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Fluorescein Diacetate Hydrolysis for Determination of Accelerated Degradation of Thiocarbamate Herbicides

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Relatively rapid assays for enzymes that degrade pesticides could be used to determine if a soil supports a microbial community capable of utilizing a pesticide as an energy substrate. Burns and Edwards (1980) suggested the detection of accumulated pesticide-degrading enzymes may indicate the presence of such a soil microorganism community. Reed et al. (1987) characterized suspect isolated strains of bacteria, actinomycetes and fungi for accelerated degradation of carbamates, organophosphates, and thiocarbamates by phosphatase, phosphodiesterase, and rhodanase analysis of pesticide spiked broth cultures. Such techniques could be useful in making recommendations for pesticide use where enhanced microbial degradation may pose a problem. Soils with a microbial component capable of accelerated degradation of a pesticide are referred to as "adapted", "aggressive", and "poised" (Kaufman and Edwards 1982). Such soils usually exhibit reduced control of microbial pathogens, insect pests, and weeds because the continuous use of certain classes of pesticides seems to select microorganisms that can rapidly tolerate or metabolize the pesticide before it can exert its toxic effect(s) upon intended targets (Felsot et al. 1981; Obrigawitch et al. 1983; Racke and Coats, 1987; Yarden et al. 1987 and Walker et al. 1986).

The compound fluorescein diacetate (FDA) is hydrolyzed by esterases, proteases and lipases in plant, mammal, fungal, and bacterial cells. The product of this reaction, fluorescein, is slowly eliminated from the cell and can be visualized in cells by fluorescence microscopy, or quantified spectrophotometrically (Schnurer and Rosswall, 1982). Quantitatively, the absorbance of fluorescein diacetate at 490 nm is indicative of the hydrolytic activity of a soil and therefore has been used as a measure of soil microbial activity (Zelles et a. 1985). We report here on the use of a spectrophotometric assay of FDA hydrolysis as a sensitive, quantitative measure of the potential of the microbiota of agricultural soils with a history of thiocarbamate herbicide use to rapidly degrade these pesticides.

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MATERIALS AND METHODS

Soil samples were collected from two cornfields with different herbicide use histories. Each sample was a homogenation of eight 1 kg subsamples; five samples were obtained from each site on March 2, 1987. The first site located near Fox, OH had a history of EPTC and butylate use with grass control failures in 1985 through 1987. The second site had no prior history of thiocarbamate use was located near Bluffton, OH. The specific soil types were both classified as mixed mesic arguidolls. Site one was a Kokomo clay loam and the second site was a Pewamo silty clay. In the laboratory, 400 ml of 5% acetone solution containing 5 or 50 ppm of EPTC or butylate were thoroughly mixed into 3 kg of soil from each site. The two concentrations of each herbicide served to "spike" the soil; additional soil mixes with only water and only with acetone solution were also prepared and tested. Sieved, 50 g aliquots of soil (2 aliquots per replicate, three replicates) were placed in 150 ml bottles and incubated to 20° C in darkness.

Spectrophotometric measurements of fluorescein diacetate hydrolysis of spiked soil samples were made 0, 4, 9, 16, and 30 days after spiking. A soil suspension consisting of 0.75 g of soil with the addition of 20 ml phosphate buffer (pH = 7.6) was shaken for 15 minutes at 24° C. A 100 ul stock solution of FDA (2 mg/ml) was added to the sample. The suspension was again shaken for 45 minutes, 25 ml acetone was added to terminate the reaction. The suspension was filtered though a N°. 2 Whatman paper filter in a buchner funnel and the filtrate was centrifuged at approximately 5,000 rpm for 15 minutes. The sample was analyzed spectrophotometrically using a Zeiss PQM II spectrophotometer. Higher absorbance values represent greater levels of microbial activity and larger microbial populations while lower absorbance values indicated smaller microbial populations and lower levels of microbial activity. All values are percent absorbance values. Factors under study were herbicide spiking concentrations (5 and 50 ppm), herbicide (butylate, EPTC, and none) and date of sampling (0, 4, 9, 16, and 30 days) were subjected to an analysis of variance. Mean separation utilizing the least significant difference method at the 0.05 level of significance was performed on all data.

RESULTS AND DISCUSSION

Concentration of spiking solution was an important factor. Table 1 shows absorbance values of butylate and EPTC respectively. However, increased concentration of solution, by an order of magnitude for both herbicides at 50 ppm was not significantly different. Significant differences were observed at the 5 ppm concentrations, this suggests that concentration may be an overriding factor in thiocarbamate biodegradation. Therefore, differences among butylate and EPTC may be observed with this assay at lower concentrations, such as the 5 ppm level.

Table 1. Mean absorbance values of 5 and 50 ppm butylate and EPTC from soils with a thiocarbamate use and non-use soil.

Concentration	Butylate	EPTC
5 ppm	0.2366 b	0.2853 a
50 ppm	0.2048 bc	0.1684 c
LSD (0.05)	0.602	

Figures 1a-c show microbial activity over the 5 sampling periods (0, 4, 9, 16 and 30 days after spiking) for butylate, EPTC poised soils and non-history soils. The non-history soil (Figure 1a) absorbance values were varied but standard deviations were fairly uniform. Further, the greatest activity was observed at the 5 ppm concentrations of butylate and EPTC at 9, 16, and 30 days after treatment. The 50 ppm concentrations of butylate and EPTC did not become active as early as the 5 ppm concentrations. This may suggest inhibition of microbial activity by the 50 ppm concentrations of the thiocarbamate herbicides, while the 5 ppm concentrations appeared not to inhibit and may have selected a small number of adapted organisms or a community of degraders in the non-history soil. In Figures 1b and c, higher mean microbial activity was observed when the same thiocarbamate herbicide was "spiked" on its poised soil. Further, absorbance values in Figures 1b and c for butylate and EPTC on their respective soils are strikingly similar, suggesting the respective soils possessed a population of degraders for butylate and EPTC. The standard deviations of absorbance values associated with butylate spiking on the butylate history soil (Figure 1b) was greater than EPTC absorbance values. Likewise, in Figure 1c we observed EPTC absorbance values varied more than butylate absorbance. Conversely, the activity of butylate in the EPTC poised soil decreased significantly at 9, 16, and 30 days after spiking (Figure 1c). Meanwhile, EPTC activity decreased significantly during the 16 and 30 day sampling dates on the butylate history soil (Figure 1b). No significant differences were observed between 5 and 50 ppm spiking concentrations in Figure 1c except at 30 days among EPTC concentrations. However, at the butylate history site (Figures 1b) at 4 and 30 days after treatment significant differences were observed among the butylate spiking concentrations. Also, the 16 and 30-day sampling dates for EPTC in Figures 1b demonstrated some disparity among absorbance values. Kaufman (1982) noticed declining lag phases of CO_2 evolution on poised soils after spiking with a susceptible pesticide substrate. Concentration was more important in determining the level of microbial activity in the non-history site while this was not as important as the actual substrate with the thiocarbamate poised soils. This may indicate a more heterogenous community of soil microbes that are not degraders while the magnitude of selective degraders in poised soils is greater than non-history soils.

The relationship between pesticide substrates, microbial communities capable of rapid degradation and substrate

FIGURE 1a. ABSORBANCE OF FLUORESCEIN DIACETATE ON A NON-HISTORY SOIL SPIKED WITH 5 AND 50 PPM BUTYLATE AND EPTC. EACH BAR IS THE MEAN + S.D. (n=3) PER GROUP.

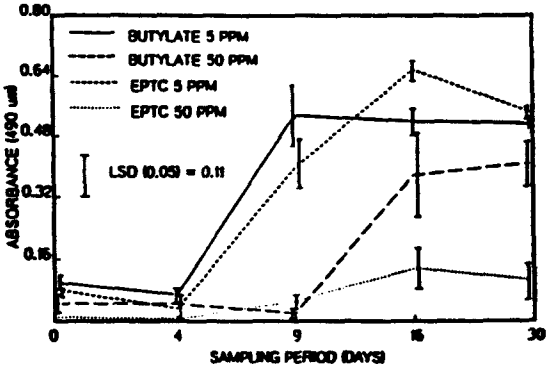


FIGURE 1b. ABSORBANCE OF FLUORESCEIN DIACETATE ON A BUTYLATE HISTORY SOIL SPIKED WITH 5 AND 50 PPM BUTYLATE AND EPTC SOLUTIONS. EACH BAR IS THE MEAN + S.D. (n=3) PER GROUP.

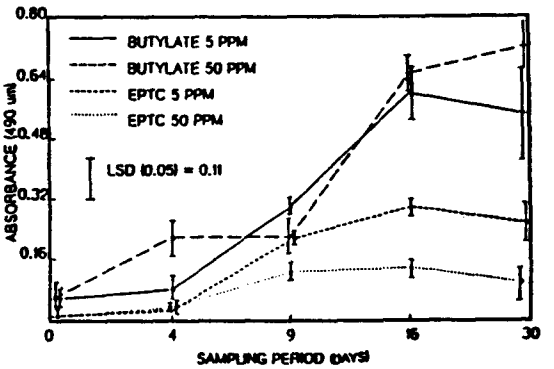
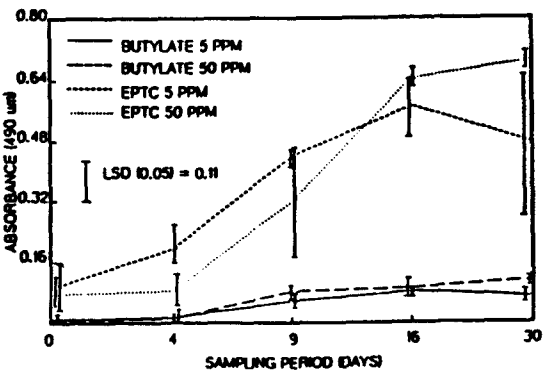


FIGURE 1c. ABSORBANCE OF FLUORESCEIN DIACETATE ON AN EPTC HISTORY SOIL SPIKED WITH 5 AND 50 PPM BUTYLATE AND EPTC SOLUTIONS. EACH BAR IS THE MEAN + S.D. (n=3) PER GROUP.



(metabolite) utilization by such a community as indicated by this assay indicates that individuals may acquire the ability to degrade the substrate. Nickerson (1984) first hypothesized selectivity of soils for degrading one pesticide faster than another is probably due to a difference among the substrates, namely the iso-butyl side chains of butylate and the propyl groups of EPTC. Our results agree with the findings of Lee (1984) that selection of a microbial community of degraders is occurring with serial applications of EPTC on soils possessing EPTC degraders. Moorman (1988) concluded metabolic activity, not numbers, was responsible for enhanced thiocarbamate degradation. Further, Senior et al. (1976) first witnessed the evolution of specific degradative enzymes by soil microorganisms. The specificity, as indicated by substrate and soil pesticide history shows the complexity of metabolic activities of a microbial community and perhaps certain individuals. Lappin et al. (1985) observed the synergistic degradation of mecoprop by a community of degraders.

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