations of these microorganisms in sugarcane soil was found, whereas under African clover a number of these organisms was 25 times lower. Earlier, Loos et al. (1979) suggested that the high numbers of 2,4-D degrading organisms in an established sugarcane field might be related to phenolic compounds from sugarcane roots.

In order to test the hypothesis that pesticides in rhizospheres undergo more intense biological influence from enhanced soil communities than in bulk soil, several experiments with corn (*Zea mays* L.) were conducted in greenhouse and field conditions.

MATERIALS AND METHODS

Experiments were conducted using soil of the Putnam-Mexico soil association, which formed in loess with a silt loam surface overlying silty clay subsoil. Soil from the A_p horizon was air dried and passed through a 1-mm sieve and was used in greenhouse experiments.

Greenhouse experiments with ^{14}C-labeled pesticides applied to soil were carried out in 2-gallon plastic containers fitted with air tight lids. Lids had a hole in the center. Corn seedlings (7-10 days old) were transplanted into containers, which were kept open until the plants were established. After establishment, the containers were covered and plants allowed to grow through the hole. Stems were sealed in the hole with the help of a non-toxic reusable adhesive (trade name Fun-Tak, produced by DAP Inc., Dayton, Ohio). In some containers, soil was divided by two concentric vertical screens (10 and 12.5 cm in diameter) of stainless steel mesh fabric with pore diameters 31.5 μm to obtain a central zone with regular roots, a middle zone with root hairs and mycorrhizal hyphae, and an external zone without roots. Control pots did not have plants. Water-alcohol solutions of ^{14}C-labelled pesticides were injected into the different zones after plants demonstrated good vigor. During the experiment, a constant flow of CO_2-free humidified air passed through the head space of sealed containers. Each container had a separate scrubbing tower with glass beads filled with lN NaOH. Total amount of evolved CO_2 was measured on a weekly basis, with a small aliquot taken for radioactivity measurement before titration. After corn plants in the greenhouse experiment entered the reproductive stage (in about 3 months), they were cut and roots were separated from the soil. All parts of plants were dried under 55°C and ground. Radioactivity was measured using a liquid scintillation counter after oxidation in a Packard sample oxidizer.

To assess the number of organisms degrading carbofuran (2,3-dihydro-2,3 dimethyl-7-benzofuranylmethylcarbamate), plants were removed from the pots or from the field and brought to the laboratory, after which soil adhering to roots was removed by vigorous shaking, and the roots severed. The roots were placed in 100 mL phosphate buffered saline (PBS; pH 7.0) and serially diluted. General microbial populations capable of degrading carbofuran were determined by dilution plating on a medium containing mineral salts and 200 $\mu g\ mL^{-1}$ of carbofuran as the only carbon source. TTC (2,3,4-triphenyltetrazolium chloride) was incorporated into the medium at 25 $\mu g\ mL^{-1}$ as an indicator of metabolically active microorganisms. Dilution plates were incubated at 28°C for 5 d. For fungi isolation, selected dilutions were plated in duplicate on Rose Bengal agar (Wollum, 1982) and incubated at 28°C for 7 d. Predominant fungal isolates were identified based on colony characteristics and morphology of conidiophores (Alexopoulos and Mims, 1979). Single isolates of fungi were maintained on Rose Bengal agar. To examine the potential of the fungi for carbofuran utilization and degradation, isolates were transferred to 125 mL flasks containing 50 mL mineral salts broth (Mueller et al., 1989) plus technical grade carbofuran added at $100\mu g\ mL^{-1}$. Three replicate flasks were prepared for each fungal isolate for each sampling date. The cultures were incubated statically in the dark at 28°C. At 0, 2, 4 and 8 d of incubation, mycelial mats were filtered onto preweighed filter papers and dried for 24 h at 105°C prior to weighing. The filtrate from each flask was analyzed for carbofuran content using HPLC. This experiment was repeated twice.

Carbofuran was extracted from each culture filtrate by shaking 5 mL of filtrate with 5 mL of HPLC-grade methanol for 30 min on a rotary shaker at 100 oscillations. Samples were centrifuged at 10,000 rpm at 4°C for 30 min. The extracts were filtered through 0.2μm membranes (Acrodisc, Gelman Sciences, Inc.) and stored at 5°C until analysis. Carbofuran was determined via reverse-phase HPLC on a Beckman Model 338 HPLC system (Beckman Instruments, Inc.) using the method described by Edwards et al. (1992). Efficiency of carbofuran recovery was 92 ± 1%.

The field experiment was carried out on six plots, each 3 x 6 m. Half of the plots were planted with corn, at a density 86,400 plants per ha. Mineral fertilizers (NPK 182/48/48 kg ha^{-1}) were applied to all plots. Carbofuran and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-S-triazine] were applied post-emergence at 3 kg a.i. ha^{-1} to all six plots. Three surface samples were taken from each plot immediately after application to verify the uniformity of application. The variability within these samples was less than 10%. Samples from 0-10, 10-20, and 20-30 cm were taken in triplicate from each plot on the 15th, 35th and 60th days. Samples from the same depth within a plot were combined and analyzed. Atrazine was determined using a laboratory robotic system (Koskinen, et. al., 1991), which has efficiency and precision of extraction of 89 ± 2%.

RESULTS AND DISCUSSION

Results of the greenhouse experiment with carbofuran (Fig. 1 and Table 1) showed that ^{14}C-labelled carbofuran added to the external zone was mineralized at a rate similar to that in soil without plants. The rate was significantly higher when ^{14}C-carbofuran was injected into the central zone. During the first 30 d, the rate of degradation in close proximity to the roots was 2-3 times higher than in soil without roots (in the external zone). The middle zone showed very low activity during the first week of incubation. After 10 d of plant development, the radioactivity of evolved CO_2 in the zone increased dramatically for a short period (10-15 d) and then dropped. In soil without plants, the rate of carbofuran mineralization slowly increased during the first month of incubation. During the first 30 d of the experiment, the amount of carbofuran degraded per gram of soil in the central zone was approximately twice as high as that in the absence of plants (Table 1). During the second part of the experiment, almost similar amounts of the pesticide were dissipated in all treatments.

Table 1. Mineralization of ^{14}C labelled carbofuran in the greenhouse experiment with corn plants (average of 3 replicates)

Labelled zone	Total ^{14}C activity lost, Bq g^{-1} soil, during days 1-29	days 30-60	days 1-75
Central	3.69	5.94	11.93
Middle	3.28	no data	no data
External	0.90	5.11	7.65
Soil without plant	1.69	4.71	8.80

Significant uptake of radiolabeled compounds was observed (Table 2). Since contact of the plant's green parts with $^{14}CO_2$ evolved from the soil was excluded, all activity found in the plants was due to absorption through the root system. Radioactivity measurements showed a significant uptake and translocation of the carbofuran and/or degradation products. The highest concentration of radioactive compounds was found in leaves, the lowest in reproductive organs. We found more than 30% of the total radioactivity applied with carbofuran in corn leaves and about 3% in the stalks. Uptake was lower when corn was subjected to water stress: 0.7-1.4% in roots, 0.25-0.37% in stalks, and 11% in leaves.

Results of the field experiment showed that corn plants have a positive effect on dissipation of applied pesticides (Table 3). In 15 days after application concentrations of atrazine and carbofuran in the upper 10 cm under corn were two times lower than in the bare soil. What is even more important, in soil planted with corn, no atrazine was detected below 20 cm (bare soil had 14.8 ng

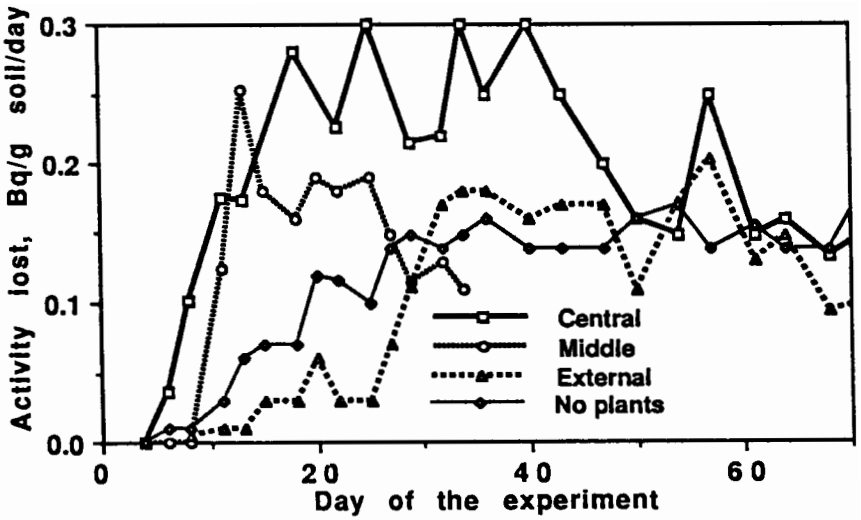

Figure 1. Dynamics of ^{14}C-labeled carbofuran mineralization in different zones of rhizosphere.

kg^{-1}), and the concentration at the 10-20 cm depth was five times lower than in the fallow soil. Fast disappearance of atrazine from the soil during this time of the year (June), when soils of central Missouri receive almost 100 mm of rain, can significantly decrease transport of atrazine to surface and ground water.

Table 2. Uptake of radioactive products by corn plants in greenhouse experiment (% of total radioactivity applied).

Plant part	Under normal water supply	Under water stress
Leaves	14.1-32.1	5.6-11.0
Stalks	2.6- 4.7	0.3- 0.4
Roots	no data	0.7- 1.4
Reproductive organs	no data	0.1- 0.2

At later stages of corn development the same trend with atrazine was observed, although the difference between corn and fallow soil was not significant. Corn plants had a similar effect on carbofuran degradation, although, due to the shorter half-life of this insecticide, its concentrations were much lower.

In the greenhouse experiment, numbers of carbofuran-degrading organisms were 3-5 times greater in rhizosphere soil than in soil without roots (Table 4). This is in agreement with our previous observations where population increases

Table 3. Concentration of atrazine and carbofuran in field experiment (standard deviations in parenthesis), ng kg^{-1} soil

Day after application	Depth, cm	Atrazine		Carbofuran	
		Corn	Fallow	Corn	Fallow
15	0-10	302.1(107.6)	604.1(116.2)*	41.5(7.2)	73.1(3.6)*
	10-20	4.5(4.9)	20.9(8.3)*	28.3(16.0)	82.0(17.2)*
	20-30	0.0(0.0)	14.8(9.0)*	62.2(9.0)	156.1(67.6)*
35	0-10	392.2(71.6)	542.2(62.2)	33.6(22.2)	15.85(10.2)
	10-20	16.8(4.4)	23.2(2.2)	26.9(3.0)	13.25(4.0)
	20-30	2.3(3.4)	9.6(4.4)	29.2(2.3)	57.45(12.2)*

*Indicates that concentration in fallow is significantly (at 0.05 level) higher than in corn plots.

Table 4. Number of carbofuran-degrading organisms found in soil and in rhizosphere of corn grown in containers (x 10^6/1g of dry weight)

Container #	Bacteria and actinomycetes		Fungi	
	Soil	Rhizosphere	Soil	Rhizosphere
1	27.5	107.1	10.1	5.3
2	25.7	84.0	10.1	5.3
3	65.0	258.7	8.7	26.6
4	39.7	154.1	9.6	21.4
5	50.7	317.4	3.2	6.3
6	40.4	73.0	7.9	19.0
7	46.6	260.3	3.7	23.3

Table 5. Biomass production and carbofuran metabolism by three rhizosphere fungi in mineral salts plus carbofuran medium.

Incubation, days	Fungal biomass, mg			Carbofuran remaining, mgL^{-1}		
	Asperg.	*Penic.*	*Trichod. viride*	*Asperg.*	*Penic.*	*Trichod. viride*
0	6.8	9.4	8.6	98.5	94.8	101.0
2	39.0	12.6	32.5	86.0	90.6	85.0
4	41.7	21.4	39.1	80.0	84.8	80.8
8	51.8	46.1	48.6	70.0	79.0	61.2
LSD (0.05)	6.2	8.5	6.0	4.0	4.0	10.2

of carbofuran-degrading microorganisms were significantly higher in the corn rhizosphere than in bulk soil (Edwards, 1990).

In the field, the dominant genera of fungi isolated from the corn rhizosphere in carbofuran-treated soil included *Trichoderma, Cladosporium, Aspergillus, Penicillium,* and *Fusarium. Trichoderma, Aspergillus,* and *Penicillium* were more prevalent in carbofuran-treated plots compared to plots not receiving carbofuran (Wootton, 1990). We previously reported the dominant genera of bacteria in carbofuran-treated soil and rhizospheres included *Pseudomonas, Flavobacterium, Enterobacter* and the actinomycetes *Streptomyces* sp. and *Promicromonospora citrea* (Edwards et al. 1992). The ability of these bacteria to metabolize carbofuran was enhanced compared to bacteria isolated from soil not treated with carbofuran.

The *in vitro* studies on potential metabolism of carbofuran by rhizosphere fungi indicated that biomass of all test fungi increased during incubation presumably due to utilization of carbofuran as a carbon and energy source (Table 5). Fungi inoculated into mineral salts broth without carbofuran did not grow during the 8-d incubation period. Biomass of *Aspergillus* sp. and *Trichoderma viride* grown on carbofuran increased rapidly compared with growth of *Penicillium* sp. The faster growth rates seemed to coincide with faster degradation of carbofuran by these two fungal species during the 8-d incubation. Carbofuran degradation by bacteria isolated from soils and corn rhizospheres receiving repeated carbofuran application was also related to utilization of carbofuran as a sole carbon source (Edwards et al. 1992). As previously reported, certain segments of the soil fungal population are able to grow more rapidly on pesticide substrates, which may be due to more efficient metabolism of certain organic groups unique to the chemical structure of a pesticide (Kaufman and Blake, 1973). The present results indicate that rhizosphere fungi may contribute to carbofuran degradation along with rhizosphere bacteria in fields planted to corn. This is supported by the ability of certain fungal genera to apparently utilize carbofuran as a carbon and energy source.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may be suitable.

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