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Influence of Cry1Ac Toxin on Mineralization and Bioavailability of Glyphosate in Soil

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The impact of transgenic plants containing *Bacillus thuringiensis* (Bt) toxin on soil processes has received recent attention. In these studies, we examined the influence of the lepidopterean Bt Cry1Ac toxin on mineralization and bioavailability of the herbicide glyphosate in two different soils. The addition of 0.25−1.0 μg g^{-1} soil of purified Cry1Ac toxin did not significantly affect glyphosate mineralization and sorption in either a sandy loam or a sandy soil. In contrast, extractable glyphosate decreased over the 28 day incubation period in both soils. Our findings suggest that the reduction in the bioavailabily of glyphosate was not influenced by the presence of Cry1Ac toxin but rather the results of aging or sorption processes. Results from this investigation suggest that the presence of moderate concentrations of Bt-derived Cry1Ac toxin would have no appreciable impact on processes controlling the fate of glyphosate in soils.

KEYWORDS: Glyphosate; Cry toxins; Cry1Ac; herbicide sorption; herbicide mineralization; genetically modified crops

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine] is an effective nonresidual herbicide used to control a wide range of annual and perennial weeds. In recent years, the increased demand for genetically modified (GM) crops, many of which are tolerant to herbicides, has further expanded glyphosate usage (*1*, *2*). However, the introduction of glyphosate-tolerant crops has renewed interest in the adoption of conservative tillage practices as a means to replace or reduce the application of preemergence herbicides (*3*). In addition to potential indirect effects, such as changes in soil management practices, the impact of glyphosate-tolerant crops on soil and water quality mainly results from alterations in the glyphosate application rate, frequency and timing of its use, its physicochemical properties, and its effects on soil microorganisms (*4*, *5*).

There is considerable information concerning the environmental fate of glyphosate. Glyphosate degrades relatively rapidly in soils, with an estimated half-life of 7−60 days (*6*), predominately by microbial-mediated processes (*7*, *8*). Despite its high water solubility, glyphosate is strongly sorbed to soil particles and consequently has low mobility through the soil profile (*9*, *10*). Organic matter, clay content, and Fe and Al oxides play important roles in the sorption of glyphosate to soil (*11*, *12*).

Aside from herbicide-tolerant crops, other major cultivated GM crops include insect-resistant and stacked-trait plants. The latter GM plants are mostly represented by crops having resistance to glyphosate and insects (*2*). Most insect-resistant varieties have been engineered to express *Bacillus thuringiensis* (Bt) toxins. Bt is a ubiquitous spore-forming bacterium that produces a crystalline parasporal body comprised of one or more insecticidal crystal (Cry) proteins. Strains of Bt that are toxic to lepidopteran and other insect orders have been known for almost 100 years and have been commercialized for more than four decades. Bt-based formulations are widely used in integrated pest management and organic farming programs due to the toxin's high selectivity and low toxicity to mammals and nontarget insects.

Bt crops may exude Cry toxin from roots into the soil or release the toxin through decomposition of crop residues (*13*−*15*). In the case of glyphosate-tolerant/Bt-protected stacked-trait crops, the joint presence of both glyphosate and Cry toxins in the soil ecosystem may occur (*8*, *16*). Although information dealing with the persistence of Cry toxins in soil is available, the potential for interaction between Cry toxins and glyphosate in the soil ecosystem is not well-understood (*8*).

In preliminary investigations, Accinelli et al. (*8*) showed that there was a significant increase in glyphosate persistence when a mixture of Cry toxins extracted and purified from a commercial formulation of *B. thuringiensis* subsp. *kurstaki* was added to soil. However, given that a mixture of Cry toxins was used in their studies and that Bt-protected plants produce a single activated crystal toxin, the authors concluded that their findings

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Table 1. Properties of IT and MN Soils

soil	textural class	particle size (%) sand	silt	clay	pH[a] (1:2.5)	organic C (%)
IT	sandy loam	63.4	22.6	14.0	8.11	0.70
MN	sand	93.5	2.7	3.8	7.20	0.94

[a] Soil pH measured in 1:2.5 (w/w) soil/deionized water mixture.

would only roughly simulate real agricultural conditions. Consequently, in this study, we investigated the influence of several concentrations of a single Cry1Ac antilepidopteran toxin on mineralization and sorption of glyphosate in two agricultural soils.

MATERIALS AND METHODS

Soils. Two soils with different physicochemical properties, representing corn production areas of the Po Valley (Italy) and of south central Minnesota, were selected for this study. Surface soil samples (0−20 cm) were taken from Ozzano, Italy (near Bologna, 44° 28′ N, 11° 28′ E) and from Princeton, MN (45° 00′ N, 93° 10′ E). The Ozzano (IT) and Princeton (MN) soils were classified as a sandy loam (Udertic Ustochrepts, fine, mixed, mesic) and as a sandy soil (Argic Udipsamments, mixed, frigid), respectively. At both locations, the soil was collected from fields that had not received glyphosate applications within the previous 5 years. Soils were air-dried and passed through a 2 mm sieve. Several physicochemical properties of the soils are given in **Table 1**.

Purification of Cry1Ac Toxin. The Cry1Ac toxin was obtained from a recombinant strain of Bt (4Q7/pPFT1Acs) that was donated by Prof. B. A. Federici (Department of Entomology, University of California, Riverside, CA). Purification of Cry1Ac toxin was done essentially as described by Park et al. (*17*). Briefly, the Bt strain was grown in 200 mL of nutrient broth (Difco) medium supplemented with 0.5% (w/v) glucose (NBG) and erythromicin (25 μg mL^{-1}). Liquid cultures were incubated for 4−5 days at 30 °C, with shaking, until sporulation and cell lysis were complete. Cells were centrifuged at 6500*g* for 15 min at 4 °C, suspended in 15 mL of double-distilled water, and sonicated for 1 min, five times. Aliquots (5−10 mL) of the samples were loaded onto a discontinuous sucrose gradient (67−72−79% w/v) and centrifuged at 20000*g* for 1 h at 5 °C. Bands containing crystal inclusions were collected using a fraction collector, dialyzed in water overnight, and lyophilized. As indicated by Park (*17*), the purity of the toxin preparation was assessed by SDS-polyacrylamide gel electrophoresis. The concentration of Cry1Ac toxin was determined using the Lowry method (*18*), with bovine serum albumin as standard.

[^{14}C]Glyphosate Mineralization Studies. A portion of the IT and MN soils was thoroughly mixed with Cry1Ac toxin powder to obtain a final concentration of 100 μg g^{-1} soil. Aliquots of these two amended soils were mixed with a sufficient mass of IT and MN soils to obtain final soil concentrations of 0.25, 0.5, and 1.0 μg Cry1Ac toxin g^{-1} soil (air-dried basis). The mixtures were vigorously blended to promote homogeneous distribution of the toxin in soils, and 20 g aliquots of each soil were weighed into 250 mL glass, screw-capped, Erlenmeyer flasks. Three replicates were prepared for each soil type and toxin concentration, and controls consisted of soils with no toxin addition.

Solutions of unlabeled glyphosate (chemical purity > 98%) and ^{14}C-labeled glyphosate (*N*-phosphonomethyl-2-^{14}C-glycine; radiopurity > 99%, specific activity = 1.18 10^6 MBq g^{-1}) were prepared in distilled water to obtain a final concentration of 5.71 μg a.i. mL^{-1} (1.48 × 10^{-3} MBq mL^{-1}). Unlabeled and radiolabeled glyphosate were obtained from Sigma Co. (St. Louis, MO). A 3.5 mL aliquot of the solution was added to each soil sample to obtain a final glyphosate concentration of 1 μg g^{-1} air-dried soil. The soil moisture in treated soil samples was adjusted to the gravimetric content at −33 kPa using distilled water and incubated in the dark at 25 °C for 28 days. The moisture content of incubated samples was checked at 7 day intervals and adjusted to the initial −33 kPa, as needed. Glyphosate mineralization was monitored every 2 days by trapping the evolved $^{14}CO_2$ in vials containing 5 mL of a 1 M NaOH solution. The NaOH solution was replaced at sampling, facilitating flask aeration. Trapped $^{14}CO_2$ in 1 mL aliquots of NaOH solution was determined by using a 1500 Tri-Carb Packard (Meriden, CT) liquid scintillation analyzer. Samples were kept in the dark for 12 h prior to analysis. No chemiluminescence was observed.

Soil Extraction. At the end of the 28 day incubation period, the distribution between solution phase and weakly sorbed and bound ^{14}C-residues in incubated soil samples was determined using a two-step procedure. Triplicate samples receiving no Cry1Ac addition (controls) and samples with the highest toxin concentration (1 μg g^{-1}) were removed, and all of the soil in each flask (20 g) was transferred to Teflon centrifuge tubes by washing with 200 mL of 0.01 M $CaCl_2$. Samples were shaken in a horizontal shaker for 14 h at 20 °C. Soil suspensions were centrifuged at 2500*g* for 30 min, the supernatant was transferred to preweighed glass vials, and 50 mL aliquots were filtered through a 0.2 μm filter. Radioactivity in1 mL aliquots of the filtered supernatants was determined by liquid scintillation counting (LSC). The remaining filtered extract was saved for later determination of the amount of glyphosate in the extracted ^{14}C residues, as described below. The soil was then extracted with 200 mL of 0.1 M K_2HPO_4, pH 8, following the same procedure used for the $CaCl_2$ extraction. Glyposate concentrations in aqueous extracts were determined by high-performance liquid chromatography (HPLC) using an HP model 1090 HPLC (Hewlett-Packard Co., Palo Alto, CA) equipped with a 0.46 cm × 25 cm Luna NH_2 column (Phenomenex Inc., Torrance, CA) and a diode array detector as previously described (*8*). HPLC fractions were collected, mixed with liquid scintillation cocktail, and quantified by LSC. Soil-bound ^{14}C residues remaining after the two extraction procedures were quantified by combusting subsamples (0.3 g air-dried basis) of the extracted soils using a Packard sample oxidizer (Packard Instrument Co.). Radioactivity was quantified by LSC.

[^{14}C]Glyphosate Sorption/Desorption Isotherms. Isotherms for sorption of glyphosate to IT and MN soils containing different Cry1Ac toxin concentrations were determined using the batch equilibrium method. Two gram aliquots (air-dried basis) of each soil−Cry1Ac combination were weighed into 50 mL glass centrifuge tubes, and a 10 mL aliquot of [^{14}C]glyphosate solution, prepared in 0.01 M $CaCl_2$, was added. Sorption isotherms were determined using triplicate samples at six initial glyphosate concentrations, ranging from 0.2 to 120 μg mL^{-1}. Radiolabeled glyphosate was added to unlabeled solutions to give an initial radioactivity of approximately 50 Bq mL^{-1}. Tubes were sealed with Teflon-lined caps and mechanically shaken at 20 °C for 14 h, and samples were centrifuged at 2500*g* for 10 min. Five milliliter aliquots of supernatant were removed and filtered through a 0.2 μm filter, and radioactivity in 1 mL fractions was determined by LSC. Preliminary investigations showed that equilibrium was reached within 14 h.

The amount of sorbed glyphosate was calculated from the concentration differences between the supernatant of the equilibrated solutions and those of the corresponding initial solutions. Sorption data were fitted to the log form of the Freundlich equation: $\text{Log } C_s = \log K_f + (1/n) \log C_e$, where C_s is the concentration of glyphosate sorbed (μg g^{-1} soil), C_e is the herbicide equilibrium concentration (μg mL^{-1} solution), and K_f and $1/n$ are the empirical Freundlich constants. Values of K_f and $1/n$ were estimated by linear regression after a log−log transformation.

Desorption isotherms for glyphosate in the two soils with no toxin addition (control) and with the highest Cry1Ac toxin concentration, 1.0 μg g^{-1}, were done using samples initially receiving 60 and 120 μg mL^{-1} glyphosate. Briefly, desorption of glyphosate was carried out by replacing a 4 mL aliquot of the glyphosate-containing solution with 4 mL of 0.01 M $CaCl_2$. After solution addition, tubes were shaking for 14 h and centrifuged as above-described. The desorption cycle was repeated four times.

Cry1Ac Persistence in Soil. The persistence of Cry1Ac toxin in samples of both IT and MN soils containing 1 μg toxin g^{-1} soil, prepared and incubated as described in the mineralization experiment, was determined. At different sampling times, the concentration of Cry1Ac toxin in soils was determined by enzyme-linked immunosorbent assay using the QuantiPlate Kit for Cry1Ab/Cry1Ac (Envirologix,

Portland, ME). Soil extraction was performed using a modification of the procedure suggested by the manufacturer. Briefly, soil samples were mixed with extraction buffer at a ratio of 1:5 and shaken at room temperature for 6 h. Samples were centrifuged at 10000*g* for 15 min, and the supernatant was filtered through a 0.2 μm filter. A 100 μL aliquot of filtered supernatant was added to each microplate well and processed as indicated by the manufacturer. Absorbance measurements were done at 405 nm using a Bio-Rad 3550 microplate reader. A standard curve was obtained using seven concentrations of purified Cry1Ac toxin, prepared in 100 mM carbonate buffer (pH 10.5). Measurements were done in triplicate, and the whole experiment was repeated using sterile soil. Sterilization was achieved by exposing soil samples to 1 MRad of γ-irradiation for 66 h using a Mark 1 68-A-3 γ-irradiator (JL Shepherd, San Fernando, CA).

Enumeration of Culturable Bacteria. At the end of the 28 day incubation period, total culturable, heterotrophic bacteria in incubated soil samples receiving no Cry toxin addition (control) and those receiving 1.0 μg g^{-1} of toxin were enumerated using the plate count procedure. Ten gram portions of each incubated soil sample were mixed with 95 mL of 0.1% sodium pyrophosphate buffer (SPB), pH 7, and shaken on a horizontal shaker for 20 min at 200 rpm. Samples were decimally diluted in sterile 0.1% SPB, and 100 μL aliquots were spread-plated onto 0.1X tryptone soy agar (Difco) supplemented with cycloheximide (50 μg mL^{-1}). Plates were incubated at 27 °C, and colonies were enumerated after 4, 6, and 10 days. Measurements were done in triplicate, and nonincubated soil samples were included as the control.

RESULTS AND DISCUSSION

[^{14}C]Glyphosate Mineralization. Glyphosate mineralization, expressed as $^{14}CO_2$ evolution, in the IT and MN control soil samples and in samples receiving 1 μg g^{-1} of Cry1Ac toxin are shown in **Figure 1**. A similar pattern of glyphosate mineralization was observed in both soils. At the end of the 28 day incubation period, total accumulated $^{14}CO_2$ in the IT and MN control soils were not significantly different ($P > 0.05$) and accounted for 36.8 and 37.5% of the total applied ^{14}C as glyphosate, respectively. No lag phase was observed, and approximately 50% of the evolved $^{14}CO_2$ occurred within the first 8 and 12 days after treatment (DAT) in the IT and MN soils, respectively. Microbial degradation is considered the predominant mechanism of glyphosate transformation in soils (*8*, *19*). Although some microbial strains have been isolated that are able to grow in artificial media using glyphosate as a sole C, N, or P source, degradation of glyphosate in natural environments, such as the soil ecosystems, has been thought to occur by nonspecific, cometabolic processes (*20*).

The addition of Cry1Ac toxin in the range from 0.5 to 1 μg g^{-1} soil did not affect patterns of glyphosate mineralization in the two soils (**Figure 1**). A slight decrease ($P > 0.05$) in cumulative glyphosate mineralization was observed with the highest toxin concentration (1 μg g^{-1}) in both soils (**Table 2**).

A previous experiment conducted with the same two soils showed that Cry1Ab toxin added as corn residues obtained from a Bt-protected hybrid did not influence glyphosate mineralization (*21*). In these previous studies, the greatest toxin concentration in soil was 14 ng g^{-1}, based on the Cry1Ab concentration in the incorporated corn residues. Besides differences among the two Cry toxins and in the modality of soil incorporation, the study reported here further supports that moderate toxin concentration ($\leq$1 μg g^{-1}) does not affect glyphosate mineralization in soil. According to Head et al. (*22*), incorporation of crop residues of Cry1Ac-encoding plants into the soil is expected to lead to a toxin concentration in the top three inches of approximately 0.65 μg g^{-1} soil. In a research study conducted throughout several agricultural areas, these authors did not observe detectable Cry1Ac toxin in soil after 3 months from the time the crop was harvested. Consequently, on the basis of the results obtained in the present study reported here and excluding bioaccumulation of Cry toxins in soil, Cry1Ac would not likely increase glyphosate persistence under field condition.

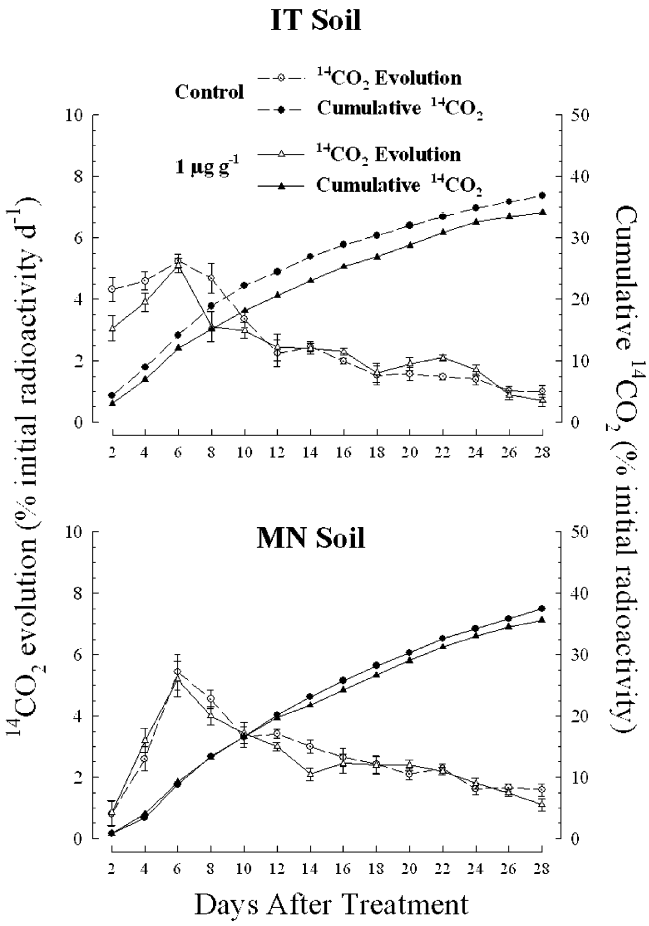

Figure 1. Effect of the highest Cry1Ac concentration on $^{14}CO_2$ evolution from IT and MN soils. Bars represent standard errors of the mean.

Table 2. Cumulative $^{14}CO_2$ Evolution during the 28 Day Incubation Period from IT and MN Soils

Cry1Ac concn (µg g^{-1})	IT soil	MN soil
– (control)	36.84 ± 0.87	37.45 ± 0.73
0.25	37.21 ± 0.91	37.51 ± 0.88
0.50	36.51 ± 0.80	36.44 ± 0.95
1.00	34.15 ± 0.93	35.75 ± 0.89

[a] Values are means of three replicates ± standard deviation.

[^{14}C]Glyphosate Sorption/Desorption. In both the IT and the MN soils, the relative amount of nonextractable ^{14}C residues, [^{14}C]glyphosate, and bound residues increased over the incubation period. This tendency was slightly greater in samples receiving the highest Cry1Ac concentration (**Figure 2**). In the present experiments, $CaCl_2$- and K_2HPO_4-extractable fractions were assumed to represent the readily available and the sorbed fractions, respectively. The mineralization of [^{14}C]glyphosate was not influenced by increasing concentrations of Cry1Ac toxin and, consequently, appeared not to be affected by the observed slight reduction of herbicide bioavailability over the incubation period. Similar to what has been found with a variety of pesticides, degradation of glyphosate in soil mainly occurs via microbial processes. It is generally accepted that microbial degradation is to some extent related to the accessibility

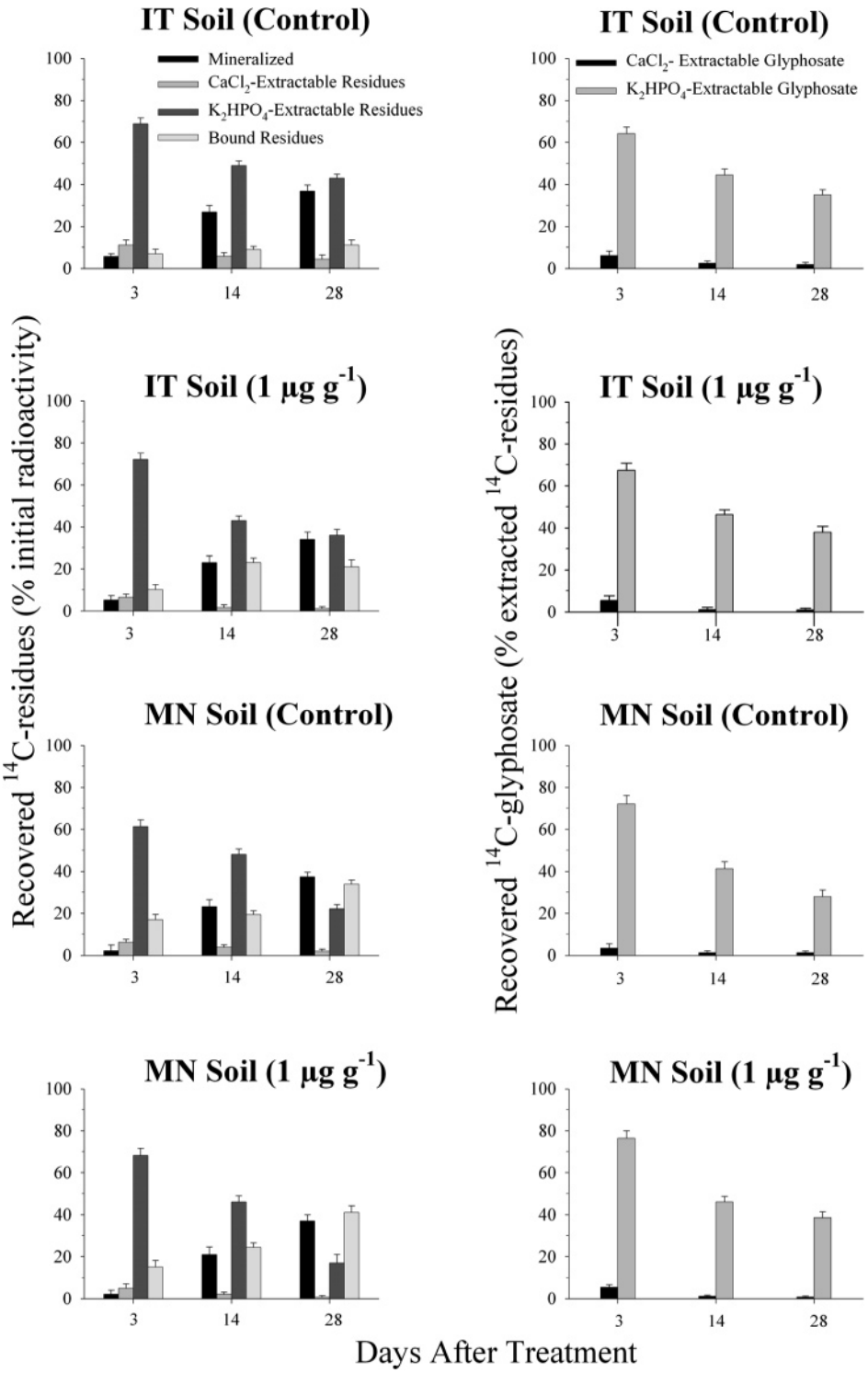

Figure 2. Distribution of ^{14}C residues into evolved $^{14}CO_2$, $CaCl_2$, and K_2HPO_4 extractable fractions, bound residues in IT and MN control soils and soils containing the highest Cry1Ac concentration, and extractable [^{14}C]glyphosate in $CaCl_2$ and K_2HPO_4 extractable fractions.

(bioavailability) of pesticide to microorganisms in soil systems. The results obtained here with this strongly sorbed herbicide suggest that the relationship between extractable herbicide fractions and mineralization intensity may be more complex than with respect to other herbicides, such as atrazine (*23*). Because most of the accumulated $^{14}CO_2$ evolution occurred within the first 8 and 12 DAT in the IT and MN soils, respectively, and Cry1Ac toxin persisted in the two soils for a relatively short time (see below), the reduction of glyphosate bioavailability at 14 DAT was most likely the result of herbicide aging, rather than exposure to the Cry1Ac toxin per se.

Glyphosate sorption to the IT and MN soils, over the initial concentration range from 0.5 to 120 μg mL^{-1}, was correctly described by the linear form of the Freundlich equation. This is similar to results reported in a previous study (*21*). However, more glyphosate was sorbed to the IT sandy loam than to the MN sandy soil. Glyphosate sorption was a concentration-dependent process, as indicated by estimated 1/*n* values (**Table 3**). Glyphosate desorption data indicated that less herbicide was desorbed from the soil than predicted by the respective sorption isotherms. Neither sorption nor desorption were affected by increasing concentration of the insecticidal toxin (**Table 4**).

Table 3. [^{14}C]Glyphosate Sorption Coefficients Determined in the IT and MN Soil as Functions of Different Concentrations of Cry1Ac

	IT soil			MN soil		
Cry1Ac concn (μg g^{-1})	K_f[a] (μg$^{1-1/n}$ g^{-1} mL$^{1/n}$)	1/n_f[b]	chdr^2[c]	K_f (μg$^{1-1/n}$ g^{-1} mL$^{1/n}$)	1/n_f	r^2
– (control)	43.01 (43.74–47.61)	0.79 (0.06)	0.99	62.16 (63.38–67.37)	0.88 (0.04)	0.99
0.25	43.55 (43.21–44.12)	0.77 (0.07)	0.96	63.22 (63.12–64.09)	0.91 (0.05)	0.93
0.50	42.97 (42.31–43.27)	0.75 (0.08)	0.98	62.81 (62.32–63.14)	0.89 (0.07)	0.99
1.00	43.71 (43.48–44.09)	0.79 (0.07)	0.95	62.49 (62.10–63.17)	0.86 (0.09)	0.96

[a] Numbers in parentheses are 95% confidence interval. [b] Numbers are mean 1/n_f ± standard deviation. [c] Correlation coefficients of linear regression of linearized Freundlich isotherms.

Table 4. [^{14}C]Glyphosate Desorption Coefficients at Two Initial Solution Concentrations (C_i) Determined in Untreated (Control) Soils and Soil Samples Containing the Highest Cry1Ac Concentration

	IT soil				MN soil			
	$C_i = 60$ μg mL^{-1}		$C_i = 120$ μg mL^{-1}		$C_i = 60$ μg mL^{-1}		$C_i = 120$ μg mL^{-1}	
Cry1Ac concn (μg g^{-1})	1/n_{f-des}[a]	r^2	1/n_{f-des}	r^2	1/n_{f-des}	r^2	1/n_{f-des}	r^2
– (control)	0.15 (0.01)	0.95	0.18 (0.05)	0.84	0.29 (0.04)	0.95	0.30 (0.03)	0.96
1.00	0.13 (0.05)	0.81	0.11 (0.08)	0.83	0.41 (0.08)	0.91	0.21 (0.05)	0.89

[a] Numbers are mean 1/n_f ± standard deviation.

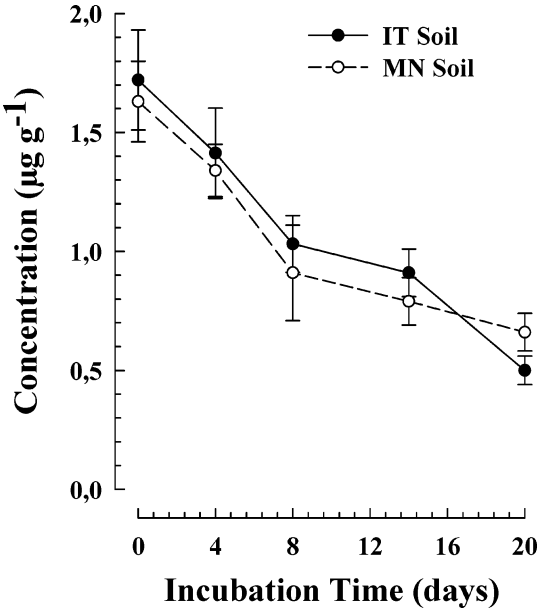

Figure 3. Concentration values ± standard deviations of Cry1Ac toxin in the IT and MN soils during the 20 day incubation period.

These findings are consistent with our hypothesis that the estimated reduction of bioavalability of glyphosate over the incubation time was not related to the presence of the toxin but probably caused by other factors or processes such as aging.

Cry1Ac Persistence in Soil and Effects on Culturable Soil Bacteria. Recovery of the applied, purified Cry1Ac toxin was 86.2 and 82.0% at time 0 in the IT and MN soils, respectively. The Cry1Ac toxin concentration declined with time according to a first-order rate equation (**Figure 3**). The average r^2 of the log-transformed first-order equation for the two soils was 0.84 ± 0.11. The two soils showed a high capacity to degrade the Cry1Ac toxin. The estimated half-lives of Cry1Ac toxin in the IT and MN soils were of 11.9 and 7.4 days, respectively. In contrast, only a slight decrease in toxin concentration was observed in both sterile soils, confirming that degradation of Cry1Ac in soils occurs mainly by microbial, rather than by chemical, processes (data not shown).

Table 5. Size of Culturable Soil Bacteria Estimated at 0 and 28 DAT in the IT and MN Soil Samples Containing 1 μg g^{-1} Cry1Ac Toxin

		soil bacteria (Log CFUs[a] g^{-1} air-dried soil)	
Cry1Ac concn (μg g^{-1})	DAT	IT	MN
– (control)	0	7.49 ± 0.32[b]	8.30 ± 0.47
1.00	28	7.94 ± 0.40	8.64 ± 0.36
1.00	28	7.55 ± 0.28	8. 60 ± 0.39

[a] Colony forming units. [b] Values are means of three replicates ± standard deviation.

Addition of purified Cry1Ac toxin to soils in the range of 0.5−1.0 μg g^{-1} did not lead to any significant variation in the number of culturable bacteria (**Table 5**). However, the number of culturable bacteria was greater in the MN than in the IT soil. Consequently, in contrast to glyphosate mineralization, degradation of the Cry1Ac seemed to be affected more by total culturable soil bacteria. Even considering that most of the soil bacteria are not culturable, these results are consistent with the glyphosate mineralization results. Reports on the effects of purified Cry toxins on soil bacteria are scarce. Under some circumstances, however, a transient stimulation of soil bacteria and fungi in soil receiving cotton tissue containing Cry1Ac toxin has been demonstrated (*24*). Moreover, most studies reported in the literature have been done using soil incorporation of crop residues or plant tissues obtained from Bt-protected plants. Venkateswerlu and Stotzky (*25*) reported that Bt toxin binds to clay minerals and becomes resistant to microbial degradation. They hypothesized that the Bt toxins released from transgenic plants into the soil may persist, accumulate over time, and present a risk to sensitive organisms. However, results from other studies (*22*, *26*) and those reported here indicate that there is little risk associated with the bioaccumulation of this group of toxins in soil. Taken together, these experiments reported no direct effects of Cry toxins contained in transgenic plant tissues or residues (*15*, *21*, *27*).

Conclusion. Results from the present laboratory investigations indicate that soil incorporation of purified Cry1Ac toxin in the range of 0.25−1.0 μg g^{-1} does not influence glyphosate mineralization or its sorption in soil. These results are in contrast to results obtained in previous investigations done using a

mixture of Cry toxins at a concentration of 10 μg g^{-1} (*21*). The concentration of Cry toxins in soil occurring during the growing season has been estimated not to exceed 1 μg g^{-1}, based on the average concentrations of Cry toxin in crop residues incorporated into the top soil or left at the soil surface (*22*). On the basis of these estimates and the results obtained here, our data indicate that concentrations of Cry1Ac comparable to those encountered under field conditions do not have the potential to increase persistence and sorption of glyphosate in soil.

ABBREVIATIONS USED

IT, Ozzano soil; MN, Princeton soil; Bt, *Bacillus thuringiensis*; C_e, herbicide equilibrium concentration; C_s, sorbed herbicide concentration; K_f and 1/*n*, empirical Freundlich constants; DAT, days after treatment; LSC, liquid scintillation counting; HPLC, high-performance liquid chromatography; SPB, sodium pyrophosphate buffer.

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