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Effects of Carbofuran and the Corn Rhizosphere on Growth of Soil Microorganisms

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Biodegradation of carbamates and carbamothioates by microorganisms in soil has been widely reported; however, the ecology of pesticide-degrading microorganisms in soils and plant rhizospheres containing these pesticides has received less attention. Some bacteria capable of carbofuran (2,3-dihydro-2,3-dimethyl-7-benzofuranyl methyl carbamate) biodegradation in soil have been isolated and identified (Felsot et al. 1981; Chaudhry and Ali 1988). Reed et al.(1987) identified several fungi and bacteria from soils with histories of pesticide application and observed that bacteria were able to metabolize the pesticides in pure culture more efficiently than were fungi. Bacteria and actinomycetes from soils with histories of carbamothioate herbicide use were found to utilize carbamothioates more efficiently than were isolates from nonhistory soils (Mueller et al. 1989). Lee (1984) found that biodegradation of EPTC (S-ethyl dipropyl carbamothioate) was significantly affected by soil fungi. Interestingly, after storage bacterial isolates lost the ability to degrade EPTC. He concluded that fungi able to degrade EPTC may retain this ability in soil longer than do bacteria. Recent studies demonstrated that fungi along with bacteria detoxify herbicide wastes during bioremediation of contaminated soils (Felsot and Dzantor 1990).

Microbial communities composed of several to numerous species are more likely to be responsible for pesticide biodegradation in soil and rhizosphere environments than are single species. Pesticides applied to soil at planting should persist during the development of plant roots. Therefore, a portion of the pesticide likely interacts with microorganisms in the rhizosphere. Few previous studies have considered the effect of plant roots on the activity of microorganisms in the presence of pesticides. A five-member microbial community from a wheat rhizosphere was able to metabolize the herbicide mecoprop [2-(4-chloro-2-methylphenoxy) propanoic acid] in liquid culture even though individual members could not (Lappin et al. 1985). Biodegradation of (2,4-dichlorophenoxy)acetic acid by microorganisms was found to be several times higher in sugarcane rhizospheres than in surrounding soils (Sandmann and Loos 1984).

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Studies examining the microbial components (bacteria and fungi) in pesticide-amended soils are needed to identifiy microorganisms involved in pesticide biodegradation and better understand relationships between soil microbial communities, crop rhizospheres and target pests. The objective of this paper is to characterize the response to pesticides of fungi and actinomycetes isolated from field plots planted to continuous corn and receiving annual applications of carbofuran and butylate [S-ethyl bis(2-methylpropyl)carbamothioate]. Responses were characterized for the selected fungi and actinomycetes both individually and combined in the absence and presence of the corn rhizosphere. A study was also conducted on the effects of carbofuran and butylate on soil fungi in pure culture.

MATERIALS AND METHODS

Fungi and actinomycetes were previously isolated from field plots which had been planted to continuous corn receiving annual applications of carbofuran and butylate for eight years (Wootton 1990).

The fungal isolates, *Trichoderma* sp. 1, *Penicillium* sp. 4, *Aspergillus* sp. 10, and *Fusarium* sp. 22 were maintained on Rose Bengal (RB) agar (Wollum 1982) at 28°C. Numerals following species names indicate accession numbers of the field-collected isolates (Wootton 1990). Isolates were transferred to 500-mL flasks containing 250 mL RB broth and grown for 4 d at 28°C on a rotary shaker at 150 rpm. Technical grade carbofuran, butylate and a combination of carbofuran + butylate were added at 100 µg/mL to separate 125-mL flasks containing 50 mL of Czapek-Dox broth (Wollum 1982). All pesticide solutions were added through 0.2-µm filter membranes. Control flasks received no pesticide. Inocula were prepared for each fungal isolate growing in RB, adjusted to contain 5 X 10^4 propagules (conidia and hyphal fragments)/mL, and added to each flask. Three replicate flasks were prepared for each pesticide-fungus combination. The cultures were placed on a rotary shaker (150 rpm) and incubated in the growth chamber at 28°C d (16 hr) and 16°C night (8 hr) temperatures. At 0, 2, 4, 8, and 16 d of incubation, mycelial mats were filtered onto preweighed filter papers and dried for 24 hr at 105°C prior to weighing. This experiment was repeated twice.

For greenhouse studies the fungal isolate *Trichoderma viride* and an actinomycete *Promicromonospora citrea*, previously shown to biodegrade carbofuran (Edwards et al. 1992), were used. A Putnam silt loam was collected from the upper 10 cm of the soil profile on an experimental field that had no history of carbofuran application. The soil had the following properties: pH_S (1:1 0.01M $CaCl_2$), 7.0; cation exchange capacity, 13.5 meq/100 g; organic matter, 3.5%. The soil was sieved, autoclaved for 1 hr on three consecutive days, and placed in plastic bags in 1-kg amounts. Technical grade carbofuran (100 mg) was added to each bag and thoroughly mixed. *Trichoderma viride* and *P. citrea* were added to separate bags of carbofuran-amended soil and

mixed thoroughly to achieve a final density of 5 X 10^4 fungal or 5 X 10^6 actinomycete propagules/g soil. Plastic pots previously surface-sterilized with 10% sodium hypochlorite were each filled with treated soil, capped with sterilized sand, set in saucers with tape sealed around the base and placed in the greenhouse according to a completely randomized design. Three replicate pots were established for each treatment. An identical experiment was prepared with the addition of surface-sterilized, pregerminated corn seeds planted 4 cm deep in each pot. Filter-sterilized nutrient solution (Miracle Grow) was provided via subirrigation. Soil samples were aseptically collected at 0, 4, and 8 d; rhizosphere samples (roots + soil) were collected at 8 and 16 d after planting. Greenhouse studies were conducted two times.

At each sampling date, pots were sampled by removing a small area of the sand layer and obtaining composite soil samples from each pot. Rhizosphere samples were obtained by vigorously shaking soil adhering to corn roots removed from pots. Soils were assayed by standard microbial techniques (Wollum 1982), using 10-fold dilutions in phosphate buffered saline. Aliquots (0.1 ml) from selected dilutions were transferred to duplicate culture plates containing tryptic soy agar (TSA) for assessment of general bacterial populations and to RB agar for assessment of fungal populations. Plates were incubated at 28°C for 7 d.

The proportion of the actinomycete population from both rhizosphere and non-rhizosphere soils capable of degrading carbofuran was estimated by plating on a mineral salts (MS) medium containing 200 µg/mL carbofuran (Mueller et al. 1989). Twenty-five µg TTC (2,3,5-triphenyl-tetrazolium chloride) per mL and 20 µg/mL cylcloheximide were added to this medium via filter sterilization through a 0.2 µL membrane (Gelman). When TTC is included with MS plus carbofuran, metabolic activity is observed as the development of red colonies resulting from reduction of TTC, thereby indicating carbofuran metabolism by microorganisms (Mueller et al. 1989). Plates were incubated in the dark at 28°C for 14 d afterwhich visible colonies were counted on each plate.

RESULTS AND DISCUSSION

Biomass (mycelial dry weight) of all test fungi increased during incubation when the fungi were grown on carbofuran, butylate, carbofuran+butylate or non-amended culture medium (Table 1). Carbofuran+butylate significantly increased the biomass of *Penicillium* at 4 d. However, at 8 d, growth of *Penicillium* on all pesticide-amended media was less than the growth on control medium, possibly indicating a decrease in the rate of pesticide metabolism due to depletion of pesticide substrate or accumulation of toxic by-products. During incubation, the apparent rate of growth of *Aspergillus* on pesticide-amended media was consistently less than growth on control medium. Biomass of *Fusarium* growth on carbofuran significantly increased at 8 and 16 d compared with growth on control culture medium. As previously reported, *Fusarium*

Table 1. Effects of carbofuran and butylate on mycelial growth of soil fungi in broth culture.

Fungal isolate	Incubation, days	Control	Carbofuran	Butylate	Carbofuran + Butylate
		Mycelial dry weight, mg			
Trichoderma	0	8 a*	11 a	12 a	10 a
sp. 1	2	12 a	15 a	16 a	12 a
	4	39 a	34 a	38 a	35 a
	8	38 a	38 a	36 a	39 a
Penicillium	0	12 a	9 a	8 a	8 a
sp. 4	2	16 a	12 a	7 a	13 a
	4	34 bB	21 bC	26 bC	41 bA
	8	68 cA	46 cB	51 cB	48 bC
Aspergillus	0	6 a	3 a	6 a	8 a
sp. 10	2	31 bA	26 bB	29 bB	17 bC
	4	39 bA	42 cA	26 bB	19 bC
	8	52 cA	58 dA	37 cB	43 cB
Fusarium	0	20 a	20 a	-----†	-----
sp. 22	2	54 bA	34 bB	-----	-----
	4	52 b	44 b	-----	-----
	8	68 cB	105 cA	-----	-----
	16	80 cB	99 cA	-----	-----

*Means followed by the same lower case letter within a column for a fungal isolate are not significantly different ($P<0.05$). Means followed by the same upper case letter within a row for a fungal isolate are not significantly different ($P<0.05$).

†Not determined for *Fusarium*.

spp. are often very efficient in metabolism of pesticides in either pure culture or in soil (Kaufman and Blake 1973; Yarden et al. 1990). Also, the ability of certain segments of the soil microbial population to grow more rapidly on pesticide substrates may be due to more efficient metabolism of certain organic groups unique to the chemical structure of a pesticide (Kaufman and Blake 1973).

Growth of the representative soil fungus in soil alone or combined with the actinomycete was relatively slow and populations did not significantly ($P<0.05$) increase until 8 d of incubation (Figure 1A). Corn rhizosphere populations of *T. viride* at 8 d was not affected by the presence of *P. citrea*; however, at 16 d, *T. viride* counts from rhizospheres containing both organisms were considerably depressed by *P. citrea*. This was likely due to development in the rhizosphere of an environment favorable for the actinomycete allowing better competition for root-exuded nutrients and/or the production of antibiotics for inhibiting fungal growth (Curl and Truelove 1986). The potential of *P. citrea*, an effective carbofuran-degrader (Edwards et al. 1992), to better establish in soil and rhizosphere environments compared to fungi may explain why fungi are generally not considered as the major organisms responsible for degradation of certain pesticides (Avidov et al. 1990).

Growth of *P. citrea* in soil alone or with *T. viride* increased rapidly by 4 d of incubation and remained steady through 8 d (Figure 1B). The proportion of the actinomycete population presumptively involved in carbofuran degradation (indicated by colonies growing on TTC) was generally stable through 8 d incubation and was approximately 80% for *P. citrea* alone and 55% for *P. citrea* combined with *T. viride*. Corn rhizosphere populations of *P. citrea* did not appear to be affected by presence of *T. viride* at either 8 or 16 d. Fungal presence in the rhizosphere appeared to decrease to 30% the proportion of *P. citrea* presumed to be carbofuran-degraders, compared with 60% presumed degraders in the rhizosphere without fungi. Actinomycete populations in soils and rhizospheres not amended with carbofuran were not significantly ($P<0.05$) different from those in soils amended with carbofuran when cultured on TSA for general actinomycete determinations (data not shown). This agrees with previous reports that increases in gross numbers of organisms for pesticide degradation is not as critical as is the increase in metabolic activity toward the pesticide by certain segments of the soil microbial population (Moorman 1988; Merica and Alexander 1990).

In conclusion, the present results illustrate the minor role soil fungi play in carbofuran and butylate degradation because, based on results of pure culture studies, fungi metabolized pesticides relatively slowly. Furthermore, the representative soil fungus *T. viride* had no apparent effect on growth of the actinomycete *P. citrea* when *T. viride* and *P. citrea* were combined in either soil or rhizosphere environments. This suggests that the majority of degradation is due to soil bacteria that have

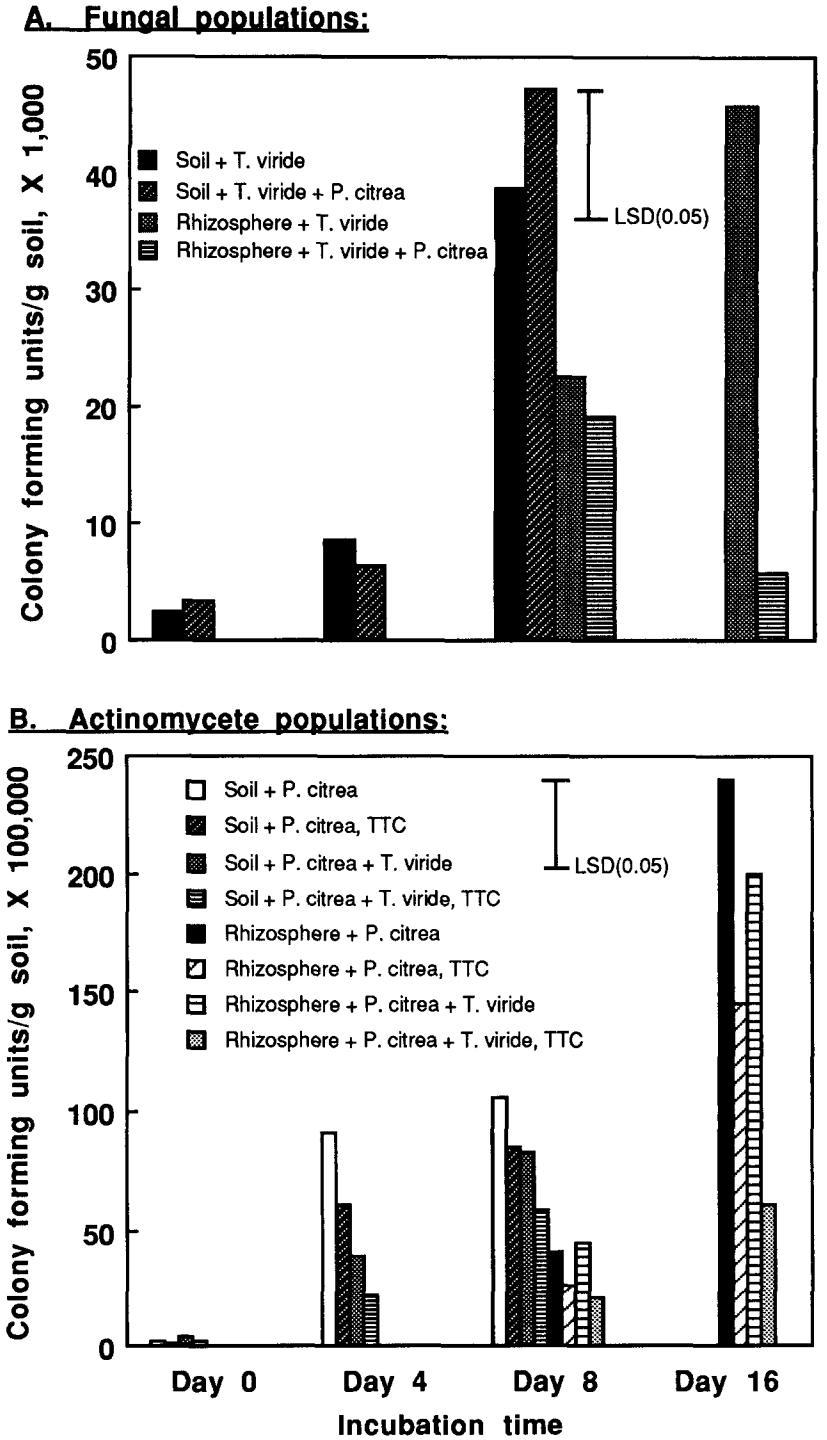

Figure 1. Soil and corn rhizosphere populations of T. viride (A) and P. citrea (B) in Putnam silt loam amended with carbofuran (100 g/kg soil).

an increased capacity for carbofuran metabolism and that are capable of readily establishing in soil and rhizosphere environments receiving pesticides applications. Pesticide degradation may be enhanced in plant rhizospheres. Thus, the plant rhizosphere should be considered in overall schemes describing fates of pesticides in the environment.

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