Adsorption and Desorption of Cry1Ab Proteins on Differently Textured Paddy Soils

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ABSTRACT

In recent years, selected cry genes from Bacillus thuringiensis (Bt) encoding the production of Cry proteins (Bt toxins) have been engineered into crop plants (Bt-crops). Through the cultivation of Bt crops and the application of Bt pesticides, Cry proteins could be introduced into arable soils. The interaction between the proteins and soils was analyzed in this study to investigate the affinity of Cry proteins in paddy soil ecosystems. Four Paddy soils were selected to represent different soil textures. Cry proteins were spiked in soils, and the amount of protein adsorbed was measured over 24 h. Desorption of Cry1Ab proteins from paddy soils was performed by washing with sterile Milli-Q water (H_{2}O_{MQ}), and subsequently extracted with an extraction buffer. The paddy soils had a strong affinity for Cry1Ab proteins. Most of the Cry1Ab proteins added (> 98%) were rapidly adsorbed on the paddy soils tested. More Cry1Ab proteins were adsorbed on non-sterile soils than on sterile soils. Less than 2% of the adsorbed Cry1Ab proteins were desorbed using H_{2}O_{MQ}, while a considerable proportion of the adsorbed proteins could be desorbed with the buffer, ranging from 20% to 40%. The amount of proteins desorbed increased with the increases in the initial amount of Cry1Ab proteins added to the paddy soils. The concentration of Cry1Ab proteins desorbed from the paddy soils was higher for sterile soils than non-sterile ones. Our results indicate that Bt toxins released via the cultivation of Bt crops, the application of Bt pesticides can be adsorbed on paddy soils, and soil texture could impose an impact on the adsorption capability.

Key Words: affinity, Bt toxin, extraction, protein solution, soil texture, sterile soil


INTRODUCTION

Bacillus thuringiensis is a gram-positive soil bacterium with the affiliation to the class of Bacilli belonging to the phylum Firmicutes, and is commonly known as Bt (Parry et al., 1983; Read et al., 2003). It is characterized by its ability to form parasporal crystals during sporulation (Read et al., 2003). The proteins in these crystals, referred to as Cry endotoxins or Bt toxins, exhibit insecticidal activity against specific insect groups, many of which are economically important crop pests. After ingestion by a susceptible insect, Bt toxins are solubilized in the insect midgut (pH > 10), and proteolytically activated into a toxin core in the insect digestive fluid (Höfte and Whiteley, 1989; Crecchio and Stotzky, 2001). The activated toxins are then bound to midgut receptors and inserted into the apical membrane, where the toxins generate ion channels or pores, thus disturbing the osmotic balance. Consequently, the cells swell and lyse (Harvey et al., 1983; Knowles and Ellar, 1987; Hoffmann et al., 1988; Schnepf et al., 1998). To date, Bt toxins are classified into four major groups and several subgroups characterized by both structural similarities and the insecticidal spectra of the toxins. The four major groups are Lepidoptera-specific (Cry1), Lepidoptera- and Diptera-specific (Cry2), Coleoptera-specific (Cry3), and Diptera-specific (Cry4) (Höfte and Whiteley, 1989; Schnepf et al., 1998; Mohan and Gujar, 2001).

The highly specific insecticidal effect led to the development of pesticides based on a mixture of cells, spores, and parasporal crystals of Bt (Beegle and Yamamoto, 1992). For decades, Bt has proven to be a useful alternative or supplement to synthetic chemical insecticides applied in agriculture, forest management, and mosquito control (Höfte and Whiteley, 1989; Schnepf et al., 1998; Crecchio and Stotzky, 2001; Dohrmann et al., 2013). Typical agricultural formulations of Bt consist of wettable powders, spray concentrates, liquid concentrates, dusts, baits, and time-release rings. Products of Bt used as microbial pest co-
control agents in agriculture and forestry are already commercially available, which are based on formulations of different Bt subspecies (Höfte and Whiteley, 1989; Icoz and Stotzky, 2008), e.g., Dipel® (DF, dry flowable Bt kurstaki), Xentari® (WG, Bt aizawai), and NEUDORFF® (StechmückenFrei, Bt israelensis).

The toxins from Bt are attractive as applied pesticides, and also for delivery via genetically modified (GM) plants. The cry genes coding for insecticidal crystal proteins have been cloned from Bt. With the insertion of exogenous cry genes in plant chromosomal DNA, Bt toxins can be expressed within plants (Schnepp \textit{et al.}, 1998; Crecchio and Stotzky, 2001; Dohrmann \textit{et al.}, 2013). Selected cry genes have been introduced into major crops, such as maize (Koziel \textit{et al.}, 1993; Kramarz \textit{et al.}, 2007), rice (Fujimoto \textit{et al.}, 1993), and potato (Adang \textit{et al.}, 1993), to produce their own Bt toxins. Transgenic Bt crops are considered as a powerful and promising alternative strategy for the protection of crops against insect infection (Höfte and Whiteley, 1989). Commercial cultivation of GM plants began in 1996 and has expanded rapidly, with 20.3 million ha of Bt crops representing 37% of all GM crops in 2007 (Helassa \textit{et al.}, 2009). The global area of GM crops continued to increase in recent years, reaching 175.2 million ha in 2013 (James, 2013). Given the rapid increase in the commercial cultivation of transgenic Bt crops, their environmental effects can’t be completely assessed (Icoz and Stotzky, 2008; Helassa \textit{et al.}, 2009). The Bt toxins are continuously produced by the Bt plants and apparently persist for some time in plant tissues (Sims and Holden, 1996; Sims and Ream, 1997). Insecticidal proteins from Bt plants are released into the soil ecosystem by different pathways, e.g., via root exudates during plant growth (Saxena \textit{et al.}, 1999; Saxena and Stotzky, 2000), via the incorporation of plant residues after the harvest of Bt plants (Tapp and Stotzky, 1998), and probably via some input from pollen (Losey \textit{et al.}, 1999; Pagel-Wieder \textit{et al.}, 2007).

Through both the application of Bt pesticides and the commercial cultivation of transgenic Bt plants, Bt toxins can be released into the soil ecosystem. Their environmental fate relies on interactions with soil; thus the effects of interactions between Bt toxins and soil particles on the persistence and insecticidal activity of the toxins should be evaluated (Crecchio and Stotzky, 2001; Hung \textit{et al.}, 2016). In controlled microcosm experiments and field studies, it has been shown that the toxins could be adsorbed and thus persist in soil for up to 8 months (Tapp and Stotzky, 1998; Saxena and Stotzky, 2002; Zwahlen \textit{et al.}, 2003). When the soil is spiked with Bt toxins, the toxins are bound to non-sterile soils and still detectable after 40 d (Tapp and Stotzky, 1995a, b). Moreover, the binding protects toxins against microbial degradation, but the insecticidal activity of the toxins is retained (Tapp and Stotzky, 1995b; Koskella and Stotzky, 1997). It is also essential to understand the reversibility of adsorption, as the ability of desorption influences the mobility and persistence of toxins in soil (Helassa \textit{et al.}, 2009). Desorption of Bt toxins has been investigated in a few studies. In general, it has been reported that Bt toxins could not be desorbed from soil or soil minerals with either water or buffer (Tapp \textit{et al.}, 1994; Crecchio and Stotzky, 2001; Chevallier \textit{et al.}, 2003). In contrast, a considerable proportion of Bt toxins could be desorbed from minerals (i.e., montmorillonite and kaolinite) with water (Saxena and Stotzky, 2000; Helassa \textit{et al.}, 2009).

The adsorption and desorption of Bt toxins on soil are a function of soil (mainly clay content but also humic substances), source of the toxins (produced by transgenic Bt plants versus Bt strains), and the particular insecticidal crystal proteins under investigation (Palm \textit{et al.}, 1996; Clark \textit{et al.}, 2005; Pagel-Wieder \textit{et al.}, 2007; Helassa \textit{et al.}, 2009; Sander \textit{et al.}, 2012; Tomaszewski \textit{et al.}, 2012). With the commercial cultivation of transgenic Bt rice (e.g., TT51-1) containing exogenous cry1Ab genes from Bt subs. kurstaki (Wu \textit{et al.}, 2013; Chen \textit{et al.}, 2014), it is reasonable to investigate the fate and behavior of Cry1Ab proteins (Bt toxins) in paddy soils. The objective of this study was to investigate the adsorption of Cry1Ab proteins on paddy soils spiked with pure proteins, and the desorption of the adsorbed proteins. Four differently textured paddy soils were selected to investigate differences in the adsorption and desorption behaviors of the proteins among soils. Sterile treatments for soils were also considered in this study in comparison to non-sterile treatments. Several concentrations of Cry1Ab proteins were introduced to investigate soil adsorption ability over time. Desorption was determined by washing with water and subsequent extraction with a specific extraction buffer.

\section*{Materials and Methods}

\subsection*{Paddy soils and Cry1Ab proteins}

Differently textured paddy soils were selected for this study, which had been described before (Eickhorst and Tippkötter, 2009). According to the World Reference Base for Soil Resources (IUSS Working group WRB, 2006) and the USDA texture classification (Soil
Survey Staff, 1996), these soils are classified as: sandy loam (Hydragric Anthrosol; LC), loam (Anthraquic Cambisol; MC), silt loam (Gleyic Fluvisol; TX), and silty clay (Stagnic Anthrosol; TL). Some physicochemical characteristics of the four selected soils were analyzed by methods at the group of Soil Microbial Ecology in University of Bremen, Germany, shown in Table I. Additional information concerning their soil matrices and particle morphologies can be obtained from thin section microscopy presented by Eickhorst and Tippkötter (2009). Both sterile and non-sterile treatments were applied to each paddy soil. For sterile treatments, the soils were sterilized by autoclaving at 121 °C for 30 min, followed by air-drying on a sterile workbench.

### TABLE I

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH(CaCl_2)</th>
<th>Electrical conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td>4.7</td>
<td>34</td>
</tr>
<tr>
<td>MC</td>
<td>4.6</td>
<td>51</td>
</tr>
<tr>
<td>TX</td>
<td>6.5</td>
<td>78</td>
</tr>
<tr>
<td>TL</td>
<td>4.7</td>
<td>174</td>
</tr>
</tbody>
</table>

(a) According to Soil Survey Staff (1996).

(b) Electrical conductivity.

Cry1Ab proteins were obtained from the Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, USA, and had been tested for the quantification of Cry1Ab toxins in Bt (Crespo et al., 2008).

### Adsorption of Cry1Ab proteins on paddy soils

A stock solution of Cry1Ab proteins (10 μg mL⁻¹) was diluted using sterile H₂O_MQ for the preparation of working solutions (500, 1000, and 2000 ng mL⁻¹). An aliquot of 1 mL Cry1Ab protein solution was pipetted into 200 mg paddy soil in test tubes (2 mL), generating an initial protein addition of 2.5, 5.0, or 10.0 μg g⁻¹ soil. To test the adsorption of Cry1Ab proteins on the walls of tubes, a treatment of pure Cry1Ab protein solutions without soil was included. In addition, sterile H₂O_MQ was pipetted into the same amount of paddy soil, set up as a control. The soil suspension was incubated and rotated in test tubes (2 mL) at 25 °C in the dark for 1, 6, and 24 h. After each incubation period, the suspension was centrifuged at 12 000 × g for 10 min. The resulting supernatants were stored for further determination of Cry1Ab protein concentrations via enzyme-linked immunosorbent assay (ELISA), which is highly preferred due to its specificity and sensitivity of analysis, allowing it to be applied in soil systems (Zwahlen et al., 2003; Baumgarte and Tebbe, 2005). The difference between the amounts of the proteins added and those detected in the supernatants was used to calculate the amount of proteins adsorbed on paddy soils. Soil pellets obtained after 24 h of incubation were prepared for desorption.

### Desorption of Cry1Ab proteins from paddy soils

After the adsorption procedures, sterile H₂O_MQ (1 mL) was added to the soil pellets. The suspensions were equilibrated by end-over-end shaking at 25 °C in the dark for 24 h before centrifugation and analysis of Cry1Ab proteins in the supernatants.

After first desorption with H₂O_MQ, the resulting pellets were then resuspended in 400 μL extraction buffer, containing 0.1 mol L⁻¹ Na₂CO₃, 0.1 mol L⁻¹ NaHCO₃, 5 mmol L⁻¹ ethylenediaminetetraacetic acid, 10 mmol L⁻¹ dithiothreitol, 50 mmol L⁻¹ Na₄P₂O₇·10H₂O, and 0.1% Triton X-100 (pH 10). Based on the results of a preliminary test, the extraction buffer (as described above and reported by Wang et al. (2006)) had a higher extraction efficiency than other two buffers which were prepared according to the protocol in a commercial ELISA kit (AP 003, Envirologix, Portland, USA) and the recipe described in Palm et al. (1994) (data not shown). The suspensions were then rotated at 25 °C in the dark for 1 h. After centrifugation (12 000 × g for 10 min), Cry1Ab proteins in the supernatants were determined via ELISA.

### Quantification of Cry1Ab proteins

The amount of Cry1Ab proteins in sample solutions was analyzed by ELISA using a commercial QualiPlate™ kit (sandwich assay: primary antibody followed by secondary anti horseradish peroxidase; AP 003, Envirologix, Portland, USA). The main procedure was the incubation of an enzyme conjugate and sample solution in a well coated with primary antibodies. After washing, a substrate was added and reacted with the enzyme to produce a stained product, indicating the presence of Cry1Ab proteins. The final solution was measured for absorbance at a wavelength of 450 nm (EMax® Endpoint ELISA Microplate Reader, Molecular Devices, Sunnyvale, USA). Inverse and linear relationships can be observed between the protein concentrations and the absorbance. A standard curve of purified Cry1Ab proteins was included in each individual analysis and thus used for calculating concentrations of Cry1Ab proteins in the samples. The sample solutions were diluted so that they were within the de-
tection range of the ELISA test. The concentration of Cry1Ab proteins was referred to 1 g soil.

**Statistical analysis**

The adsorption and desorption experiments were conducted in triplicate. The data are presented as means and standard errors (SEs). Statistical analyses were performed using SPSS Statistics 20 (International Business Machines Corp., Armonk, USA) using one-way analysis of variance (ANOVA) and Duncan’s test.

**RESULTS AND DISCUSSION**

**Adsorption of Cry1Ab proteins on the silt loam soil**

The Cry1Ab proteins were rapidly adsorbed on the silt loam soil when the soil was spiked with Cry1Ab proteins at three different concentrations (Fig. 1). Adsorption of Cry1Ab proteins on the silt loam soil tended to increase over time. More than 98% of the proteins were adsorbed on the soil after 1 h; thereafter, only a small amount of proteins was adsorbed. When the Cry1Ab protein concentration increased from 2.5 to 10.0 g g\(^{-1}\) soil, the amount of proteins adsorbed on the soil increased. The amount of Cry1Ab proteins adsorbed on the silt loam soil were close to 2.50, 4.98, and 9.98 g g\(^{-1}\) soil when the initially added proteins were 2.5, 5.0, and 10.0 µg g\(^{-1}\) soil, respectively.

Concentrations of proteins adsorbed on the non-sterile soils were slightly higher than those adsorbed on the sterile soil. The protein concentrations in the control tubes containing Cry1Ab protein solutions without soil did not change during the experiment, which were 500, 1000, and 2000 ng mL\(^{-1}\) (data not shown), indicating that there was no adsorption of Cry1Ab proteins on the walls of the tubes.

The adsorption of Bt toxins on paddy soils is important for assessing the environmental risk associated with the commercial cultivation of Bt crops (Pagel-Wieder et al., 2007). Thus, the effect of contact time on the adsorption of Cry1Ab proteins on paddy soil was investigated in this study. Most of the Cry1Ab proteins added were adsorbed on the silt loam soil after 1 h (Fig. 1). Previous studies have also shown that Cry1Ab proteins are adsorbed on clay minerals within 1 h, and the adsorption is completed after 4 h (Tapp et al., 1994; Pagel-Wieder et al., 2007). Sundaram (1996) investigated the adsorption of Bt toxins on the sandy loam and clay loam soils, and found that the amount of Bt toxins adsorbed on the sandy loam soil reached a maximum after 3 h, in contrast to the maximum on the clay loam soil after 4 h. In addition, a longer contact time does not result in an increase in Bt toxin adsorption (Crecchio and Stotzky, 2001). The adsorption isotherms of Bt toxins on the clay soil are the L-type (Giles et al., 1974), suggesting a low affinity interaction as reported in several studies (Crecchio and Stotzky, 2001; Fu et al., 2007; Helassa et al., 2009). The adsorption of Cry1Aa proteins on kaolinite and montmorillonite could be fitted to the Langmuir isotherm (Helassa et al., 2009). Adsorption of Bt toxins on a constant amount of humic acids increases with increasing toxin concentrations, until a plateau is reached (Crecchio and Stotzky, 1998).

However, Sundaram (1996) investigated the adsorption pattern of Bt toxins on forest soils using the Freundlich adsorption isotherm and a linear isotherm equation instead of the Langmuir equation, because of the heterogeneity of the soil surface. Moreover, at higher concentrations, the linear model showed deviations. It has been reported that the adsorption of Bt toxins on the sandy and clay loam soils can be up to

![Fig. 1 Adsorption of Cry1Ab proteins on a silt loam paddy soil with an initial Cry1Ab protein addition of 2.5 (a), 5.0 (b), or 10.0 (c) µg g\(^{-1}\) soil. Vertical bars indicate standard errors of the means (n = 3).](image-url)
301 and 474 μg g⁻¹ soil, respectively (Sundaram, 1996). In this study, the high (> 98%) and rapid absorption of Cry1Ab proteins on the silt loam paddy soil could be explained by the low concentrations of Cry1Ab proteins used, which resulted in large numbers of unoccupied surface sites on the adsorbent, that were easily accessible for molecules of Cry1Ab proteins (Pagel-Wieder et al., 2007).

Autoclaving was conducted in this study to inhibit microbial activity. We detected lower concentrations of Cry1Ab proteins in supernatants from the non-sterile treatment than the sterile treatment, indicating higher adsorption of Cry1Ab proteins on the non-sterile soils than the sterile soils. Douville et al. (2005) suggested that sterilization could significantly inhibit the degradation of Bt toxins in soil and water. Our study indicated that the inactivation of indigenous microorganisms in the silt loam soil could lead to less degradation and thus higher amounts of free Cry1Ab proteins in the supernatants, which resulted in a decreasing adsorption of Cry1Ab/Ac proteins on the sterile soil.

**Adsorption of Cry1Ab proteins on differently textured paddy soils**

The adsorption of Cry1Ab proteins on differently textured paddy soils was measured using two initial concentrations of proteins added to the soil, i.e., 2.5 and 5.0 μg g⁻¹. When the paddy soils were spiked with the Cry1Ab proteins, more than 98% of the proteins were adsorbed (Table II, Fig. 2). The adsorption was almost completed within 1 h, despite a slight increase in the amounts of proteins adsorbed afterwards. The amount of adsorbed Cry1Ab proteins on the sandy loam soil was more for the non-sterile treatments than the sterile treatments. Similar trend for the differences between the sterile and non-sterile treatments were found.

### TABLE II
Concentrations of Cry1Ab proteins adsorbed on four differently textured paddy soilsa) with an initial Cry1Ab protein addition of 2.5 or 5.0 μg g⁻¹ soil

<table>
<thead>
<tr>
<th>Cry1Ab proteins added</th>
<th>Paddy soil</th>
<th>Cry1Ab protein concentration in sterile soil</th>
<th>Cry1Ab protein concentration in non-sterile soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μg g⁻¹ soil</td>
<td>μg g⁻¹ soil</td>
</tr>
<tr>
<td>2.5</td>
<td>Sandy loam</td>
<td>2.4907 ± 0.0008a)</td>
<td>2.495 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>Loam</td>
<td>2.4938 ± 0.0007</td>
<td>2.497 ± 0.0001</td>
</tr>
<tr>
<td></td>
<td>Silt loam</td>
<td>2.4927 ± 0.0004</td>
<td>2.497 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td>Silty clay</td>
<td>2.4964 ± 0.0007</td>
<td>2.497 ± 0.0008</td>
</tr>
<tr>
<td>5.0</td>
<td>Sandy loam</td>
<td>4.9406 ± 0.0015</td>
<td>4.976 ± 0.0005</td>
</tr>
<tr>
<td></td>
<td>Loam</td>
<td>4.9581 ± 0.0073</td>
<td>4.995 ± 0.0001</td>
</tr>
<tr>
<td></td>
<td>Silt loam</td>
<td>4.9439 ± 0.0032</td>
<td>4.987 ± 0.0008</td>
</tr>
<tr>
<td></td>
<td>Silty clay</td>
<td>4.995 ± 0.0004</td>
<td>4.997 ± 0.0001</td>
</tr>
</tbody>
</table>

a) Means ± standard errors (n = 3).

Fig. 2  Adsorption of Cry1Ab proteins on differently textured (sandy loam, loam, silt loam, and silty clay) paddy soils with an initial Cry1Ab protein addition of 2.5 (a) or 5.0 (b) μg g⁻¹ soil after 1 and 24 h. Vertical bars indicate standard errors of the means (n = 3).
on the other three paddy soils.

The proportion (> 98%) and rapidity (within 1 h) of adsorption observed indicated that paddy soils have a strong affinity for Cry1Ab proteins at a low Cry1Ab protein concentration (2.5 µg g\(^{-1}\) soil). When the initial concentration of Cry1Ab proteins was increased to 5.0 µg g\(^{-1}\) soil, there was a slight difference in protein adsorption among the four paddy soils: adsorption was lower on the sandy loam soil than the silty clay soil. Doerr et al. (2000) pointed out that sandy soils are particularly prone to hydrophobicity as expressed as soil water repellency, due to their smaller surface area in comparison to clayey soils. Therefore, sandy soils are more easily saturated with hydrophobic organic molecules (Helassa et al., 2011). This is consistent with the greater amount of Cry1Ab proteins adsorbed on the silty clay soil than the sandy loam soil (Table II).

Ideally, sterilization should inhibit microbial activity and have no effect on soil physicochemical properties, but such a sterilization method does not exist (McNamara et al., 2003). The heat from autoclaving may release labile carbon and carbon dioxide from soil organic matter and also induces mineralogical changes (Egli et al., 2006). Shaw et al. (1999) stated that abiotic changes to the soil during the sterilization process may complicate the interpretation of comparisons between sterile and non-sterile soils. Therefore, differences in the adsorption of Cry1Ab proteins between sterile and non-sterile paddy soils could be caused by the inactivation of microorganisms, and consequently less degradation (as discussed above), as well as by other abiotic changes during autoclaving.

**Desorption of Cry1Ab proteins adsorbed on paddy soils**

After adsorption, the desorption of Cry1Ab proteins from paddy soils was performed by washing with sterile H\(_2\)O\(_{MQ}\), and subsequent extraction with extraction buffer. The desorption efficiency of the water was much lower than that of the extraction buffer. No more than 2% of the adsorbed Cry1Ab proteins were desorbed from the four paddy soils by washing with sterile H\(_2\)O\(_{MQ}\) (Fig. 3).

A considerable proportion (20%–40%) of the adsorbed proteins was desorbed from the silt loam soil with the extraction buffer (Table III). Comparing desorption of Cry1Ab proteins from the silt loam soil, the amount of the desorbed proteins differed due to different initial concentrations of the proteins that were spiked into the soil (\(P < 0.05\)). The more the Cry1Ab proteins that were initially added, the more that proteins were desorbed. The concentrations of proteins desorbed from the non-sterile silt loam soil were ca. 0.7, 2.0, and 3.0 µg g\(^{-1}\) soil for the initial protein concentrations of 2.5, 5.0, and 10.0 µg g\(^{-1}\) soil, respectively (data not shown). However, when expressed as the percentage of adsorbed proteins desorbed, the desorption did not increase with increasing initial protein concentration (Table III). When the concentrations of proteins added to the soil increased from 5.0 to 10.0 µg g\(^{-1}\) soil, the relative desorption decreased from 40.3% to 31.5%, respectively. Desorption of Cry1Ab proteins was significantly higher from the sterile silt loam soil than the non-sterile silt loam soil (\(P < 0.05\)).

Studies on the desorption of Bt toxins using water have reported contrasting findings. Crecchio and Sto-
TABLE III
Desorption of the Cry1Ab proteins adsorbed on a silt loam paddy soil with an initial Cry1Ab protein addition of 2.5, 5.0, or 10.0 µg g\(^{-1}\) soil by washing with sterile Milli-Q\(^{\circledR}\) water (1st desorption) and subsequent extraction with a specific extraction buffer (2nd desorption)

<table>
<thead>
<tr>
<th>Cry1Ab proteins added</th>
<th>Cry1Ab proteins desorbed</th>
<th>Non-sterile soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st desorption</td>
<td>2nd desorption</td>
</tr>
<tr>
<td></td>
<td>Sterile soil</td>
<td></td>
</tr>
<tr>
<td>µg g(^{-1}) soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.14 ± 0.02(^{a,b})</td>
<td>26.2 ± 1.6a</td>
</tr>
<tr>
<td>5.0</td>
<td>1.16 ± 0.07b</td>
<td>39.1 ± 3.3c</td>
</tr>
<tr>
<td>10.0</td>
<td>1.12 ± 0.13b</td>
<td>30.4 ± 1.9b</td>
</tr>
</tbody>
</table>

\(^{a}\)Means ± standard errors (n = 3).

\(^{b}\)Means followed by different letters within each column are significantly different at P < 0.05.

Cry1Ab proteins were tightly bound on the soil. The toxins can remain in the soil and retain their insecticidal properties for up to 8 months (Tapp and Stotzky, 1995b, 1998; Palm et al., 1996). This could elicit and enhance the selection of target organisms, constituting a risk to the soil environment (Heckel, 1994; Alstad and Andow, 1995; Crecchio and Stotzky, 2001).

The paddy soil-Cry1Ab protein interaction was partly reversible, as the sterile H\(_2\)O\(_{MQ}\) and the specific extraction buffer desorbed a considerable amount of Cry1Ab proteins. Desorption of adsorbed Cry1Ab proteins from the other three paddy soils was far lower with H\(_2\)O\(_{MQ}\) than that with the specific extraction buffer (P < 0.05), which is consistent with the data obtained for the silt loam soil, as described above.

Different amounts of the Bt toxins (Cry1Ab proteins) were desorbed from the differently textured paddy soils. Desorption of the adsorbed proteins was lower from the silty clay soil than from the sandy loam soil. When the concentration of added Cry1Ab proteins was maintained at 2.5 µg g\(^{-1}\) soil, more Cry1Ab proteins were desorbed from the sandy loam soil than from the silty clay soil (Fig. 3a). When the Cry1Ab proteins were added at a concentration of 5.0 µg g\(^{-1}\) soil, the amount of Cry1Ab proteins desorbed from the sandy loam soil was 2.5 times as much as that from the silty clay soil (Fig. 3b). Desorption of Cry1Ab proteins from each paddy soil increased with increasing amounts of added proteins. When the initial concentration of Cry1Ab proteins increased from 2.5 to 5.0 µg g\(^{-1}\) soil, the amount of proteins desorbed from the sandy loam soil increased from 0.76 to 2.28 µg g\(^{-1}\) soil (data not shown), indicating an increase in relative desorption from 30.7% to 45.9%, respectively (Fig. 3). In addition, desorption with sterile H\(_2\)O\(_{MQ}\) was more at higher concentrations of Cry1Ab proteins in comparison to lower ones. Similar trends of the relative desorption were obtained for the other three paddy soils, i.e., the loam, silt loam, and silty clay soils.

The data indicated that the Cry1Ab proteins were more strongly bound on the silty clay soil than on the sandy loam soil, indicating a greater affinity for the silty clay soil. It is known that Bt toxins react with the organomineral surfaces of soils, and clay minerals play a dominant role due to their large specific surface areas and surface charges (Quiquampoix, 1987a; b; Quiquampoix and Burns, 2007; Helassa et al., 2011).
intensity of adsorption differed in the affinity for paddy soils with different textures. Once Cry1Ab proteins were adsorbed on the silty clay soil, the interaction between the Cry1Ab proteins and the silty clay soil became stronger with increasing time, and the proteins adsorbed were less desorbed in comparison to the sandy loam soil.

CONCLUSIONS

The affinity of Cry proteins has important implications for soil monitoring. The persistence of proteins adsorbed, which cannot be desorbed by either the sterile H2O_MQ or extraction buffer suggested that Cry proteins released from the cultivation of GM crops would accumulate in soil ecosystems. Soil properties, due to their complexity, should not be considered a simple factor affecting the fate of Cry proteins in paddy soil ecosystems.

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